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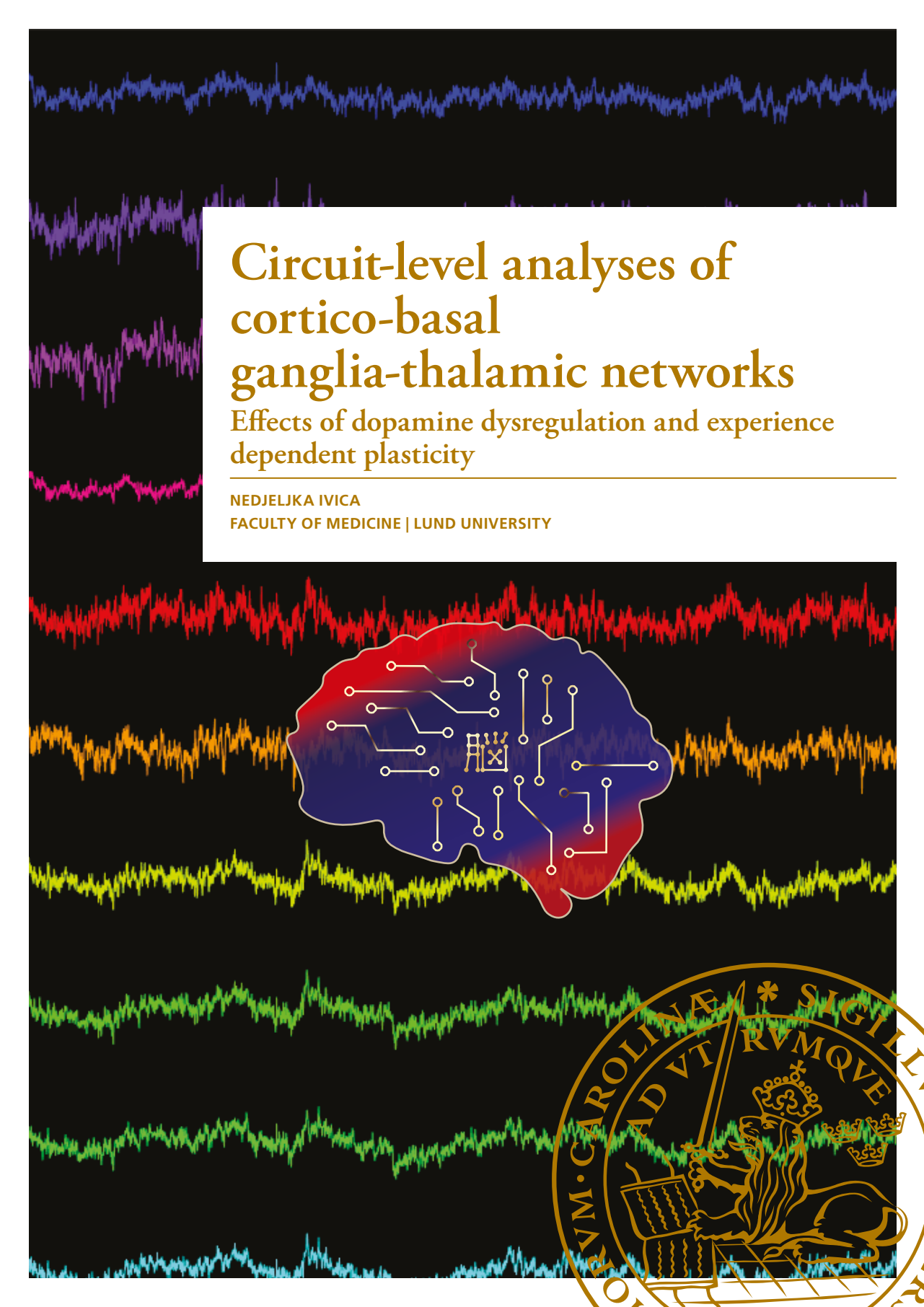
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Circuit-level analyses of cortico-basal ganglia-thalamic networks

Effects of dopamine dysregulation and experience
dependent plasticity

NEDJELJKA IVICA

FACULTY OF MEDICINE | LUND UNIVERSITY



Circuit-level analyses of cortico-basal ganglia-thalamic networks

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Effects of dopamine dysregulation and experience dependent plasticity

Nedjeljka Ivica



LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Abstract The cortico-basal ganglia-thalamic (CBT) circuit is thought to be involved in control of voluntary and goal-directed movements and action selection. Dopamine is known to play a crucial role in this circuit and regulating its activity. The important role of dopamine is particularly evident in Parkinson's patients, where dopaminergic cells are dying and motor impairments follow. While dopamine replacement is an effective therapy, satisfactory alleviation only lasts for a limited number of years, after which patients frequently develop side-effects in the form of levodopa-induced dyskinesia. In order to clarify the neurophysiological consequences of dopamine dysregulation we have here investigated the electrophysiological activity of each part of the CBT-loop in rats during different experimental conditions, using custom made multi-channel electrodes. Neuronal activity changes in 16 CBT structures were characterized upon acute pharmacological dopaminergic manipulations and firing rate changes of subgroup of cells within different structures in the CBT circuit were shown to potentially be responsible for the severe akinesia induced by the drugs. We have also developed a novel method to monitor the global state of the CBT circuit in a rat model of levodopa-induced dyskinesia and showed how this approach can be used to help developing new pharmacological therapies. Lastly, to investigate how somatosensory input is affecting motor circuits, we have recorded activity of the whole CBT-loop in rats before and after extensive skilled forelimb reaching and grasping training. Preliminary results show that only the motor cortex display experience-dependent changes due to the reaching training.		
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Nedjeljka Ivica



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Integrative Neurophysiology & Neurotechnology
Neuronano Research Center
Department of Experimental Medical Science
Faculty of Medicine
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Original papers included in this thesis

Paper I

Design of a high-density multi-channel electrode for multi-structure parallel recordings in rodents

Ivica N*, Tamté M*, Ahmed M, Richter U, Petersson P.

Conf Proc IEEE Eng Med Biol Soc. 2014; 2014:393-6. *Shared first authorship.

Paper II

Changes in neuronal activity of cortico-basal ganglia-thalamic networks induced by acute dopaminergic manipulations in rats

Ivica N, Richter U, Sjöbom J, Brys I, Tamté M, Petersson P.

Eur J Neurosci. 2017 Dec 18.

Supplementary material to Paper II

Paper III

Systems-level neurophysiological state characteristics for drug evaluation in an animal model of levodopa-induced dyskinesia

Tamté M, Brys I, Richter U, **Ivica N**, Halje P, Petersson P.

J Neurophysiol. 2016 Mar; 115(3):1713-29.

Paper IV

The organization of somatosensory information to cortico-basal ganglia motor circuits change as a consequence of intense motor practice

Nedjeljka Ivica, Ulrike Richter, Joel Sjöbom and Per Petersson. *Manuscript 2018.*

Supplementary material to Paper IV

Popular science text

The brain has always intrigued us. As it turns out quite often, the more we know about it, the more we are puzzled. Its complex processes and activity are crucial for choosing the best behavior from the repertoire of all behaviors when needed in different circumstances. In accordance with that, one of the most significant circuits involved in selection, initiation and evaluation of motor behavior is probably the cortico-basal ganglia-thalamic loop (CBT). Structures of the CBT loop are in a complex anatomical and functional relation to each other and are proposed to act on one another, such that when one increases activity it will affect the next structure in the line. Dopamine appears to have an important role in the normal function of this circuit in the control of motor actions. On top of that, there are two types of dopamine receptors which are suggested to direct the course of activity from striatum, an entry point of the basal ganglia structures, to subsequent structures. These different types of dopamine receptors appears to set a specific pathway when activated, called the direct and indirect pathway, which will, respectively, promote certain motor actions and inhibit unwanted actions. However, during years of research it was shown that the activity of this loop is not as simple as it was first suggested, primarily due to dopamine receptors being distributed in most of the structures of this circuit. This came to be obvious when observing pathophysiology behind Parkinson's disease (PD). In Parkinson's disease many motor and some non-motor symptoms are caused by the lack of dopamine in the CBT loop. Studies have found a rather intriguing appearance of certain brain oscillations, called beta oscillations ranging from 15-30 Hz, in both parkinsonian humans and in animal models that seemed to be pathological. While some concluded these beta oscillations were possibly causing the PD like motor symptoms, others have questioned if these oscillations are actually directly inducing symptoms of disease. Additional to that, while in some parkinsonian animal models, neuronal activity has been reported to follow the proposed diagram of the CBT loop, others have found discrepancies.

A common therapy for PD patients is externally supplemented dopamine in the form of levodopa which is then converted to dopamine in the brain by a specific enzyme; tyrosine hydroxylase. However, this therapy has a limited life-span due to inadvertently causing uncontrollable movements called dyskinesia within a few years of treatment. Considering that most of the physiological processes behind all these issues are still unknown, the focus of my research was the importance of this circuit in will-governed motor control. We decided to investigate the role of dopamine more in detail in the CBT circuit and how it works in animals lacking dopamine but is healthy otherwise. In order to inspect this, we developed a method where we can record and later examine neuronal activity from the whole CBT circuit in parallel in rats that were allowed to move freely. This was done by a chronic

implantation of hundreds of microelectrodes in all the parts of the CBT loop. Using these electrodes, we were then able to identify patterns of activity as the basis of specific behaviors. In particular, we focused on the animal's motor activity during different parts of their natural and drug induced behaviors. We investigated the role of dopamine by pharmacologically inducing depletion of dopamine in this circuit. This allowed us to continuously monitor changes in cell activity and communication in circuit connections associated with the transition between various pharmacologically induced states. Meanwhile, we also experimented with chronic animal models of Parkinson's disease. In this way, we studied both immediate and chronic effects of reduced dopamine levels. We found that in pharmacologically treated animals, beta oscillation and motor symptoms were not coupled and causative as some studies previously suggested, and that cells change their activity significantly under effect from almost all drugs, which were differently targeting either dopamine receptors or inducing a near complete depletion of dopamine. However, the change in neuronal activity did not resemble the proposed activity of the CBT loop in the most widely accepted conceptual model of PD. Nevertheless, we propose that change in neuronal activity occurring in single cells could be sufficient to cause motor symptoms, but needs to be clarified in further detail in future studies. Furthermore, we also decided to investigate appropriate methods to help us finding new therapies to overcome dyskinesia. We developed a novel method that could monitor global neurophysiological states that we could display as dots in coordinate system, greatly facilitating an intuitive interpretation of these complex data-sets.

While the CBT circuit is heavily affected under the dysregulation of dopamine and multiple structures change their activity, we also wanted to investigate how the healthy CBT circuit modifies its activity and perception of the outside world based on a new experiences. For any given motor action, we receive sensory information from the surface of the skin. Thus, we wanted to investigate whether the cells that are part of the motor control in the brain, such as cells in the CBT structures, change their processing of external stimuli under the influence of learning a new motor skill which inevitably affects received sensory information. This was accomplished by tactile stimulation of the rat's forepaw, while lightly anesthetized, and simultaneously recording the neuronal activity from all of the CBT circuit, both before and after skilled reaching and grasping motor training. Even though the results we present herein are preliminary, we indeed can see changes that we believe happen due to the skilled motor training. These changes are most obvious for motor cortex, in how it perceives different tactile stimuli after the training. The studies presented here are adding new bits of information to the function and physiology of the CBT circuit, however, much more is left to unravel and many more researchers will undoubtedly investigate CBT circuits for at least next few decades.

Znanstveno-popularni tekst

Mozak je oduvijek bio predmet interesa. Kako se ispostavilo vrlo često, što više znamo o mozgu, to nam je zagonetniji. Njegovi složeni procesi i cerebralna aktivnost ključni su za odabir optimalnog ponašanja iz repertoara ponašanja kada je to potrebno u različitim okolnostima. U skladu s tim, jedan od najznačajnijih krugova uključenih u odabir, pokretanje i vrednovanje motoričkog ponašanja je vjerojatno kortiko-bazalna ganglijsko-talamička petlja (KBT).

Strukture KBT petlje su u kompleksnom anatomskom i funkcionalnom odnosu i pretpostavlja se da djeluju jedna na drugu, na način da povećanje aktivnosti jedne strukture utječe na sljedeću strukturu u nizu. Izgleda da dopamin ima važnu ulogu u normalnoj funkciji ove petlje u kontroli motoričkih radnji. Povrh toga postoje dvije vrste dopaminskih receptora za koje se smatra da reguliraju tijek aktivnosti strijatuma, koji je ulazna točka bazalnih ganglijskih struktura, do sljedećih struktura u nizu. Čini se da ove različite vrste dopaminskih receptora uspostavljaju specifičan put kad se aktiviraju, nazvani izravan i neizravan put, od kojih prvi navedeni promiče određene motoričke aktivnosti, a drugi spriječava neželjene radnje. Međutim, tijekom godina istraživanja pokazalo se da aktivnost ove petlje nije tako jednostavna kao što je bilo prvi put predloženo, prvenstveno zbog dopaminskih receptora koji se nalaze na površini većine struktura KBT petlje, ne samo na površini strijatuma. Navedeno je postalo jasnije prilikom promatranja Parkinsonove bolesti (PB). U Parkinsonovoj bolesti mnogi motorički i neki ne-motorički simptomi su uzrokovani nedostatkom dopamina u KBT petlji. Studije su otkrile prilično zanimljive pojave određenih oscilacija u mozgu, nazvane beta oscilacije u rasponu od 15-30 Hz, koje su nađene i u čovjeka sa PB-om i u životinjskim modelima Parkinsonove bolesti, za koje se smatra da su patološki uvjetovane. Dok su neki zaključili da navedene beta oscilacije uzrokuju motoričke simptome Parkinsonove bolesti, drugi su doveli u pitanje da li ove oscilacije izravno induciraju simptome bolesti. Pri tome, dok je u nekim parkinsonskim modelima životinja zabilježeno da aktivnost živčanih stanica slijedi predloženi dijagram KBT petlje, drugi su pronašli odstupanja.

Uobičajena terapija za bolesnike s PB-om je supstitucija dopamina u obliku levodope koja se u mozgu pretvara u dopamin pomoću određenog enzima; tirozin hidroksilaze. Međutim, ova terapija ima ograničen vijek, jer unutar nekoliko godina liječenja dovodi do pojave nevoljno izazvanih nekontroliranih pokreta koji se nazivaju diskinezije. S obzirom da je većina fizioloških procesa iza svih ovih simptoma još uvijek nepoznata, fokus mog istraživanja je bila važnost KBT petlje u upravljanju motoričkim radnjama koje možemo kontrolirati.

Odlučili smo detaljnije istražiti ulogu dopamina u KBT mreži i kako funkcionira kod životinja bez dopamina, koje su inače zdrave. Da bismo bili u mogućnosti istražiti KBT mrežu, razvili smo metodu koja nam omogućava snimanje i kasnije

usporedbu neuronske aktivnosti iz cijele KBT mreže u isto vrijeme, u štakora koji se mogu slobodno kretati. To je izvedeno pomoću kronične implantacije stotine mikroelektroda u svim dijelovima KBT petlje. Koristeći ove elektrode, bili smo u stanju identificirati obrasce aktivnosti kao temelj specifičnih ponašanja. Posebno smo se usredotočili na motoričku aktivnost životinja tijekom različitih dijelova njihova prirodnog ponašanja i ponašanja induciranog lijekovima. Istražili smo ulogu dopamina pomoću farmakološki induciranog nedostatka dopamina u ovoj neuralnoj mreži. Ovako smo kontinuirano mogli pratiti promjene aktivnosti živčanih stanica i komunikacije između različitih struktura KBT petlje, a koje su mogle biti zbog farmakološki induciranih stanja. U međuvremenu smo eksperimentirali na kroničnim životinjskim modelima Parkinsonove bolesti. Na ovaj smo način proučavali neposredne i kronične učinke smanjene razine dopamina. Utvrdili smo da, kod farmakološki tretiranih životinja, beta oscilacije i motorički simptomi nisu bili povezani kao što su neka istraživanja prethodno predložila. Također smo pronašli da živčane stanice značajno mijenjaju svoju aktivnost pod djelovanjem gotovo svih primjenjenih farmakoloških manipulacija, koje su bile usmjerene na različite dopaminske receptore ili su dovele do gotovo potpunog osiromašjenja dopamina u KBT petlji. Međutim, promjena neuronske aktivnosti nije odgovarala predloženoj aktivnosti KBT petlje prihvaćene u najraširenijem konceptualnom modelu Parkinsonove bolesti. Ipak, mi predlažemo da je promjena neuronske aktivnosti koja se javlja u pojedinačnim stanicama mogla dovesti do motoričkih simptoma, ali da su potrebna daljnja istraživanja. Nadalje, odlučili smo istražiti i odgovarajuće metode za pomoć u pronalaženju novih terapija za svladavanje diskinezije. Razvili smo novu metodu koja može pratiti globalna neurofiziološka stanja koje možemo prikazati točkama u koordinatnom sustavu, što uvelike olakšava intuitivno tumačenje ovih složenih skupova podataka.

Dok je KBT mreža iznimno neuravnotežena pod disregulacijom dopamina i mnoge strukture mijenjaju svoju aktivnost, također smo željeli istražiti kako zdrava KBT mreža modificira svoju aktivnost i percepciju vanjskog svijeta pod utjecajem novih iskustava. Za bilo koju motoričku aktivnost mi primamo osjetne podražaje s površine kože. Stoga smo željeli istražiti da li živčane stanice koje su dio motoričke kontrole u mozgu, kao što su stanice u KBT strukturama, mijenjaju svoju obradu vanjskih podražaja pod utjecajem učenja nove motoričke vještine koja neizbježno utječe na primljene osjetilne informacije. To je postignuto taktilnom stimulacijom štakorske prednje šape, pod laganom anestezijom, dok smo istodobno bilježili neuronsku aktivnost iz cijelog KBT kruga prije i poslije treniranja štakora kako da posegne za hranom i ugrabi je, slično kao što bi čovjek. Iako su rezultati ovdje predstavljeni preliminarni, mi doista možemo vidjeti promjene koje se, vjerujemo, događaju zbog vještog motoričkog treninga prednje šape. Ove su promjene najočitije u primarnom motornom korteksu, u načinu na koji ova struktura percipira različite taktilne podražaje nakon treninga. U ovoj doktorskoj disertaciji sve prikazane studije pridonose novim spoznajama o funkciji i fiziologiji KBT mreže,

međutim, mnogo toga se još treba otkriti i mnogo više istraživača će nesumnjivo istraživati KBT mreže barem sljedećih nekoliko desetljeća.

Abbreviations

5-HT, 5-hydroxytryptamine, serotonin

6-OHDA, 6-hydroxydopamine

AADC, aromatic L-amino acid decarboxylation

AIM, abnormal involuntary movement

AMPT, alpha-methyl-p-tyrosine

AP, anteroposterior

BG, basal ganglia

CBT, cortico-basal ganglia-thalamic circuit

CM/Pf, centromedian and parafascicular nuclei

CNS, central nervous system

D1R/2R, dopamine 1/2 receptor type

DA, dopamine

DBS, deep brain stimulation

DLS, dorsolateral striatum

DMS, dorsomedial striatum

DV, dorsoventral

EN, entopeduncular nucleus

EP, evoked potential

GABA, γ -aminobutyric-acid

Glu, glutamatergic

GPI/e, globus pallidus pars interna and externa

INs, interneurons

ISI, inter-spike interval;

Levodopa (L-DOPA), L-3, 4-dihydroxyphenylalanine

LFP, local field potential

LID, levodopa-induced dyskinesia

M1, primary motor cortex

MHC, magnitude-squared coherence
ML, mediolateral
MSN, medium spiny neuron
PCs, principal cells
PD, Parkinson's disease
PSTH, peri-stimulus time histogram
RF, receptive field
RFA, rostral forelimb area
RT, reticular thalamic nucleus
SNc/r, substantia nigra pars compacta and reticulata
STA, spike-triggered average
STN, subthalamic nucleus
Thal, Thalamus
VL/VA, ventrolateral/ventroanterior nuclei
VPL, ventral posterior lateral nucleus
VPM, ventral posterior medial nucleus
VTA, ventral tegmental area

Summary

To learn about how the nervous system controls and guides our behavior has always been of interest, in particular, what role the brain and its different regions have. Humans and most animals physically move in space. Hence, observing behavior and monitoring at the same time neuronal activity can help us elucidate how movements originate in the brain. The basal ganglia, are tightly interconnected with thalamus, cortex and brainstem and have been suggested to be particularly relevant in the control of voluntary motor actions. The cortico-basal ganglia-thalamic (CBT) circuit has been proposed to be involved in goal-directed behavior and action selection. To observe activity in all parts of CBT circuit, firstly there was a need to improve the technology used to collect the electrophysiological data from all the structures of this circuit. For this purpose, a custom made multi-channel recording electrode was designed and tested *in vivo* to record from eight structures of the CBT circuit in each brain hemisphere. This electrode recorded both low-frequency activity of the population of the neurons (local field potentials, LFPs) and single or multi-cell unit activity. Additionally, it was made adjustable for different numbers of recording channels in different structures and implanted as a one piece electrode. This technological platform for neurophysiological recordings was used in all the subsequent studies.

In the CBT circuit, the neurotransmitter dopamine has a key role in regulating its activity, and lack of it can cause abnormal neurophysiological and behavioral patterns firstly observed in patients with Parkinson's disease (PD). Externally added dopamine, or rather the molecular precursor levodopa (L-DOPA), is one of the most used therapies in alleviating motor symptoms in PD patients. However, the therapeutic window lasts for only a few years, after which patients often develop another type of abnormal motor behavior, in the form of levodopa-induced dyskinesia (LID).

There are two proposed major pathways funneling information through the basal ganglia - the direct and indirect pathway, that are differentially influenced by the specific type of dopamine receptors (D1 or D2) which are primarily expressed by certain cells in the respective pathway. The direct pathway is believed to be involved in promoting and sustaining an action, while the indirect pathway is thought to be active in parallel to inhibit alternative actions. To observe in detail the consequences of different types of dopaminergic pharmacological interventions, we have here studied the effects of four different acute pharmacological manipulations involving both dopamine depletion and the blocking of each type of dopamine receptors separately and together. During these different experimental conditions, time electrophysiological and behavioral measurements were examined at the same time. We found that specific changes in firing rates in single cells might play the most crucial role in the most conspicuous type of motor dysfunction that are typically

observed in dopamine depleted animals - akinesia. Next, we used a well-established chronic model of LID, i.e. 6-hydroxydopamine (6-OHDA) unilateral lesions in rats which develop dyskinesia after L-DOPA treatment, to test for neurophysiological biomarkers in order to help treat LID. We confirmed previously reported neurophysiological patterns found in LID rats and expanded our analyses to characterize global neurophysiological states of the CBT network after treatments with experimental drugs that have previously been suggested to alleviate LID. The method we presented could be of great use for evaluating novel drug therapies, especially targeting conditions that do not show clear behavioral characteristics in animal models of the disease.

Lastly, to appropriately perform a movement in a given space, we rely on external sensory information from the surface of our skin. This suggests that sensory systems feed into the motor system and this notion has certain support from previous studies. To explore if we could observe experience dependent plasticity in cortico-basal ganglia motor circuits, rats were trained for skilled forelimb use, reaching and grasping for a small food pellet. In addition, the evoked responses to tactile stimuli applied to the glabrous skin of both forepaws was recorded from all parts of the CBT circuit before and after the training. Only one forepaw was trained, while other one served as a control. We also examined evoked activity both ipsilateral and contralateral to the stimulated paw. Similarly to sensory areas, preliminary results show that the side contralateral to the stimulated forepaw displays stronger activation than the ipsilateral side and cortico-striatal structures have the fastest response to the stimuli in the CBT circuit. Lastly, but most importantly, primary motor cortex is the only structure that shows sensorimotor changes that appear to arise as a consequence of the skilled forelimb training.

Introduction

Most creatures on this planet have an ability to physically move their body (beyond digestion movements), including species ranging all the way from microorganisms to larger animals. Individuals that are able to move have an evolutionary advantage over sessile organisms. They can in the most elementary form adapt their body posture toward beneficial environmental conditions or ultimately move distances in a pursuit for a better habitat. Most animals start to move physically from one place to the other early on in their maturity, some even as soon as they are first out in the world.

Humans also start to exhibit limb movements early on in their development. This happens already during the time in the womb, as fetuses, from eight week of life (de Vries et al. 1982), but it will take us several months after we are born before we are able to actually move by using our whole body, rather than just producing simple movements of our limbs. Though movement is ever present in human life and scientist have for long been investigating which systems of our body that are involved in the production of movements, and how we decide which movement we will make etc., much more is left to discover yet. In order to investigate more in depth the mechanisms underlying control of voluntary movements in vertebrates, scientist regularly employ animals in their experiments. Although much can be learnt from behavioral studies, there are also studies that involve more invasive methods probing functions of the nervous system, such as electrophysiology where we can measure electrical potential differences that occur during a movement both at the level of muscles and at the level of brain.

In this thesis I will introduce parts of the motor system of relevance for the control of voluntary movement, and compare the healthy to the diseased state in rat as experimental animal. In particular I will discuss disease conditions where the important neurotransmitter dopamine is not maintained at normal physiological levels. Furthermore, this thesis will provide some insights into the mechanisms behind the learning of a complex and novel motor skill and how the sensory input to motor systems in the brain is modified by learning of a novel motor skill.

Building blocks of the motor control system

Movements are a product of organized spatial and temporal patterns of muscle contractions that lead to a motor actions, whether they are voluntary or involuntary. Movements are coordinated by the neural circuits within brain and spinal cord. Thus, smooth and coordinates movement, in order to be executed properly, need several important sub-systems working together and in balance.

Periphery and Spinal cord

Muscles are controlled by the central nervous system together with peripheral nerves. This includes both voluntary movements and autonomous control. Autonomic actions such as intestine movements, breathing, respiratory rate, heart rate, etc. have independent control mechanisms and can only partly be modified by voluntary control. Several reflexes are controlled via relays through spinal cord and brainstem, generating stereotypic responses to the same type of stimulus even before the information about the stimulus actually has a chance to reach the brain. There are also motor patterns that are controlled by small control circuits that can be called into action by higher motor centers when needed (e.g. locomotion, chewing, scratching etc.). Typically for these types of movements they occur without conscious thought about the precise motor act.

Voluntary actions, on the other hand, are processed at higher motor centers under conscious control. These voluntary movements are then passed down and executed by the same parts of the musculo-skeletal system that also handles the more automatic actions. Muscles contract their muscle fibers after the stimulus delivered by lower motor neurons has passed a certain threshold. The axons of lower motor neurons are innervating muscle fibers, while their cell bodies are located in the ventral horn of spinal cord or in the brainstem motor nuclei. One lower motor neuron innervates many muscle fibers by its branching axons and creates a so called motor unit - the smallest unit that can be activated to produce a movement. Lower motor neurons normally get input from local circuit neurons, and more rarely directly from upper motor neurons. Local motor circuits are located in the spinal cord and they receive sensory input from skin, joints and muscles, as well as descending projections from supraspinal systems. Upper motor neurons have their cell bodies in the higher motor centers within the brain (brainstem and motor region of cerebral cortex), while their axons connect to local circuit neurons within spinal cord. They essentially carry information from all the higher motor centers such as motor cortex, brainstem, basal ganglia and cerebellum. The peripheral innervation of the skeletal muscles and its connections in spinal cord are organized in topographical manner. This means that the lower motor neurons that innervate muscles of a shoulder are

placed more medially within the spinal cord, while elbow muscles and more distal muscles in hand and fingers are positioned laterally to those (Purves 2008).

Somatosensory information is funneled to motor circuits

Sensory information from the skin, arising for example by activation of a certain muscle, is topographically organized in the projection to supraspinal centers in a corresponding way as the motor output. That means that peripheral nerves in a given skin area that reacts to a sensory stimulus will send this information forward to a particular part of the sensory cortex. Importantly this information will also reach motor cortex as well as the premotor network of the lower motor neurons in spinal cord and thereby potentially directly modify motor output.

This sensory information is collected by somatic sensory afferents, with cell bodies located in the dorsal root ganglia and with axons sending information to the dorsal horn of spinal cord. The stimulus at the skin surface alters permeability to cation channels in the nerve endings which ultimately develops a depolarizing current known as a receptor potential. The magnitude of the stimulus is proportional to the magnitude of depolarization, once the threshold reached its potential the signal is actively conducted in the afferent fiber. Special receptor cells (mechanoreceptors) are often encapsulating afferent fibers which are sensitive to different stimuli (touch/ proprioception). Afferent fibers that lack mechanoreceptors are called free nerve endings and they are in particular relevant in the sensation of pain and temperature (Purves 2008).

Brainstem and its descending motor tracts

The relevance of brainstem control centers is evident in the maintenance of balance, posture and in orienting visual gaze. These movements are controlled by the vestibular complex, the reticular formation and the superior colliculus. Even though these centers can operate without supervision of higher motor centers in cerebral cortex, they usually work together with motor cortex and the cerebellum in organization of volitional movements. Vestibular nuclei that receives information from the semicircular canals and the otolith organs specifies the position of the head and its tilt as well as translation and angular acceleration. Certain parts of this system projects to spinal cord, via the medial vestibulospinal tract where it sends information for the regulation of reflexive neck muscle activation in response to the movements of the head; and via the lateral vestibulospinal tract where it supervises proximal limb muscles in order to regulate balance and upright posture. Part of the projections from vestibular nuclei is also to cranial nerve motor nuclei that controls eye movements (the third, fourth and sixth cranial nerve).

The reticular formation encompasses clusters of neurons situated among axon bundles. It controls respiration, cardiovascular, coordination of eye movements, regulation of sleep and wakefulness, sensory motor reflexes, and temporal and spatial coordination of limbs and the trunk motor actions including modes of locomotion. In contrast to the vestibular nuclei which regulates rapid compensation to any postural instability via vestibular system in the inner ear, the reticular formation helps initiating rapid adjustments that stabilize posture during ongoing movements. It accomplishes this by feedforward mechanism by comparing the predicted change in posture due to a future planned movement with the current posture of the body before movement happened. Whereas the new movement is initiated by motor cortex that directly sends this information to spinal cord, the postural compensation for it comes indirectly to spinal cord by reticular formation which received the same information from motor cortex (cortico-reticulospinal pathway).

In non-humans the rubrospinal tract is relevant for adjusting distal musculature of the upper extremities (together with direct pathway from motor cortex). In adult humans nucleus ruber has an important role in the communication with cerebellum but, the role of the rubrospinal tract in direct control of lower motor neurons is less clear. The superior colliculus is influencing axial neck musculature and is of particular importance in generating orienting head movements, as well as eye movements (Purves 2008).

Motor cortex

The structure thought to be involved in planning, initiating and directing movement is the primary motor cortex. The direct projections from motor cortex to spinal cord provide speed and agility of movements, and directs highly skilled finger movements (Purves 2008).

The basic functional organization of the motor cortex has been known for a hundred years when G.Theodor Fritsch and Eduard Hitzig in 1870 showed that electrical stimulation of motor cortex evoked muscle contractions on the contralateral side of the body (Fritsch and Hitzig 2009). Later it was proposed that motor cortex in fact contains full spatial representation of the body's musculature. Whether this organization is directly linked to single muscles is however not clear. The experiments by Wilder Penfield, show that humans have a spatial body representations (or motor maps) where the movements that require greater details and finer motor control are represented by a greater area in the motor cortex (Penfield and Boldrey 1937). Primary motor cortex is functionally organized so that muscle groups moving the same part of the body are located more closely to each other in the primary motor area. It differs from the rest of motor cortex (premotor/supplementary) by its cytoarchitecture (Zilles and Amunts 2010, on

Brodmann's areas) as well as by a lower activation threshold for electrical stimulation to elicit muscle response in contralateral side of the body. Primary motor cortex consists of 6 layers, and pyramidal cells in the 5th layer send descending axons to the spinal cord. These cells are also called upper motor neurons. Some of these cells are referred to as Betz cells, which have the biggest soma of all nerve cells. These cells however account only for about 5 % of the projections to spinal cord, the rest comes from non-Betz pyramidal cells (Purves 2008). As pointed out the functional organization of the motor cortex is largely unknown and some more recent findings suggest that is actually organized based on the function of movements rather than individual muscle representation (Penfield and Boldrey 1937; Barinaga 1995; Graziano et al. 2005). According to this notation (see e.g. (Graziano et al. 2005) this representation of movements (elicited by somewhat longer current pulses) might be related to particularly important behaviors such as inspecting something in front of the eyes or putting it in the mouth.

Premotor cortex

Multiple regions of the frontal lobe cortices that are positioned rostral to primary motor cortex are involved in motor production in one way or another. The role of these interconnected premotor areas is complex and not fully known. These areas receive extensive multisensory information from the parietal lobes. They are believed to be involved in selecting and planning purposeful actions from the repertoire of possible relevant actions in a certain context, whether it is toward external or internal cues. This process of action selection is thought to also involve the basal ganglia and there are dense direct projections from the frontal cortex to the input nuclei of the basal ganglia (Purves 2008). These areas in interaction with parietal areas are also involved in imitational learning of motor skills by observing other performing a goal directed movement via 'mirror' motor neurons (Rizzolatti et al. 1996; Rizzolatti and Craighero 2004). Notably, Broca's area, involved in speech production is also located in this area.

Cerebellum

Densely packed cortex of cerebellum contains over 50% of neurons in the brain (layered in three layers). It consists of highly structured circuit of cells and axons, and one of the biggest cells of the whole brain - Purkinje cells are the providing the output. Another cluster of cells that is deep within white matter of cerebellum are deep cerebellar nuclei and they are the main output from cerebellum to the rest of the brain.

Cerebellum receives sensory input, input from spinal cord, muscles and other parts of the brain, and processes this information to in return regulate the

smoothness, correct timing of different muscles contractions and precision of the movement. It plays a big role in coordination and posture, as well as fine-tuning movements. It compares ongoing movement with the intended movement and corrects those motor error on-line. As a modulator of the movements, it has ability to also learn from previous motor errors in order to improve the future performance. Furthermore, cerebellum is relevant not only for motor control, but also has function in some cognitive processes, language, attention and some emotional responses (Purves 2008).

Basal ganglia structures

The basal ganglia (BG) are subcortical nuclei that are comprised of several different structures that are mutually interconnected and share related functions. These structures are striatum, pallidum, subthalamic nucleus (STN) and substantia nigra (SN). Their basic structure has been preserved throughout vertebrate evolution since cyclostomes such as lamprey (oldest group of vertebrates) to humans (Grillner et al. 2013; Steiner and Tseng 2017). They are known to be involved in action selection, the control of goal directed movements and the learning of a novel motor skills, but also in the evaluation of actions in terms of their potential reward (Balleine and O'Doherty 2010).

In the physiological control of movements, the basal ganglia are greatly cooperating with motor cortex, thalamus and brainstem. The basal ganglia are also involved in non-motor functions, which has become particularly evident in disorders of basal ganglia that typically lead to motor signs, but also to some non-motor problems such as cognitive and emotional dysfunction. This is perhaps not surprising, given that the basal ganglia have both motor and cognitive/limbic circuits. The motor components of the basal ganglia are striatum (caudate and putamen), globus pallidus, substantia nigra pars reticulata (SNr) and subthalamic nucleus (STN). They are involved in normal course of activation of voluntary movements and for smooth switching between commands that initiate and terminate movements. More limbic/cognitive components are the nucleus accumbens, ventral pallidum. With respect to dopaminergic innervation the substantia nigra pars compacta (SNc) primarily projects to the motor related parts of the striatum whereas the ventral parts of striatum are innervated by the ventral tegmental area (VTA) and are thought to have role in reward-associated learning.

Striatum

As previously mentioned, in primates the dorsal striatum is what is primarily motor related and it consists of the caudate and putamen, while ventral striatum consists

of nucleus accumbens and the olfactory tubercle, and belongs to the limbic system (Purves 2008). Because this thesis is focused on motor systems, those components will here be described more in depth.

Striatum meaning “striped” is the input zone of the basal ganglia (together with the STN), where all incoming axons from cerebral cortex, thalamus and brainstem deliver information onto the large dendritic trees of medium spiny neurons (MSNs). They, in turn, release inhibitory γ -aminobutyric acid (GABA) neurotransmitters via synapses located in globus pallidus and SNr (and SNc). A single cortical axon typically contact several MSNs. But at the same time a very large number of cortical axons contact each MSN (Kita 1996). Staining striatum with acetylcholinesterase, which inactivates acetylcholine, shows differentially stained regions, referred to as “striosomes” and “matrix” (Graybiel et al. 1991). Caudate and putamen receive different projections from cerebral cortex which reflect functional differences between these two nuclei. The corticostriatal pathway thus may be partly segregated based on different functions and this segregation may be kept via parallel but competing channels sending different types of information out of basal ganglia (Alexander et al. 1986).

The MSNs are the projection neurons out of striatum. They also receives innervation from thalamus (the intralaminar nuclei) and intrinsic interneurons in striatum. These inputs, in contrast to cortical inputs, tend to make synapses close to cell soma which can modulate information coming from cortical synaptic activation. The spontaneous activity of MSNs is low due to potassium channels that are open near resting potential. They close when the cell is sufficiently depolarized, typically via an abundance of excitatory cortical and thalamic inputs. Dopamine is thought to directly control the likelihood of transitioning between these down- and up-states via activation of D1 and D2 type receptors (Purves 2008).

Except MSNs, in striatum there are also 1) cholinergic interneurons that release acetylcholine, which is thought to modulate the activity of MSNs, and react to release of dopamine from SNc, and 2) other inhibitory interneurons, including fast-spiking interneurons that express parvalbumin, tonically active neurons (TANs) and other interneurons (further divided by the specific neurotransmitters or neuropeptides contained in them, Silberberg and Bolam 2015).

Dopamine and dopamine receptors

Dopamine is an organic chemical that acts as neurotransmitter and plays an important role in our brain. Abnormal signaling of this catecholamine and dysfunction of dopaminergic cells functions is implicated in several neurologic and neuropsychiatric disorders. In particular, it is crucial for appropriate functioning of basal ganglia. Indeed, its role as a neurotransmitter was recognized after it was identified as being essential to normal motor function. For example, in Parkinson’s disease (PD) lack of dopamine is known to be causing both motor and non-motor deficits in afflicted patients. The specific signs in each patients may differ somewhat

but typically patients are akinetic, rigid and with tremor, and may also have problems with gait and speech. Dopamine replacement therapy results in great improvement of symptoms of this disease but treatment effects are gradually complicated by side-effects.

Dopamine receptors are belonging to the group of G-protein coupled receptors. There are five distinct but closely related dopamine receptors (D1, D2, D3, D4 and D5) that are grouped into two groups: D1 type receptor and D2 type receptor. These were first characterized based on the biochemical reactions of dopamine being coupled with adenylyl cyclase (AC) activity and that only one subgroup was positively coupled to AC, while the other was independent of AC (Spano et al. 1978). Later on, distinctions of these two groups arose from their different pharmacological properties (Kebabian and Calne 1979). Subsequent studies involving genetic cloning of dopamine receptors showed that many dopamine receptors subtypes have depolarizing effect on the cells (Bunzow et al. 1988). Based on their pharmacological, biochemical and structural properties these receptors were classified as D1 type receptors (D1 and D5) (Tiberi et al. 1991) or D2 type receptors (D2, D3 and D4) (Andersen et al. 1990).

Dopamine receptors are present in many brain structures (Smith and Villalba 2008) as well as outside the central nervous system, with different abundance of each subtype (Beaulieu and Gainetdinov 2011). In the brain, dopamine receptors come in varying but relatively high level of density in the areas innervated by the nigrostriatal, mesolimbic, and mesocortical projections, such as the caudate-putamen (striatum), nucleus accumbens, substantia nigra, frontal cortex, etc. D1 type receptors are thought to be postsynaptic, while D2 type are both pre- and postsynaptic receptors giving them a more complex role. (Jaber et al. 1996). In general, D1 and D2 type receptors are suggested to have differential role within basal ganglia which will be further discussed below.

The indirect, direct pathway and hyperdirect pathways

The basal ganglia control of voluntary movements is thought to involve different pathways which act differently in the feedback loops onto cortex. There are two proposed main pathways and an additional one that is more modulatory/general. The so called direct pathway is said to have a role in allowing for movement to occur, while the indirect pathway stops certain movements from happening. These different pathways are activated by dopamine binding to different type of dopamine receptors in such way that dopamine binding to D1 type receptor is coding for activation of the direct pathway, while D2 type receptor is coding for inhibition of the indirect pathway.

The direct pathway ultimately excites cortex through disinhibition of thalamus by striatal MSNs directly inhibiting GPi/SNr, thus promoting cortical activity. The indirect pathway disinhibits STN which in turn send excitatory signals to GPi/SNr, so that thalamus is inhibited and ultimately cortex will not be excited by thalamus

(DeLong 1990). The hyperdirect pathway connects cortex directly to STN and is thought to allow for an immediate pause in motor actions (Nambu et al. 2002). This pathway thus acts in parallel to the other two striatal pathways

These complex loops become even more complex when considering that different dopamine receptors are located in many of these brain regions so that in the end direct dopamine binding in the different structures will influence how each structure will perform and thereby the outcome of the activation of these pathways.

Globus pallidus

In primates, globus pallidus includes globus pallidus interna (GPi) and externa (GPe, separated by a fibre bundle, internal medullary lamina; DeLong 1971). In rats, globus pallidus (GP) is equivalent to globus pallidus externa, while globus pallidus interna is entopeduncular nucleus (EN). Pallidus means pale which reflects the many myelinated axons in this nucleus. EN in rats and GPi in primates contain the same type of neurons as SNr and work closely together as an output of basal ganglia. For the easier reading I will refer to globus pallidus segments only as primate naming.

Globus pallidus cells have similar properties as substantia nigra cells, they are largely, tonically active and they exhibit inhibitory GABAergic output. GPi and GPe are classically considered to be part of the direct and indirect pathways (Albin et al. 1989; DeLong 1990). For the sensorimotor part of the basal ganglia-thalamic loop, GPi projects with inhibitory GABAergic connection to the ventroanterior and ventrolateral nuclei (VA/VL) of thalamus, which subsequently projects to motor areas of cerebral cortex. GPi receives inhibitory GABAergic input from striatum and, this circuit from the cortex via striatum and GPi constitutes the direct pathway. GPe projects with inhibitory GABAergic projection to the subthalamic nucleus (STN) and GPi but also back to striatum, while it receives inhibitory input from MSNs, primarily of D2R type and glutamatergic input from STN. This somewhat more elaborate circuit from cortex to thalamus constitutes the indirect pathway. Furthermore, GPe also projects to the reticular thalamic nucleus (RT), which is responsible for GABAergic input to other thalamic nuclei rather than projecting to cortex (as the rest of thalamus), hence modulating information in thalamus. Thus, by disinhibition of the thalamus via inhibition of the RT, GPe can indirectly influence thalamic output to cortex (Hazrati and Parent 1991a).

GPe has become an increasingly hot topic during the last years after more has been revealed about its anatomy, cell types and functionality (Gittis et al. 2014). It is known now that GPe consists of few different cell types and anatomical segments that contribute differently to motor and non-motor behavior. Already in 1971. DeLong in recordings in monkeys found that GPe and GPi neurons have distinct firing patterns, and that GPe patterns have two distinct neuron populations, while

GPe has one. He also found that there was a fourth neuronal population which was positioned along the borders of both GPi and GPe and also toward ventral pallidum, but concluded that it should not be regarded as part of neither GPi nor GPe. In GPe, two distinct neuronal firing patterns were described, one group that fires repeatedly with high frequency, followed by silent moments up to few seconds, while the other neuronal group fires with low frequency combined with bursts (DeLong 1971; Kita 2007). However, GPe was even after this finding treated as a homogenous structure that had same type of cells and had straight-forward role in the indirect pathway. The same way as the basal ganglia have, together with the role in sensorimotor integration, also an associative and limbic role, it has been confirmed that GPe itself is functionally subdivided (François et al. 2004; Grabli et al. 2004). In an anatomical study (Sato et al. 2000) it was shown that GPe has 4 types of projection neurons, respectively projecting to: 1) GPi, STN and SNr (<20%), 2) STN and SNr (> 50%), 3) GPi and STN (<20%), 4) striatum (<20%). This shows that GPe has a bigger influence on activity of the output of basal ganglia than previously thought.

However, electrophysiological recordings in rats still show two distinct firing patterns of GPe neurons (Benhamou et al. 2012). Based on this physiological division, GPe neurons have been divided into arkypallidal and prototypic GPe neurons. Arkypallidal cells construct about a fourth of GPe cells, express preproenkephalin (PPE), have a lower firing rate with bursts and they project to striatum, while prototypic cells fire tonically at high frequencies *in vivo*, often express parvalbumin (PV) and project to STN (Mallet et al. 2012; Abdi et al. 2015). From a broader functional perspective, basal ganglia are thought to be involved in action selection and the choosing of correct sets of motor actions to be performed but also in the generation of start and stop signals that initiates and terminates action sequences. It has, for example, been shown that arkypallidal neurons are relevant for the stop cues to be successfully carried out during a go/stop task (the rat was trained to place its nose in central point until the onset of a cue “go” that directed to move its head rapidly leftwards or rightwards, while on 30% of the trials, the cue “go” was followed by a “stop” cue that instructed the rat to leave its nose in central point) (Mallet et al. 2016).

Lastly, but importantly, GPe has been implicated heavily in BG dysfunction in Parkinson’s disease (PD) (Filion and Tremblay 1991a; Filion et al. 1991). In particular due to its projections to STN, a pathological over-active striatal inhibition of GPe that subsequently disinhibits STN would in Parkinson’s disease lead to abnormal STN and GPe oscillatory activity (Bevan et al. 2002), where effects of dopamine depletion are that GPe neurons begin to express low-frequency oscillatory activity and the STN exhibits more intense oscillatory activity (Magill et al. 2001). Notably, GPe neurons express abnormal bursting activity with reduced mean firing rate activity (Filion et al. 1991; Magill et al. 2001) compared to regularly tonic activity, which could bring potentially to synchronized or oscillatory neuronal firing patterns (Kita 2007). It is not fully understood how these changes in firing patterns

lead to 10-30Hz oscillatory activity found in GPe-STN (Bevan et al. 2002). However, STN and GPe are reciprocally connected nuclei and their glutamatergic and inhibitory network could likely be a cause of oscillatory activity and act as a pattern generator. This has to be further investigated as now we can look more specifically into how different GPe neurons affect dopamine depletion and PD pathophysiology.

Thalamus – motor nuclei

Thalamus is a main circuit node and a relay structure of sensory and motor information to cerebral cortex (Haber and Calzavara 2009). It consists of many nuclei, but the motor and somatosensory nuclei are covered in this thesis.

The subdivision of motor thalamus is different in different vertebrates. For example in cats there are four regions ventroanterior (VA), ventromedial (VM), ventrolateralanterior (VL_a) and ventrolateralposterior (VL_p) nuclei. In rats it is hard to distinguish between VA and VL, so they are often considered together (VA/VL). In primates motor thalamus is divided into further nuclei but the terminology used to refer to the different structures is not consistent (Hirai and Jones 1989; Bosch-Bouju et al. 2013). Motor thalamus is interconnected with cerebral cortex, and receives major inputs from cerebellum (dentate and interposed nucleus) and from output nuclei of the basal ganglia, SNr and GPi. While input from cerebellum and cortex are glutamatergic, input from BG is solely GABAergic. Additionally motor thalamus also receives input from reticular thalamic nucleus (Hazrati and Parent 1991b). While all neurons of motor thalamus receive input from all motor cortex and motor related areas, a neuron in motor thalamus receives either BG or cerebellar input (Yamamoto et al. 1984; Nambu et al. 1988). This displays how there is relatively little convergence between BG and cerebellar input in thalamus, but higher level of integration of different cortical inputs. Associative input from cortex comes mainly to VM and partially to VA, while premotor and motor cortices project to VA and VL. SNr gives input mainly to VM and VA, GPi to anterior part of VL, and cerebellum to posterior part of VL (Bosch-Bouju et al. 2013). It is likely that this complex organization of afferents coming to motor thalamus is needed for processes related to movement preparation adequately to be delivered to motor cortex. Cells in motor thalamus exhibit tonic firing but also have intrinsic properties for high frequency bursts for spikes, called low threshold calcium spike (LTS) bursts that happen due to T-type calcium channel of distinct properties (Jahnsen and Llinás 1984). These high frequency bursts mainly happen during slow wave activity or when animal is drowsy (Llinás and Steriade 2006; Bosch-Bouju et al. 2013).

The role of motor thalamus is regarded to be in control of posture, motor learning and controlling general movements. These conclusions come from lesion studies (reviewed in Bosch-Bouju et al. 2013), for example lesions of VA, VL_a and VL_p in

primate produce ataxia and dysmetria of contralateral arm (Bornscheigl and Asanuma 1987). Physiology of motor thalamus has often been speculated to be similar to what is known about sensory thalamus because of their proximity and similar interconnection with cortex. Major theory about sensory thalamus is distinction of its afferents to “drivers” and “modulators” (Sherman and Guillery 1998), which says that drivers significantly affect sensory thalamic neurons firing, while modulators don’t have such impact. These “drivers” and “modulators” are defined with several characteristics (Sherman and Guillery 1998, 2011). By what we know today, it is not obvious that motor thalamus acts the same way as sensory thalamus (Bosch-Bouju et al. 2013). Indeed, in the review by Bosch-Bouju et al. 2013, it is proposed that motor thalamus acts like a “super-integrator” assimilating information coming from cortical layer V, BG and cerebellum and actively processes it, and that there may be multiple drivers or “driver-like” inputs. BG and cerebellum on their own process and modify information coming from cortex before forwarding it to motor thalamus. Motor thalamus, after integrating all the incoming information and making sure that motivational and proprioceptive instructions are integrated, sends out highly refined motor plans to cortex to update preparation and performance of motor program. Since BG activity is significantly altered in PD, it is expected that motor thalamus also acts differently namely because of BG input but also due to dopaminergic receptors in thalamus (Sanchez-Gonzalez et al. 2005). However, reports of changes in motor thalamus activity are minor, but existent. For example, lesions of motor thalamus could abolish tremor and rigidity, as well as deep brain stimulation (DBS) applied to thalamic nuclei (reviewed in Bosch-Bouju et al. 2013).

Thalamus – somatosensory nuclei

Somatosensory pathways originating in the spinal cord, trigeminal complex and brainstem converge to ventral posterior complex of the thalamus (Gauriau and Bernard 2004) which is composed of the ventral posterior lateral nucleus (VPL) and ventral posterior medial nucleus (VPM).

Organization of this complex is completely somatotopic in such a way that somatosensory information arriving from trunk, limbs and posterior head terminate in VPL, while those from the face end up in VPM. VPL is proposed to be divided into rostral (rVPL), middle (mVPL), and caudal zones (cVPL), which are distinct non-overlapping regions processing different types of specific information (Francis et al. 2008). In specific, rVPL receives mainly proprioceptive input, mVPL cutaneous information with detailed representation of fore- and hindlimbs, while cVPL receives mainly nociceptive information and visceral stimuli. In the same study, the authors also found that size of receptive fields (RF) changes substantially along rostrocaudal axis of VPL. Receptive field is a specific region of a sensory

space (e.g. skin, visual field) where a neuron's firing rate is modified by the stimuli. The RF of rVPL and cVPL are broad, while those of mVPL are well-defined and somatotopically organized with the forelimbs represented medially and hindlimbs laterally. Motor cortex receives sensory information directly from thalamus, relayed through the border area between VPLo (pars oralis) and cVPL (Bornschlegl and Asanuma 1987). This was proven on a functional level, as there was a difference in hand orientation and finger manipulation recovery depending on if this region was lesioned or not, combined with somatosensory cortex removal.

Subthalamic nucleus

The subthalamic nucleus is located ventral to the thalamus. The principal neurons are glutamatergic, however there is also very small amount of GABAergic interneurons that could play an important role in the intrinsic functionality of STN (Lévesque and Parent 2005).

The STN, as mentioned above, participates in the indirect pathway where it receives inhibitory input from GPe, it is also part of the hyperdirect pathway receiving excitatory input directly from cortex. In addition it also receives glutamatergic projections from thalamus. STN projects to SNr, reticular formation, possibly striatum (Smith et al. 1990) and cortex (Jackson and Crossman 1981), GPi, as well as back to GPe (this is essentially creating a negative feedback loop after which GPe increases firing rate, which subsequently further inhibits STN). STN as GPe have similar subdivisions where the dorsolateral part projects to sensorimotor areas of the BG, ventromedial to association areas, while the medial part of nucleus is concerned with limbic areas (Parent and Hazrati 1995).

From the perspective of network dynamics, it has been proposed that the connection between GPe and STN has the role of a basal ganglia pacemaker and that this pacemaker could be the source of normal and pathological synchronized oscillatory activity in basal ganglia (Plenz and Kital 1999; Bevan et al. 2002, 2006; Sharott et al. 2005). In PD patients, pathological oscillations in the beta range (<30Hz) has been found in STN (Brown et al. 2001). For example, certain patients with tremor exhibited beta range frequency oscillations (15-30 Hz) in STN which could be reduced by voluntary movements and exogenous dopamine (Levy et al. 2000, 2002). STN oscillations may thus play a key role in motor symptoms of PD (Kühn et al. 2009).

STN is one of the most successful targets for neuromodulatory therapy in PD. Electrodes are implanted in STN where it is possible to re-set the abnormal oscillations that STN exhibits by high frequency stimulation, called deep brain stimulation (DBS) (Benabid et al. 1994; Limousin et al. 1998; Hammond et al. 2007). The mechanism behind DBS is not completely known. Because DBS (Benazzouz et al. 1993; Benabid et al. 1994) showed very similar results to lesioning

of STN (Bergman et al. 1990) in alleviating motor symptoms of PD, it was hypothesized that DBS reduces the firing rate of an excessively increased activity in STN (Filali et al. 2004; Meissner et al. 2005). However, this “inhibition” model of DBS is only one out of several hypotheses. It has also been proposed that “excitation” and “disruption” of pathological activity patterns may be the underlying mechanisms, this model assumes that DBS could differently activate both the implanted and neighboring structures (Chicken and Nambu 2016). Interestingly, it has also been shown that DBS can help patients not only in alleviating motor symptoms but also non-motor symptoms (Kurcova et al. 2018). Though STN is usually the main target, DBS is performed on other targets as well such as GPi (Chicken and Nambu 2013) and SNr (Tai et al. 2003).

Substantia nigra

Substantia nigra (in Latin black substance) consists of two parts, substantia nigra pars reticulata (SNr) and pars compacta (SNc). It is relevant in motor planning, reward seeking, eye movements etc. Both structures are directly involved in motor control.

While SNr is the output structure of BG, SNc provides dopaminergic input to all BG structures, cortex and thalamus. A relevant feature of SNc dopaminergic neurons is therefore that they are autonomous pacemakers bringing dopamine regularly to all innervated structures (Guzman et al. 2009). The death of dopaminergic neurons in PD, leads to many motor and non-motor symptoms, which shows how relevant SNc is in these aspects. SNc dysfunction therefore affects several functions such as fine motor functions involving the paw (Pioli et al. 2008), reward and motivational behavior (Ljungberg et al. 1992), temporal processing (Matell and Meck 2000), etc.

SNr receives the majority of its input from striatum (inhibitory GABAergic) directly or indirectly through GPe and STN (glutamatergic). Having an important role in motor control, SNr together with GPi is projecting to VA/VL thalamus, which finally projects to motor cortex. SNr is known to be involved in visual and oculomotor functions by projecting directly to superior colliculus allowing for saccades (Hikosaka and Wurtz 1983). However, direct SNr projections to brainstem nuclei are not limited only to eye movements, these projections also affect centres in brainstem relevant for postural control, muscle tone and locomotion (Takakusaki et al. 2003, 2011; Stephenson-Jones et al. 2011; Grillner and Robertson 2015, 2016).

As proposed in PD that indirect pathway becomes dominant over direct, SNr has a major role there as a tonically active output basal ganglia structure inhibiting VA/VL thalamus. Studies have found that SNr exhibits synchronized low beta power oscillations and increase in coherence of increase in power of low beta oscillations with motor cortex (Avila et al. 2010; Brazhnik et al. 2012). Besides

STN, SNr is one of the possible targets for DBS, although in combination with STN mainly in treatment for gait complications in PD (Weiss et al. 2011; Scholten et al. 2017).

The cortico-basal ganglia-thalamic loop – connecting the dots

The above discussed structures together make up the cortico-basal ganglia-thalamic loop. This loop form a motor circuit where information coming from cortex is returned to cortex through thalamus after being processed and modified in basal ganglia. It is through thalamocortical projections that basal ganglia finally affects motor cortex (Parent and Hazrati 1995).

As discussed, thalamus further sends massive projections to striatum from caudal intralaminar nuclear group which, in primates, comprises the centromedian and parafascicular nuclei (CM/Pf) which are providing entrance to attention-related stimuli to BG (Smith et al. 2009). These terminals are located at dendritic shafts on striatal neurons rather than on the heads of dendritic spines like other thalamic nuclei, thus anatomically evading any relation with dopaminergic terminals. While CM projects mainly to dorsolateral sensorimotor striatum, Pf projects to associative part of striatum. The output of BG also projects to CM/Pf creating reciprocal closed loop with BG that is suggested to be largely implicated in physiology of BG, together with input from cortex providing glutamatergic input, and having a distinct functionality (Smith et al. 2014).

As earlier mentioned BG also projects to brainstem nuclei directly, skipping thalamus in order to control for posture and locomotion, while also getting the same input as brainstem from cortex creating another level of modifier that goes through BG (Grillner 2015).

Furthermore, basal ganglia are also central structures in circuits that are involved in modulating non-motor aspects of behavior. These parallel ongoing loops originate from different regions of cerebral cortex that subsequently employs different subdivisions BG and thalamus, and finally influence areas of frontal lobe not directly related with premotor or motor cortices. Functional topography is largely conserved from cortex throughout BG then to thalamus and lastly back to cortex. This type of topographic organization of cortico-BG-thalamic circuit has led to a model of parallel and segregated loops involving discrete motor, limbic and cognitive pathways (Alexander et al. 1986, 1990; Alexander and Crutcher 1990; Middleton and Strick 2002). It has been proposed that there are five parallel but segregated pathways: two regarding skeletomotor and oculomotor areas of cortex (M1 and frontal eye field; FEF), and another three related to non-motor areas in frontal lobe which are dorsolateral prefrontal cortex, lateral orbitofrontal cortex and

anterior cingulate cortex (Alexander et al. 1986). These authors also speculate whether different cortico-BG-thalamic loops work in similar manner as sensorimotor part of the loop that is very well investigated. Most of the non-motor cortical areas have been proposed to be implicated in cognitive behavior, including memory, planning, attention (Goldman-Rakic 1987; Petrides 1995). BG projects largely to different prefrontal areas, volume similar to projection toward motor cortical areas, and these projections were segregated from those to motor areas and are also topographically organized. This displays BG's big influence on the cognitive operations of the frontal lobe (Middleton and Strick 2002). Even though of the mentioned anatomical pathways where topography from cortex to BG is preserved, there is evidence that these separate cortico-BG-thalamic pathways can affect each other (François et al. 1994; Haber et al. 2000; Kolomiets et al. 2001). This idea that for example the limbic loop could influence motor loop has been demonstrated in rats, where limbic striatum (ventral striatum) influences motor striatum (dorsolateral striatum) via striato-nigral-striatal (SNS) pathway and through dopaminergic neurons (Nauta et al. 1978; Haber et al. 2000).

Furthermore, different cortical areas show some convergence in their projections to striatum, more specifically it was demonstrated that reward and cognitive area in primate striatum receive converging input from different reward-processing and cognitive cortical area, which is proposed to mediate different aspect in incentive learning (Haber et al. 2006).

Finally, it is suggested that particularly thalamus could potentially be relevant structure for integration of these different information in order to deliver input to cortex about most appropriate behavior, whether it is motor or non-motor (reviewed in Haber and Calzavara 2009).

Learning a new motor skill

Learning in general requires integration of various inputs such as emotional, motivational, cognitive and motor functions, which is critical for developing new learned behaviors such as the execution of smoothly performed, goal-directed movements. The development and execution of appropriate behaviors to environmental stimuli requires constant updating and learning.

When one is learning a novel motor skill it most likely requires a thoughtful effort in improving the performance, but at some level during learning this is not necessary anymore and new motor skill is performed effortlessly. It has been suggested that plastic changes in basal ganglia influence this automation and learning process. For example, reaching and grasping is done in healthy people readily in everyday life and the time that is spent on thinking about is minimal. Rodents can also learn how to reach for the food and grasp. This has been a subject of scientific interest since

1930s where the for example purpose of handedness was discussed in this context (Peterson 1931). This work was continued by Whishaw and colleagues in a series of studies where he has investigated skilled forelimb reaching in rats with regard to kinematics, neural control and evolutionary aspect of reaching in the rat and other animals (Whishaw and Pellis 1990). He also confirmed that BG and sensorimotor cortex lesions are exhibiting similar deficits in skilled forelimb reaching performance and it is suggested that skilled forelimb use depends on shared neural management of these two systems (Whishaw et al. 1986).

Comparisons of the rat model of skilled forelimb reaching and grasping to humans have shown that the two are strikingly similar in hand/paw shaping during reaching despite some differences during grasping, where rats use the whole paw to grasp while humans use pincer grasp (Sacrey et al. 2009). Skilled reaching movements have been scored based on end point measure of attempts and success, biometric measures and rating movements from formal description of movements, and it has been shown that there exist remarkable similarities in reach-to-eat between rodents and human. This has enabled translational research where the rat model of skilled forelimb reaching is used to investigate pathology of BG, and more general brain damage and disease (Klein et al. 2012).

Integration of sensory information in motor systems

Movement control is greatly depending on sensory input and feedback, both for on-line correction while the movement is performed and off-line evaluation of the end result of a certain action. Thus, it has been logically assumed that sensory input must be fed back to the motor system for better control and performance of movements.

It has been proposed that sensory feedback is received by a given motor control area in the brain in a functional manner such that each area receives sensory input from the same region of the body that it is mechanically activating. This has been shown in Cebus monkeys in Rosén and Asanuma 1972 where the microstimulated area of the motor cortex that caused flexion of the digits was shown to also exhibit tactile receptive fields on the same side of the hand (glabrous skin) where the flexion occurred. This shows how the portion of the skin that was affected by the muscles involved in flexion is also sending a sensory feedback to the same neurons that were involved in creating the flexion movement initially. In the previously mentioned skilled reaching task, it is presumably very important that proper sensory input is available to enable successful reaching and grasping for food, since this type of movement involves translation of the hand/paw to the place where food is, shaping of the digits for grasping, grasping and subsequent withdrawal of the hand/paw to bring the food to the mouth.

It has been proposed that there are at least two sensory attention processes in this task, hand transport to the target and hand shaping that is temporarily coupled by visual guidance, and somatosensory feedback for grasping, and then withdrawal and release (reviewed in Sacrey and Whishaw 2012). This sort of economical use of sensory attention would enable efficiency in eating, provide enhanced neural control and reduce time between different components of this movement.

There are mainly two sensorimotor systems that were investigated more in detail on this matter, spinal cord and cerebellum. Both of these have been shown to have a modular organization such that each specific module relates to a specific muscle. In spinalized, decerebrated, unanesthetized rats, the spatial organization of cutaneous input to hindlimb withdrawal reflexes was monitored upon noxious stimulus. Nociceptive receptive fields of each muscle corresponded to the areas of the skin flexed by the muscle, which expanded later to slightly wider area of the skin not affected by muscle contraction after the threshold for reflexes decreased (Schouenborg et al. 1992). When sensorimotor transformations were studied in greater detail in the spinal nociceptive withdrawal reflex system in another study, it was confirmed that the withdrawal reflex system consists of reflex modules which are corresponding to the action of involved muscle (Schouenborg and Weng 1994). The authors have shown a strong relationship between the withdrawal pattern and the reflex gain distribution. That is, the relative sensitivity of each reflex pathway from different skin sites is adapted to the efficiency of each involved muscle to unload, or withdraw, the skin in the standing position.

This suggests that experience dependent processes in association with movements have had an imprint on this motor system allowing for the adequate sensorimotor transformation (Schouenborg and Weng 1994). Thus withdrawal reflexes appeared to be organized in a modular fashion with different modules, each acting mainly on one muscle. Furthermore, it was suggested and confirmed in young rats that each of these withdrawal reflex modules are self-organized in a manner that they learn what their performance is by spontaneous muscle activity, thus governing network plasticity (Pettersson et al. 2003). This spontaneous activity is reflected in muscle twitches that occur on particular atonic muscle in tactile contact with the environment during sleep and it is most likely arriving from spinal cord itself. Likewise, in the studies of climbing fiber projecting to the forelimb area of the anterior cerebellar lobe, detailed nociceptive topography and receptive fields in cats were found (Ekerot et al. 1991). The nociceptive receptive fields of these climbing fibers that receive information from certain muscles or groups of synergistic muscles through spinal cord reflex arcs, were ending in narrow sagittal strips called “microzones”. Moreover, the same organizational principle was shown here, where these specific “microzones” were influencing the same muscle or muscle group they were receiving the information from. Thus, a spinocerebellar functional organizational model was proposed where each of these discrete microcomplexes is represented in the cerebellar cortex (Apps and Garwicz 2005).

However, by now, there is no study that monitors the organization of sensory information in the motor system that encompasses the whole CBT circuit which is absolutely necessary to know in order to understand how this motor system in the brain learns from sensory information.

The need for improved treatment of Parkinson's disease

In this thesis I will discuss how the neuronal control of movements by cortico-basal ganglia-thalamic circuits changes in Parkinson's disease (PD), and point to possible pathophysiological mechanisms underlying motor symptoms.

Approximately 41 in 100 000 people are diagnosed with PD between 40 to 49 years old, but the prevalence increases steadily with age resulting in a total prevalence of 1-2% in the population above the age of 65 (Pringsheim et al. 2014). The most common therapy that alleviates motor symptoms is dopamine replacement in the form of levodopa (L-DOPA) administration, which is dopamine precursor. However, the therapeutic window of L-DOPA is frequently becoming more limited over the years as patients develop dyskinesia (uncontrollable movements) at the doses needed to alleviate the PD symptoms. Hence, there is an urgent need to investigate other therapeutic possibilities.

PD was described by James Parkinson in 1817, and even though there were a few new discoveries throughout decades (like the finding of Lewy bodies in the brains of diseased patients), it wasn't until 1950s after finding the crucial role of dopamine in this disease (Carlsson et al. 1958; Hornykiewicz 2006) that PD gained wider attention in the scientific world.

Fast-forward, today we know a lot more about PD and the neuronal circuits involved in it, but not nearly enough to say goodbye to this disease. Research on animal models involving dopamine depletion resulted in the proposal that abnormal activity within basal ganglia could arise from oscillations (Brown et al. 2001), synchronized activity of population of cells reflected in local field potentials (LFPs).

Another important aspect of the disease, are the side-effects of the most common therapy used. As low L-DOPA doses becomes less effective in alleviating the motor symptoms of PD, typically after several years of treatment, therapeutic L-DOPA doses instead commences to create a new set of motor problems, in the form of abnormal involuntary movements (AIMs). These are characterized by jerky moves, chorea (dance-like moves) or dystonia. It is not clear why this type of levodopa-induced dyskinesia (LID) appears, but it may be due to the combination of the exogenously added L-DOPA together with gradual DA-ergic cell degeneration (Cenci 2014). Several researchers concentrate on solving this problem, but to this date it is still not clear what exactly causes it and how to avoid it. A novel finding that may point to a mechanistic explanation, is a neurophysiological phenomenon

first found in the rat model of LID. As DA levels peak in the striatum this promotes the display of involuntary movements (Cenci 2014). Halje et al. 2012 proposed that in this situation the emergence of 80 Hz oscillations, especially in cortical regions and partly in striatal, leads to LID. The use of this phenomenon as a biomarker in the treatment of LID is further discussed in this thesis.

On the pathophysiology of Parkinson's disease

Degeneration of dopaminergic cells in SNc (and eventually VTA) leads to lack of dopamine in the projection areas of these cells. The impact of dopamine shortage is evident in PD patients, with abnormal basal ganglia function associated with noticeable motor symptoms. Patients experience troubles in moving normally, more specifically rigidity and bradykinesia and often tremor. Additionally, it was shown that beyond loss of dopaminergic cells, there is abundant loss of noradrenergic and serotonergic cells progressively into the disease, in locus coeruleus and dorsal raphe, respectively (Jellinger 1991; Delaville et al. 2011; Buddhala et al. 2015; Politis and Niccolini 2015). All these characteristics may also be associated with limbic and cognitive deficits.

In 1989 Albin and colleagues proposed a model of cortico-basal ganglia-thalamic network function that explained how different parts of the BG are involved in motor control. The model also explained what happens in different motor disorders and what lies behind their pathophysiology. These disorders involved Huntington disease (HD), hemiballism and Parkinson's disease, that is, both hyperkinetic and hypokinetic disorders. Two major features in this revised model of BG function were suggested compared to earlier views, the presence of different subgroups of striatal projection neurons that project to different targets and the idea that DA has different influence on these subtypes of striatal projections neurons (Albin et al. 1989). This model was further explained by Mahlon. R. DeLong in 1990 and it has remained textbook material that we all learn about in basic neuroscience courses (DeLong 1990). During normal physiological conditions, it has been suggested that the direct pathway is rather choosing which motor action will be performed, while indirect pathway stops any competing motor actions. This model, which is fundamentally based on changes in firing rate of neurons in different parts of the BG suggests that the activity of each structure affects the next structure in line that is connected anatomically and functionally (Fig. 1), while one of the two pathways is more or less activated based on which dopamine receptor type was stimulated. In the parkinsonian state, when the lack of dopamine is apparent, it is suggested that the activity of the indirect pathway is increased and, in this way, the inhibition of the competing movements is promoted. Thus, after striatum inhibits the GPe, this will subsequently disinhibit STN, which is going to stimulate GPi/SNr and ultimately inhibit thalamus that would excite cortex which would in the end lead to

even less action due to cortex not exciting striatum. The CBT circuit has been heavily investigated throughout years and has gotten slight modifications but the main idea has remained the same (Mink 1996).

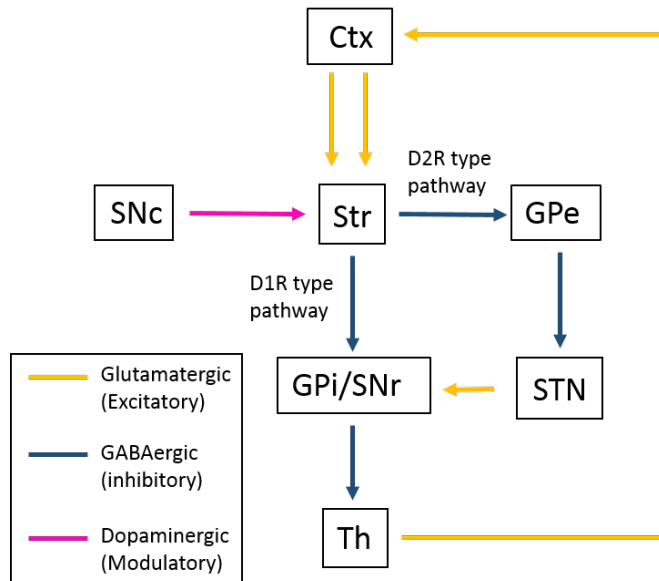


Figure 1. The classical model of the cortico-basal ganglia-thalamic circuit. Ctx: Cortex, Str: Striatum, GPi/e: Globus pallidus interna and externa, STN: Subthalamic nucleus, SNC/r: Substantia nigra pars compacta and reticulata, Th: Thalamus.

According to an alternative view, parkinsonism is caused by the disruption of normal cortical activities because of excessive beta-band oscillations and decrease in gamma-band oscillations (Hammond et al. 2007). Even though, some oscillatory states are relevant for normal functioning in the brain and in this case of CBT (reviewed in Singh 2018), exaggerated beta oscillation is suggested to be abnormal and possibly the cause of motor symptoms shown in PD (rigidity, bradykinesia, akinesia, tremor). More specifically, in humans, monkeys and rats, after dopaminergic depletion (or in PD) motor symptoms are present and it is noticeable in most of the CBT network that increase in power in beta frequency < 30Hz occurs; thus, this neurophysiological characteristic has been suggested to be the reason behind characteristic PD or PD-like motor symptoms (Sebban et al. 1999; Brown et al. 2001; Levy et al. 2002; Sharott et al. 2005; Avila et al. 2010; Dejean et al. 2011; Brazhnik et al. 2012; reviewed in Hammond et al. 2007; Singh 2018). This beta frequency is usually split to a lower (12- 20 Hz) and a higher portion (20 – 30 Hz), of which it is suggested that low beta is associated with parkinsonism while high

beta might not even be related to PD and possibly physiological, considering that inactive but healthy subjects exhibit it (reviewed in Hammond et al. 2007). In parkinsonian animals low beta oscillation is reduced slightly before and during the movement (Avila et al. 2010, reviewed in Hammond et al 2007), while high beta seems to appear during more active states (Sharott et al. 2005; Avila et al. 2010; Brazhnik et al. 2012).

Even though abnormal beta oscillations as a potential pathophysiological mechanisms has gained substantial support over the last ten years it has also been questioned by a few studies where akinesia and parkinsonian-like motor symptoms have been shown to appear before beta-band oscillation, and oscillations have appeared only at the later stage of dopamine depletion in lesioned hemiparkinsonian rats (Mallet et al. 2008; Degos et al. 2009). Deviating findings were also reported in the same studies when the authors compared the lesioned parkinsonian rat model to pharmacologically induced dopamine depleted rat model and found that pharmacological models did not exhibit beta oscillations, in urethane-anesthetized rats (Mallet et al. 2008) or in awake behaving rats (Degos et al. 2009), although the animals developed akinesia. Conversely, other studies have shown that even after acute pharmacological manipulations and dopamine depletion, animals indeed display abnormal beta oscillations as well as akinesia (Sebban et al. 1999; Dejean et al. 2009, 2011). This inconsistency between physiological findings and motor deficits is quite intriguing and thus needs to be investigated further.

Furthermore, when looking at the firing rates in the CBT circuit, there are also clearly differences from expected changes based on Albin-DeLong model in chronic animals (Miller and DeLong 1987; Fillion and Tremblay 1991b; Bergman et al. 1994) and acute pharmacological dopamine manipulated animals where mixed results have been reported (Fillion 1979; Costa et al. 2006; Burkhardt et al. 2009).

Clearly, a further physiological characterization of the cortico-basal ganglia-thalamic system is needed to clarify in greater detail how the different structures of this circuit influence the activity of others and pinpoint how malfunctioning of certain cell groups eventually leads to symptoms of the disease. This has been the main objective of this thesis.

Aims

- I. To create a technological platform for high-density recordings in CBT networks in rodents.
- II. To characterize the role of dopamine in CBT circuits by acute pharmacological manipulation.
- III. To characterize the role of dopamine in CBT network that is super-sensitized to dopamine stimulation.
- IV. To characterize plasticity in the organization of somatosensory input to CBT networks as a consequence of extensive training.

Methods

Electrophysiology as research methodology

Until now, I have been describing the cortico-basal ganglia-thalamic (CBT) circuit and its involvement in motor control. Thus, if we would want to observe control of motor actions in these brain structures we need to grasp how to achieve this and what could be the best methods to use in order to reach our goals. For decades, the structures that I have described in the Introduction of this thesis have been investigated in order to understand how they all come together when choosing a certain volitional act, or when learning a new action or simply to be able to control a certain behavior.

For some of these brain structures it was more straightforward to conclude which physiological functions they mainly are involved in. For example, motor cortex is involved in learning a novel motor skill (lever pressing twice with prescribed temporal sequence), but not executing it once the skill was learned, which was evident after motor cortex lesion; still, the rats were only able to learn the skill if the motor cortex was intact (Kawai et al. 2015). However, most of CBT structures still have complicated operation to understand entirely their role in this matter (for example motor thalamus with questionable changes under Parkinson's disease compared to other cortico-basal ganglia structures (reviewed in Bosch-Bouju et al. 2013), or GPe with already mentioned more complex structure and projections than previously thought). There are potentially, multiple ways (methods) to gather knowledge about cortico-basal ganglia-thalamic network, and beyond that also different levels of depth we could investigate each structure from this circuit and the circuit itself. This can range from observing mechanisms at their smallest molecular level within the cells, receptors, proteins, etc. (e.g. observing metabolic activity in (Rolland et al. 2006) to those observing millions of cells at once at the macro level in order to record their activity (such as fMRI, (Oguri et al. 2013)).

It would be optimal to be able to observe the physiology of these structures while they at their most natural state in a live organism during the movement. Most interesting and ultimately informative is collecting the data on exact activity of the neural cells together with the activity of populations of the cells in the structures of interest. To measure the activity of the cells, reflecting the communication that happens between them, we can measure electrical voltage changes that occur due to changes in concentration on a very small scale of distinct ions which carry positive

or negative charges. This particular method called electrophysiology was somewhat introduced in the 1660s when Dutch scientist Jan Swammerdam discovered excitable properties of frog's leg muscle and could in principle use needles attached for the muscle for contraction recording (although it is not known if such recording was ever made on a charcoaled paper). However, Luigi Galvani demonstrated first generation of action potential and discovered "animal electricity" by experimenting over decades on tissues reacting to external stimuli and creating electrical signals and so starting up true electrophysiology era (reviewed in (Verkhatsky et al. 2006). Experimental electrophysiology has since matured and has been developed for both in vivo and in vitro studies. Both of these alternatives are invasive, but on the other hand they offer a closer picture of all the cells behavior in a local volume than non-invasive methods such as fMRI or electroencephalography that collect change in electric potentials reflecting the activity of much larger populations of cells. Furthermore, in vivo electrophysiology can offer an image of what is really happening at the neuronal level in an animal that is able to behave ordinarily.

Hence, for my research questions, the only viable option was to use electrophysiological techniques for recording brain activity at the cell level in freely moving animals. When I started my PhD-studies, ordinarily, scientists were not able to record from multiple structures of CBT circuit in parallel. Electrophysiological studies, thus, often show bilateral recordings of single cell activity and LFPs from one structure (SNr) (Avila et al. 2010) or two structures in parallel (GPi and STN, Bergman et al. 1994) in the CBT circuit, here particularly, with an aim to monitor changes in CBT occurring after induction of a hypodopaminergic state. Importantly, there was no existing technology that would allow me to perform recordings in all parts of the cortico-basal ganglia-thalamic circuit in parallel. Consequently, the development of such a technology became my first research goal.

Rat as experimental animal model

While a lot of fruitful research in Neuroscience have been carried out in invertebrates (e.g. *Caenorhabditis elegans*, *Drosophila melanogaster*), or in fishes (e.g. *Danio rerio*, lamprey) and birds (e.g. *Taeniopygia guttata*), mammals are much more widely used, primarily since there are closer in evolutionary line to humans. Cats and monkeys were very popular animal models in Neuroscience in the past and even though they are still in use as animal models, rodents have gained more in popularity over last few decades. Furthermore, rodents are also widely used as animal models in other scientific fields beyond Neuroscience. They are preferred due to their low cost of maintenance and small size so they can be kept in confined spaces, high reproductive speed, and life span of about two years which is sufficient

for many scientific questions to be investigated. Especially mice, and more seldom rats, can be genetically engineered to precisely fit certain research questions.

Rodents are also able to learn certain skills similar to humans (e.g. skilled forelimb reaching -mentioned in Introduction) and beyond that, they have a similar brain structure and functions to humans in several ways and thus are often used as translational model for human diseases affecting the central nervous system (CNS), and for many other diseases (McGonigle 2014; McGonigle and Ruggeri 2014). Hence, the rodent was selected as a preferred animal model for scientific studies I was working on throughout my PhD education. More specifically, considering that electrophysiology in a live organism was the preferred method to investigate neuronal activity from cortico-basal ganglia- thalamic regions, rat was the more suitable choice over mouse considering their larger brain size and their milder composed behavior.

6- hydroxydopamine (6-OHDA) hemiparkinsonian rat model

As already mentioned in Introduction, Parkinson's disease is one of the most abundant neurological disorders that increasingly affects aging population and it is tightly connected with abnormal functions of the CBT network. There is consequently a strong interest to understand this disease better and to develop new approaches to how we can cure it. Hence, it has been of considerable interest to create animal models of PD which could help us investigating this matter. Even though there are multiple models used in different contexts (for example: 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) in non-human primates and mice, or alpha-synuclein model in rats), the 6-OHDA model of PD has become one of most favored (reviewed in Francardo 2017).

It has been about 50 years since Ungerstedt first showed 6-OHDA to be affecting both noradrenergic and dopaminergic neuron degeneration (Ungerstedt 1968). This notion motivated researchers to perfect this model as a rat model of PD to resemble the human disease better by mapping which region is the best to lesion. Nowadays, it is a common rat model of PD. In the unilateral PD model lesions are typically directed to the medial forebrain bundle, which results in loss of the majority of dopaminergic cells in SNc and VTA. This model involves nigrostriatal DA degeneration, partial degeneration of serotonergic and noradrenergic system, PD-like motor deficits, and is therefore regarded as a satisfactory model to observe early and, in particular, late stages of PD (reviewed in Cenci 2014 and Francardo 2017). The 6-OHDA toxin induces an acute but permanent damage, but also causes long-term plastic changes that, resemble human PD. These changes seem to occur in a somewhat biphasic manner, where the majority of DA innervation is lost within 1

week post-lesion in rat, while subsequently having another step of mild DA innervation loss during next months (Björklund et al. 1997).

Even though, unilateral 6-OHDA rat model shows instant motor deficits on the contralateral side of the body to the lesion such as limb-use asymmetry, some studies report that it is only after several days, that they notice neurophysiological characteristic signatures of PD symptoms (Mallet et al. 2008; Degos et al. 2009) e.g. increase in power of <30 frequency in LFPs (Brown et al. 2001 in human; Sharott et al. 2005 in rat). However, in those studies (Mallet et al. 2008; Degos et al. 2009) it was reported that akinesia appeared before exaggerated beta synchronization has been recorded. This animal model of PD was hence optimal to use in some of presented studies in this thesis.

Acute pharmacological dopamine depletion rat model

Acute pharmacological models that exhibit some PD symptoms are used in research, such as reserpine or alphas-methyl-p-tyrosine (AMPT). Both of these affect more than just the dopamine system in the brain. Reserpine inhibits vesicular monoamine transporter (VMAT2), and this way it depletes storages of monoamines (dopamine, serotonin, noradrenaline) and in this sense resembles PD biochemistry (Jellinger 1991; Delaville et al. 2011; Buddhala et al. 2015; Politis and Niccolini 2015). Its effect is transient (recovery within 24 h) and it is not reflecting the long-term changes in network properties thought to be an integral part of PD and thus is mainly beneficial to look into symptoms of PD (such as rigidity, akinesia) and their possible alleviation through different treatments (reviewed in Duty and Jenner 2011). AMPT however acts by inhibiting tyrosine hydroxylase, an enzyme that converts the amino acid L-tyrosine to the dopamine precursor L-DOPA, and thereby depleting endogenous dopamine. It also affects noradrenaline levels due to these two transmitter substances sharing the same pathway of synthesis. Its effect is also transient and there is an appearance of PD-like symptoms, such as rigidity and akinesia (which can be measured by a catalepsy test - time measured where animals are unable to move away from a horizontal bar on which they are placed with their forepaws; Sotnikova et al. 2005).

In Paper II we have shown what happens in LFPs and on the single cell level in parallel recordings in the CBT circuit after a rat has been injected with AMPT. We could here observe the temporal course of acute dopamine depletion and its effect on these brain structures. At the same time, we validated and compared the observed symptoms and neurophysiological patterns with the 6-OHDA chronic model. Furthermore, considering that dopamine binds to both D1 and D2 type of receptors, we also investigated the same features after injecting drugs that are more specific for one or the other type of dopamine receptor (SCH23390 as D1R antagonist,

haloperidol as D2R antagonist). These drugs also induced transient rigidity, akinesia and increase in power in beta frequency (< 30 Hz), and thus also showed similarity to PD symptoms (Sebban et al. 1999; Dejean et al. 2009, 2011). Including drugs that particularly aim at dopamine receptors was to help us elucidating how the two major pathways of the basal ganglia react to dopamine depletion and what symptoms we can observe. For yet another comparison, and as a control, we have also compared the effects of injection of a combination of these two drugs (D1/D2R antagonists).

While all of these pharmacological manipulations of dopamine levels are transient and acute, and in this way not resembling PD in the long-term plastic changes (reorganization of synaptic connections or changes in cell activation occurring due to dopaminergic cell death) of the disease, they still have the advantage that we can monitor changes that occur during the decrease in dopamine in one animal multiple times against an untreated baseline and we can elucidate the time course of reactions to the drug for each monitored structure. Therefore, acute pharmacological models aim more at explaining the occurrence of symptoms that resemble symptoms in PD, than to study pharmacotherapy (Duty and Jenner 2011).

Levodopa-induced dyskinesia (LID) rat model

The most common treatment of PD is externally added dopamine in the form of L-DOPA. L-DOPA has limited power in treating PD after a few years as many patients exhibit LID. The 6-OHDA unilateral lesion in rats is currently the favored animal model in most studies investigating processes around LID (reviewed in Francardo 2017). Rats that are lesioned are given daily therapeutic dose of L-DOPA and within a few days start to show abnormal involuntary movements (AIMs) affecting the muscles of the body contralateral to the lesion side. These AIMs involved limb, trunk and orofacial region, and they are measured by various tests. The tests score for AIMs, observing rotations and assessments of spontaneous limb use such as cylinder test, grid test, etc.

When these animals are treated with the drugs that are used for their antidyskinetic properties in PD patients, it was reported that some of the AIMs have decreased (axial, limb and orolingual), however at the same time this did not affect L-DOPA induced rotations. Thus, it has been validated that this model represents good rat model for LID (Cenci et al. 2002; Lundblad et al. 2002). Additionally, when recording electrophysiologically M1 and DLS in this rat model of LID, it has been shown that dyskinetic state exhibits significant increase in power in fairly narrow band around 80 Hz, especially in M1 (Halje et al. 2012). This particular oscillation, at around 80 Hz, was stopped by topically administrating D1 antagonist to M1. Moreover, mentioned newly found oscillation under the dyskinetic state, has become a valuable neurophysiological feature to look for in dyskinetic animals and

evaluating therapeutic effects of experimental drugs in order to stop dyskinetic movements.

In Paper III, we used this rat model for exactly that goal, to experiment with different experimental pharmacological substances that could potentially lead to abolition of dyskinetic symptoms and that could help us identify neurophysiological different states after effects of each drug.

Improving research method and techniques

Conventional electrophysiological recordings in rats are somewhat limited, due to challenging implantations and size of electrodes. We wanted to overcome the limitation of recording activity from only a few implanted electrodes and therefore only a few cells at once, thus to be able to record from many cells in different structures of CBT circuit at the same time. For this, we designed a custom built multi-channel electrode that is capable of chronic recording of data *in parallel* from multiple structures in freely behaving rats (Paper I). This would allow us to gather extensive amount of data from several CBT structures that are recorded at the same time during the same behavior. This electrode design is flexible with respect to number and position of electrodes arranged in different structures, implanted as one-piece and it is tailored for implantation in small experimental animals such as rodents. It is supporting recording of both local field potentials (LFPs), which reflect activity of population of the cells, as well as single cell activity. This type of electrode was used not only in all the described projects in this thesis, but in all subsequent projects in our lab. Its specific design has been crucial for obtaining this unique type of simultaneous electrophysiological recordings in rats that can bring a new level of understanding to how interconnected networks in multiple structures work jointly. Hence, introducing novel findings to the scientific community in demonstrating the underpinnings of physiological processes at a systems level.

In Paper I, it is described in detail how such a custom build electrode is constructed, as well as an example demonstration of one functional recording with this electrode. This electrode supports 128 recording channels, 16 reference channels and 6 stimulating channels when making full usage of our recording system (*Neuralynx Inc.*). Considering the amount of channels available and the relative sizes of structures, we were able to record from eight different structures in each hemisphere of the CBT circuit at the same time (16 structures in total) in these experiments. These structures were Rostral Forelimb Area (RFA - a rodent supplementary motor area), primary motor cortex (MI) encompassing area of the forelimb representation, dorsolateral striatum (DLS), dorsomedial striatum (DMS), globus pallidus (GP), ventrolateral/ventroanterior nuclei of the thalamus (VL/VA), subthalamic nucleus (STN) and substantia nigra pars reticulata (SNr) (Fig. 2).

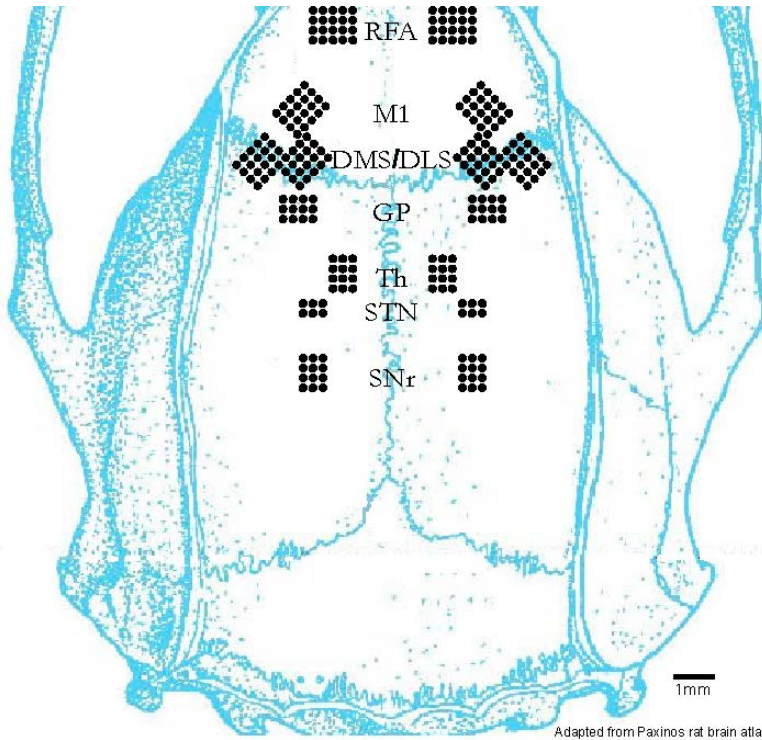


Figure 2. Dorsal view of the rat skull with overlaid antero-posterior (AP) and medio-lateral (ML) coordinates of the CBT for implantation and building of the multi-channel recording electrode. Th- VLVA nuclei of thalamus

The layout of the electrode consisted of minimum five recording channels, with up to 10 recording channels for bigger structures and one reference channel in each of these structures. This means that there was in total 144 tungsten wires (33 μm) that needed to be correctly positioned by antero-posterior (AP), medio-lateral (ML) and dorso-ventral (DV) coordinates for the respective structures. These wires were connected by a custom made printed circuit board (PCB) that subsequently connected to Kyocera connectors (Fig. 3). The electrode was grounded by silver wire which was also connected to the skull screws. Ultimately, implanted electrodes were connected via custom made adaptors to pre-amplifiers and cables to the hardware.

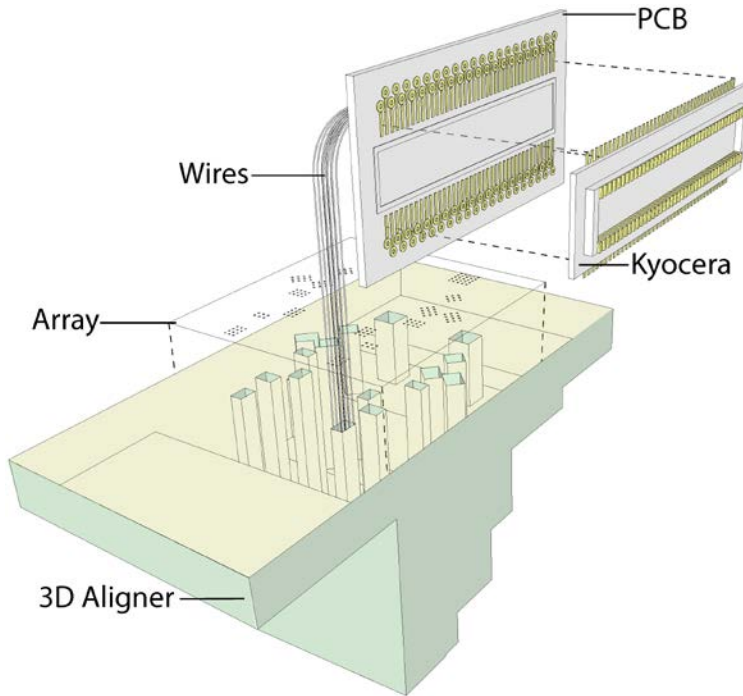


Figure 3. Schematic overview of the building process of the custom made multi-channel electrode.

This electrode was successfully used in an example experiment where parallel coherence measures are shown for all pairs of structures (Fig.4). This type of measurement is in principle relevant for measuring relation between two anatomically and physiologically connected structures, where phase-locking of oscillations at different frequencies is monitored to assess causality. Importantly, it was not possible to measure connectivity between multiple structures in such way before due to inability to record from multiple structures in parallel. In this experiment, 6-OHDA unilaterally lesioned rat was recorded with this newly designed electrode during the baseline and during the levodopa effect from both intact and lesioned hemisphere (notably, after injection of levodopa this animal displayed levodopa-induced dyskinesia).

Spectral power of each LFP signal and coherence measurement of pairs of LFP signals were computed with multitaper method implanted in Chronux 2.0. Power spectrograms were normalized to its pink noise signal (Halje et al. 2012), and finally mean power spectrum was created for each structure. Since there were also several measures of coherence due to multiple pairs of channels in each structure, mean magnitude-squared coherence (MHC) was created by variance-stabilizing transform for the coherence (arc-tanh, Pesaran 2008).

When looking at mean (MSCs) between RFA and DLS (Fig.4), there are two marked arrows (black and red) in the intact and lesioned hemisphere. These arrows indicate two distinct frequency bands, lower <30 Hz and higher >80 Hz, in the lesioned hemisphere but not in the intact, showing distinguishable difference between these two hemispheres and between two different states (on/off levodopa). As discussed, lower frequency oscillations (<30 Hz) are commonly shown in PD patients (Brown et al. 2001) and 6-OHDA lesioned rats (Sharott et al. 2005), while the frequency band around 80 Hz is displayed in dyskinetic state (Halje et al. 2012).

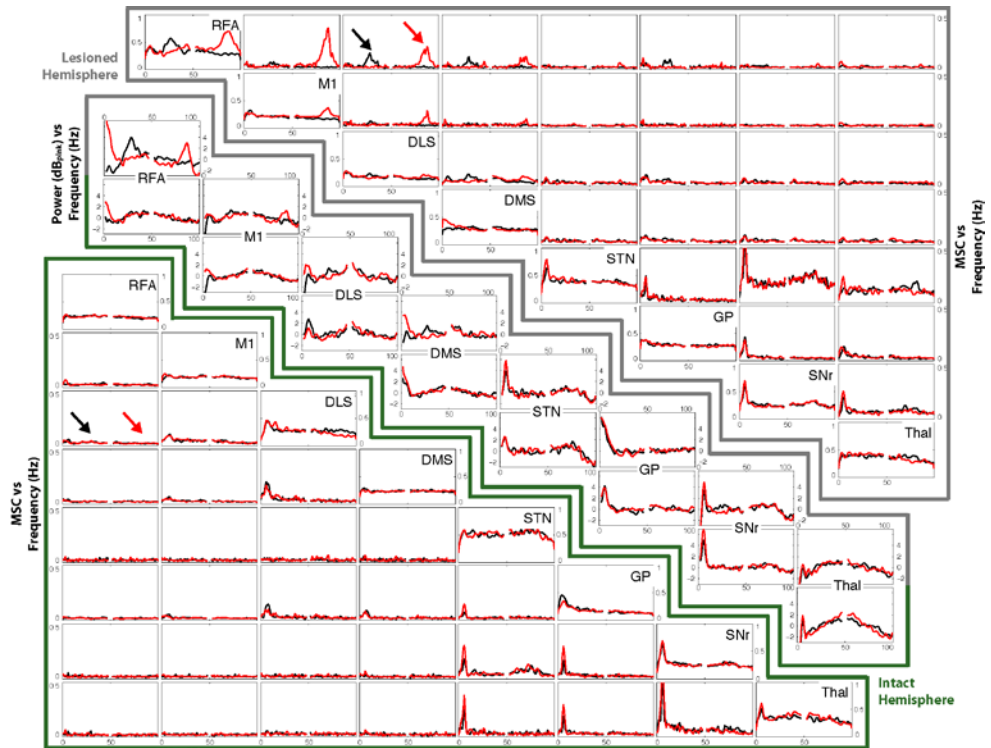


Figure 4. Power and mean magnitude-squared coherence (MSC) versus frequency displayed for the intact and lesioned hemisphere during baseline (healthy/parkinsonian state, black traces) and levodopa treatment (levodopa/dyskinetic state, red traces). The narrow interval at 50 Hz is not shown because of power line interference.

Thus, taken together, this electrode could be used in investigating systems activity in both healthy and diseased animals on a single cell level or LFP level. Its chronic functionality enables us to monitor changes over time in neural circuits due to, for example, learning of a novel skill and, additionally, its arrangement can be adjusted easily to different coordinates for diverse structures of interest.

Reaching paradigm

After observing these abnormal states of the CBT network, we wanted to inspect the healthy CBT network. Additionally, we also wanted to investigate how prone the network is to plastic changes as a result of experience-dependent mechanisms.

The main goal was to decode what role CBT circuits have in learning of a novel motor skill and subsequently, does the sensory representation in the motor parts of CBT change due to learning a new motor skill. The rat was trained in skilled reaching for a food pellet with the forelimb, resembling human reach and grasp for the food (conferred in Introduction). Naïve, implanted rats with described multi-channel electrode in the same CBT regions as above, were thought to reach for the food pellet on a shelf in an experimental apparatus (see details on training in the Supplementary material to Paper IV). The animals were trained for about two to four weeks after which they became proficient in reaching and grasping for the food pellet within milliseconds and would typically retrieve 50-80 pellets within an hour and a half. However, before animals have learned how to properly reach and grasp for the food pellet they are usually clumsy and miss the pellet, but once they learn they are proficient in this task and this action is extremely fast and presumably requires fast coding in CBT network. Considering that both neurophysiological data and videos were simultaneously recorded for all of the steps of learning this task, we could monitor neurophysiological and kinematic changes that occur due to learning of the reach and grasp task (Supplementary material to Paper IV). Despite this project not being ready to present in my thesis other than the data included in Supplementary material to Paper IV, the experimental model described here is a template for Paper IV.

Experimental model to investigate changes in tactile representation in CBT network due to prolonged training of a skilled movement

Considering that sensory feedback is crucial in forming a movement, a goal was to investigate if the sensory information delivered to motor systems is modified due to extensive training of a novel motor skill (reaching and grasping for the food pellet, Supplementary material to Paper IV).

For this purpose, in Paper IV tactile stimulus was delivered to the plantar skin of the forepaw in lightly isoflurane anesthetized rats before and after reaching training. This tactile stimulus was delivered 150 times to 16 different sites of each paw, even though only one paw was trained in skilled reaching. Because the tactile stimulation experiments were performed under anesthesia, great care was taken to monitor

anesthetic depth (observing cortical LFPs during the experiment as well as breathing rate, for details see Paper IV). Performing a number of stimulation experiments before and after the training should enable us to directly observe changes in activity patterns of individual recorded cells to stimulation of certain areas of the skin.

Furthermore, since only one of the paws was trained we have a very good control state where we can compare cells reacting to stimuli delivered to the trained paw, before and after training, to the untrained paw. Additionally, we can compare reactions of the cells that are ipsilateral or contralateral to the stimulated paw, whether it is trained or untrained paw. This enables us to monitor most of changes that could occur due to the training.

Results

Having developed large-scale recordings for each subsequent project (Paper I), we next looked at role of dopamine in CBT circuit by using acute pharmacological manipulation (Paper II). After the rats were injected with either AMPT, D1R, D2R or D1/D2R antagonists, it was very obvious that all these drugs caused severe akinesia which was indistinguishable between the different drugs. This akinesia was experimentally confirmed by a catalepsy test where animals were deemed to be cataleptic if they stayed with their forepaws placed on a 10 cm high horizontal bar over 30 s. This type of akinesia was not observed for the unilateral 6-OHDA rat model. The most obvious difference depending on the type of pharmacological intervention was the difference in length of akinetic symptoms for each drug which varied with hours (shortest effect being for the D1R antagonist –around two hours, and longest for AMPT and D2R antagonist, from about four hours and longer). Furthermore, different drugs affected the animal's behavior at different times post injection, with D1R antagonist causing akinesia with the fastest onset (around 20 min), followed by D2R antagonist (around 90-120 min) and AMPT (200-300min). Combination of D1/D2R antagonists was showing mixed effect of both drugs, thus causing akinesia at about 20 min after injection and lasting longer, as the D2R antagonist itself.

As we recorded both LFPs and single units from each CBT circuit structure and each hemisphere, there was an abundance of data to analyze and we firstly looked at LFPs.

To analyze LFP signals we created power spectrograms for each structure and each experiment based on the mean of the time-varying potential differences between all pairs of electrodes within the same structure. Spectrograms displayed power of all frequencies present in the LFP signal, however, we concentrated our analyses on frequencies below 45 Hz due to previous reports of exaggerated power in beta frequency following dopamine depletion (<30Hz).

In these experiments we could conclude that akinetic symptoms aren't caused by the increase in power in beta frequencies in LFPs even though they generally show up together as symptoms of lack of dopamine in CBT circuit. In specific, even though all drugs caused very similar symptoms we could clearly see that akinesia after D1/D2R antagonist injection (Fig. 5a, black line marks first positive catalepsy test - appearance of akinesia) appeared before we could detect any increase in power in beta frequency (in our case 12-20Hz).

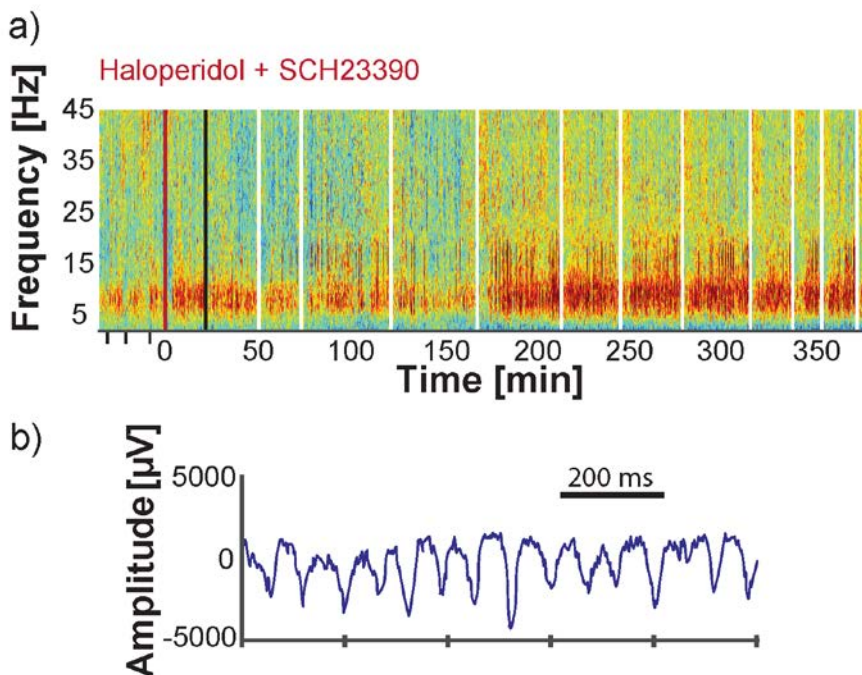


Figure 5. Power spectrum and raw LFP signal from an example experiment with D1/D2R antagonist.
a) Spectrogram showing relative changes in frequency content before and after D1/D2R antagonist injection ($t=0$, red vertical line). Black vertical line represents first positive catalepsy test where akinesia is present, and the white vertical lines show catalepsy tests that still confirm ongoing akinesia. **b)** Raw LFP trace from one recorded channel from DLS at the time of visible increase in beta oscillations (about 185 min after the injection).

Interestingly, when zooming in on the LFP signal from one channel at the time of increased beta power (Fig. 5b), we can see that the envelope of the oscillation is increasing in amplitude rather transiently, rather than appearing as a steady oscillation. It also shows distinct differences in sharpness of peaks and troughs. Similarly, when looking at the firing rates in the same experiment, we can see that they are modulated after the drug application for majority of the cells, but again with somewhat different delays in relation to the time of injection.

Next, when looking at the time-averaged power density spectra for all drugs and experiments and comparing these with 6-OHDA lesioned rats (Fig. 6), we noticed that AMPT and D1/D2R antagonists showed similar spectral changes in the low beta range compared to the baseline (orange and blue traces against green, respectively), most pronounced in cortex and striatum. However, considering that D2R antagonist showed similar results to these two (maroon traces), while D1R showed very mild spectral changes in low beta frequency (yellow traces), it is possible that this difference in beta power could be due to reduced dopamine binding to the D2R. Asterisks mark significant changes in spectral power at the frequency

of 12-20 Hz compared to the baseline. This finding once again was interesting since all drugs produced very similar motor symptoms. Comparing acute dopamine depletion to the chronic model (black traces), we could see similar increases in power in low beta frequencies. For the chronic group these differences did not reach significance but this could be due to that the baselines were constructed from averages of all the baselines from acute pharmacological dopamine depleted rats, thus not constituting paired datasets like in the acute experiments.

We thereafter looked into the rate changes induced by the drugs across all of the CBT structures recorded. We found that in our case it was close to impossible to match the findings to the original Albin-DeLong model of CBT in hypodopaminergic state. Instead, we found that most of the structures after treatment with any of the drugs exhibited firing rate changes. Somewhat surprisingly, fractions of cells showing increase and decrease in firing rate were rather balanced within a single structure resulting in minimal net rate changes (Fig. 7). This complex pattern could perhaps be explained by the fact that dopamine receptors are placed in all the structures of the CBT network (Smith and Villalba 2008) and so the drugs are directly affecting each structure by altered dopamine levels or binding to one or the other type of dopamine receptor.

In addition to changes in firing rates, the temporal pattern of firing may also be of importance. In particular, we wondered whether a single cell rather fires at a specific phase of the beta oscillations. Since there has been evidence that many cells tend to become entrained to cortical slow oscillations (Magill et al. 2000, 2001), we looked at all the cells individually and if their firing is somehow entrained to cortical oscillations. We found that a substantial amount of cells were entrained to either theta or beta frequency after the drug injection period. However, a few cells were entrained also during the baseline state (before drug injection), but these cells were mainly entrained to theta frequency and they mainly stayed entrained to this same frequency even after drug injections. These theta entrained cells show us that not all entrainment is pathological since they occur both in naïve and in drug induced states. While mainly cells from cortico-striatal structures showed higher level of entrainment to M1 LFPs for both theta and beta frequency, deeper structures showed slightly higher level of entrainment under D2R antagonist for beta frequency compared to other experimental conditions.

Furthermore, after realizing that many of the cells are showing entrainment, we investigated whether this oscillatory pattern of firing could perhaps come from these cells themselves acting as drivers of the oscillations. Autocorrelograms showed us very few cells showing a strong oscillatory pattern (only 121 of 3048 cells). These oscillatory cells exhibited mainly either theta or beta oscillatory firing patterns. Theta oscillatory cells appeared both before and after drug injections, while beta oscillatory cells mainly appeared after the drug injections (with a most common peak frequency at 15 Hz). Similarly to entrainment analyses, cells that displayed these characteristics came from all recorded structures of the CBT circuit.

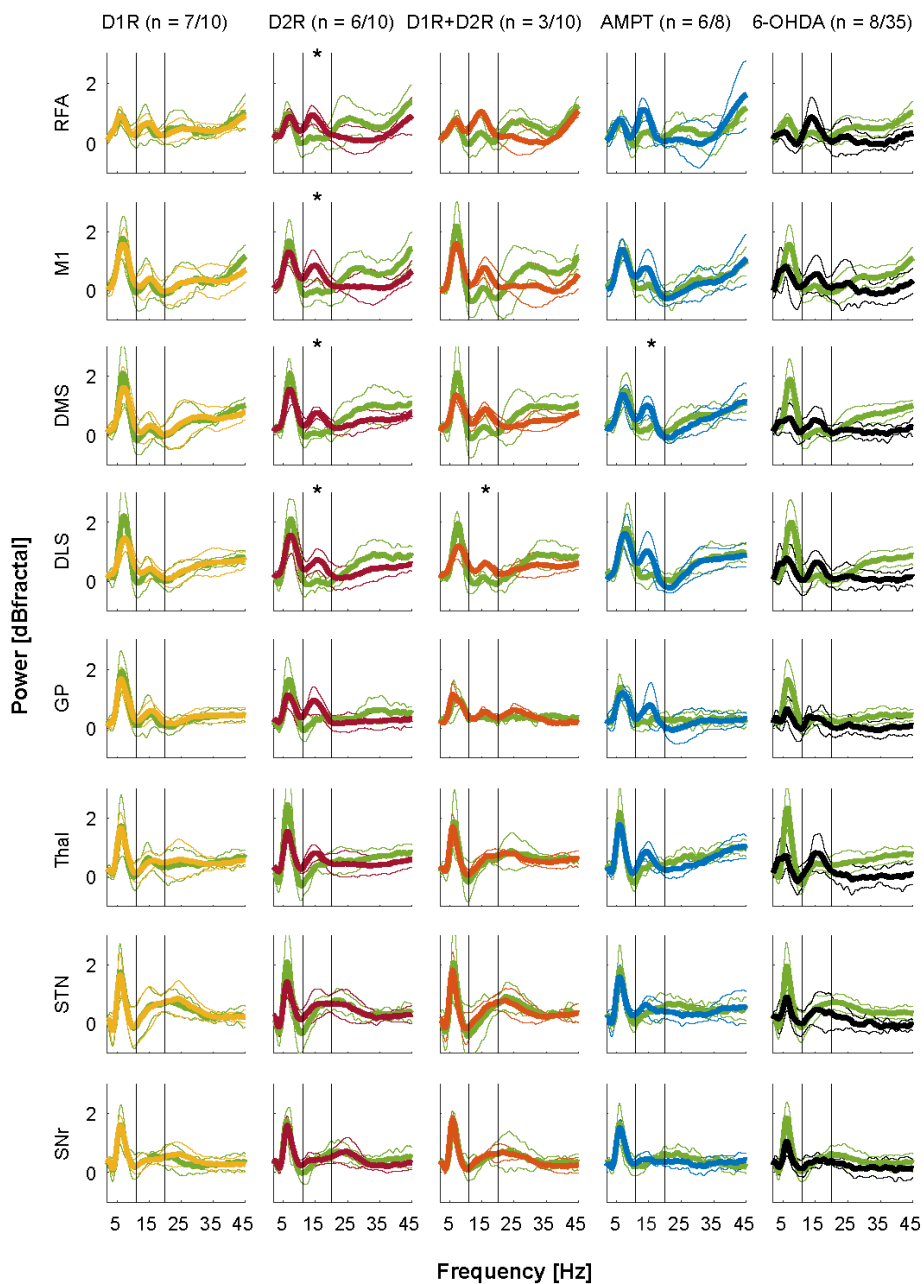


Figure 6. Changes in LFP spectral content following dopaminergic manipulations. Mean power spectral density across animals during baseline (green traces) and drug-treated periods (D1R antagonist/yellow, D2R antagonist/maroon, D1/D2R antagonist/orange, AMPT/blue). Drug-treated animals were compared to 6-OHDA unilaterally lesioned animals (black).

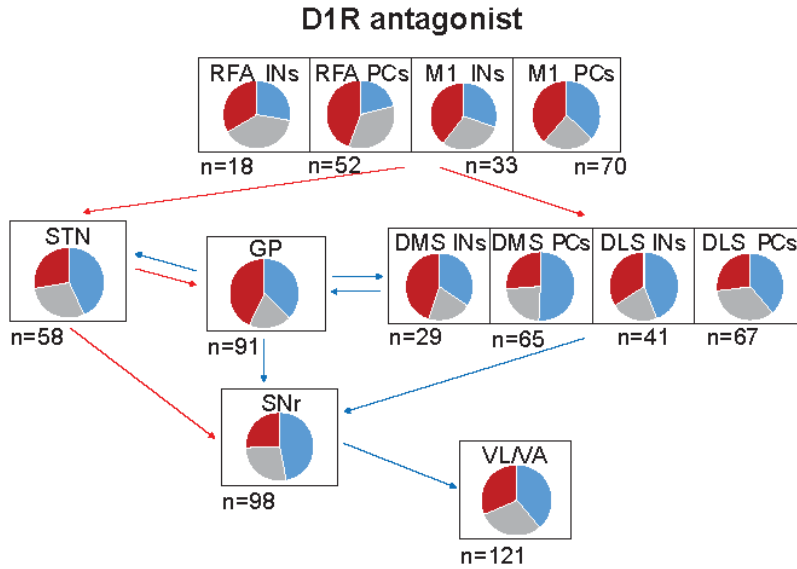


Figure 7. Firing rate changes following D1R antagonist divided by structure and cell-type (interneurons (IN) and principal cells, PCs). Fraction of cells showing a significant increase/decrease in firing rate (drug vs. baseline) is represented by red/blue slices, and the gray area represents non modulated cells. Red and blue arrows between boxes denote the principal glutamatergic and GABAergic anatomical connections between CBT structures.

As we initially showed that the appearance of increase in power of low beta frequency did not match appearance of motor symptoms, we decided to further examine temporal aspects of the neurophysiological changes that occurred in these experiments.

In fact a closer inspection of the onset of beta oscillations revealed not only that the appearance of increased low beta power after treatment with D1R antagonist poorly matches the onset of the motor symptoms, but that this was generally true also for the D2R antagonist (Fig. 8). However, we can see that after D2R antagonist treatment, the power in low beta starts to appear rather selectively in cortical structures very shortly after the injection and then gradually increase and appear in deeper structures too.

Finally, once we looked at the firing rate changes for cells that either increased or decreased their firing rate following drug treatment we realized there could be an explanation for and the occurrence motor symptoms (Fig. 9). The D1R antagonist caused significant change in firing rates already 10 min after injection. The D2R antagonist caused similarly immediate changes in firing rates after the injection, however, most of the observed changes happened in the latter part of the recordings in this case.

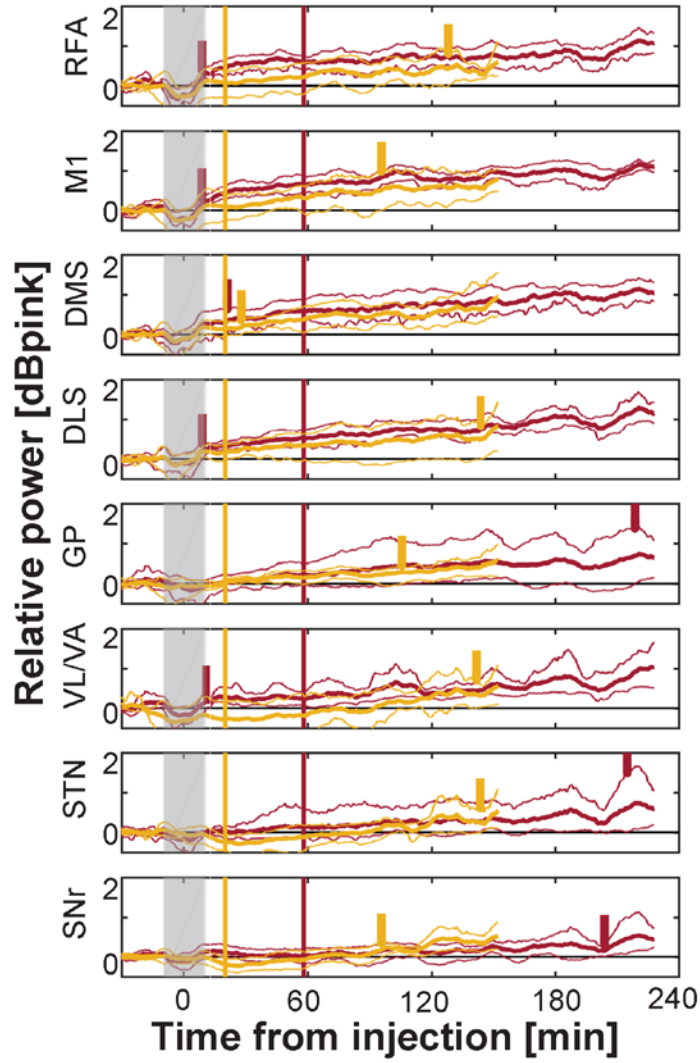


Figure 8. Temporal development of changes in relative band-power in the low beta over time in relation to the onset of motor symptoms. Drug injection at $t = 0$; vertical lines denote the median time point of first positive catalepsy test, and vertical bars mark the time point after which beta power is consistently higher than baseline confidence interval (CI).

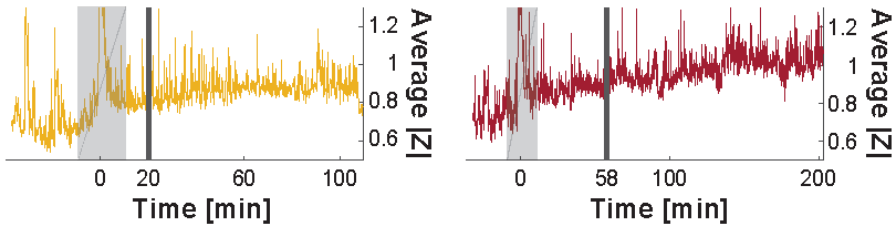


Figure 9. Averaged absolute deviations from mean baseline firing rates for all experiments with D1R antagonist (left, yellow) and D2R antagonist (right, maroon). Significant rate changes are observed shortly after administration of drugs preceding the first evidence of catalepsy.

No firing rate changes were however observed after the first injection of AMPT. This finding, served as an important control, showing that drug injections *per se* did not induce rate changes.

Taken together, even though we could not see any difference in net firing rates for each structure individually, the increase and decrease of firing rate of different fractions of cells within the different structures could, in fact, be one of the first manifestation of pathological changes that eventually lead to motor symptoms. Indeed, when plotting the temporal development of both firing rate changes and increase in power in low beta frequencies for AMPT, D1R and D2R antagonist (Fig. 10), we can clearly demonstrate that rate changes reach their observed highest level when the catalepsy appears for all three drugs (note asterisk as significance mark). In contrast, beta power emerges slower and gradually increases for most of the drugs actually after catalepsy developed (n.s. as non-significant and # as significant).

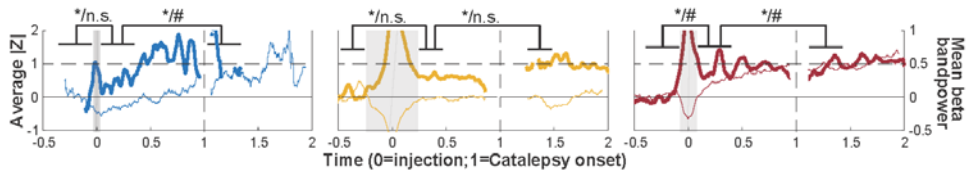


Figure 10. Changes in beta power (thin line; right y-axis) and firing rates (thick line; left y-axis) plotted with an x-axis scaling adapted to latency of catalepsy [drug injection at $t = 0$ (first injection for AMPT) and catalepsy onset at $t = 1$]. The average absolute Z-score on the left y-axis is normalized from baseline ($y = 0$) to the early cataleptic period ($y = 1$). The beta power on the right y-axis is shifted to average baseline power ($y = 0$). Gray shaded and empty (white) areas denote periods excluded from further analyses because of manual handling of the animals or locomotion (nine D1R, eight D2R and four AMPT experiments were included). Statistical differences mark changes between the indicated time periods (late baseline, early postinjection and early catalepsy).

After showing how dopamine depletion affects the CBT circuit in acute pharmacological models, I will present neurophysiological states of the CBT-loop in the chronic parkinsonian unilateral 6-OHDA lesion and LID rat model.

Furthermore, in the LID rat model, behavioral and electrophysiological effects of experimental and clinical drugs used to ameliorate dyskinesia will be presented (Paper III). Lesioned animals (displaying 75 -100 % reduction in immunohistological staining of tyrosine hydroxylase in striatum ipsilateral to lesion) that were severely dyskinetic after L-DOPA treatment were chosen for these experiments. The same type of electrode was used to record from all of the CBT circuit which enabled us to record both LFPs and unit activity.

In Fig.11 the difference in spectral content of example LFPs are shown for a parkinsonian rat that becomes dyskinetic following L-DOPA injection. The previously reported narrow band gamma oscillation around 80 Hz in M1 in dyskinetic animals (Halje et al. 2012) was confirmed here (Fig. 11 Top right), as well as an increase in power in theta band oscillation in STN (Fig.11 Bottom left, as previously reported in humans, Alonso-Frech et al. 2006). Power changes in these bands proved to be consistent between recordings, showing significance for gamma oscillation in M1 (65-100Hz) and significance for theta oscillation in STN (3-9 Hz). These animals show a typical increase in beta power (<30 Hz) in M1 in the parkinsonian state, but after L-DOPA this is not the case anymore. It can be speculated that theta oscillation is possibly more related to the increased motor activity after L-DOPA, while gamma oscillation is more specific for dyskinesia. This is because of the lack of significant differences in theta power increase in STN when comparing lesioned and non-lesioned hemisphere after L-DOPA administration, whereas the difference in increase of gamma oscillation in M1 is significantly different for the lesioned versus non-lesioned hemisphere.

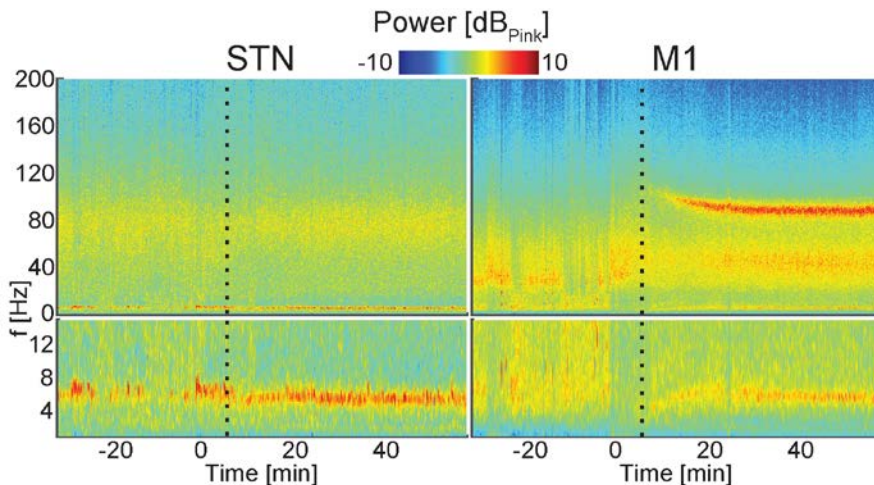


Figure 11. Neurophysiological signature of STN and M1 during the parkinsonian and dyskinetic state, off/on levodopa, from an example experiment (black dash line is injection event). Top panel: Power spectrogram showing the interval 0-200 Hz. Bottom: close-up of the low-frequency range from the top row. Power is expressed in decibels relative to the estimated pink-noise floor (dBpink).

Even though the majority of the dopaminergic terminals are lost in the telencephalon of the lesioned hemisphere, some levodopa is converted to dopamine and released from dopaminergic cells that are still intact. However, in addition to this release, serotonergic cells are known to be able to convert levodopa to dopamine by the same enzyme as in dopaminergic neurons - enzyme aromatic L-amino acid decarboxylation (AADC), which is also responsible for converting 5-hydroxytryptophan (5-HTP) to serotonin (5-hydroxytryptamine, 5-HT) (Ng et al. 1970; Maeda et al. 2005), – this way serotonergic cells greatly participate in release of dopamine from their terminals (Nicholson and Brotchie 2002; Carta et al. 2007). Serotonergic cells are sending their axons from dorsal raphe nucleus to all areas of BG (Lavoie and Parent 1990), and even though there is also loss of these cells in PD, relatively abundant amount stays intact (Carta et al. 2007). It is suggested that an unregulated release of dopamine is therefore happening due to lack of dopamine autoreceptors on serotonergic cells, which are usually present on dopaminergic cells and regulate dopamine release from dopaminergic cells by negative feedback. Perhaps the therapeutic role of L-DOPA is largely maintained by release of dopamine from remaining dopaminergic cells, while dyskinetic and dysregulated release of dopamine comes from serotonergic cells that end up releasing this “false transmitter” from their axons (Carta et al. 2007). As this effect especially arises as PD progresses and there are increasingly less of dopaminergic cells, it is proposed to be causing AIMs as a consequence of dopaminergic dysregulation in CBT circuits (Carta et al. 2007; Cenci 2014). Thus, as a possible novel therapy for treatment of dyskinesia 5-HT (1A, 1B, 2C) receptors agonists were proposed that could decrease the uncontrolled release of dopamine from serotonergic cells (Nicholson and Brotchie 2002).

Regardless the molecular mechanisms, our lesioned animals which had lost the majority of dopaminergic cells in one hemisphere of the brain all became dyskinetic after L-DOPA treatment and, furthermore, exhibit abnormal neurophysiological patterns in the CBT network. Both theta and gamma oscillations that appeared after L-DOPA in lesioned hemisphere are possibly valid neurophysiological markers of dyskinesia.

However, there could be more unknown neurophysiological markers that would thoroughly describe diseased and dyskinetic states which could help us finding new appropriate antidyskinetic therapies. Furthermore, displayed motor behavior gives us only indirect information about neural states we are trying to treat, thus, it is hard to deduce which neurophysiological effects originate from the new therapy. Consequently, search for this type of drug is rather challenging if we were to observe effects of new experimental drugs on the whole CBT circuit.

So, as a next objective, we wanted to identify more global biomarkers of LID in rat and to find if we can use these newly found biomarkers to find new treatments for dyskinesia. Therefore, we observed which neurophysiological changes we can see as result of different drugs given to alleviate dyskinesia. We show

neurophysiological features of the LID rat model before and after it has been injected with one of four chosen drugs that were suspected to alleviate LID. These drugs were: Amantadine which is a (N-Methyl-D-aspartate) NMDA-receptor antagonist, Levetiracetam which is drug usually used for treatment of epilepsy, Diazepam which is benzodiazepine with sedative, muscle relaxant actions and 8-OH-DPAT which is 5-hydroxytryptamine- 1A (5-HT1A) presynaptic autoreceptor agonist used to reduce serotonergic release of dopamine. Lastly, we used the experimental drug WAY-100,635, which is 5-HT1A receptor antagonist, to reverse the effect of 8-OH-DPAT. Additionally, we also performed behavioral assessment of the severity of the displayed dyskinesia during the whole experiment by manually scoring AIMS.

Power spectrograms created from the average of all the electrodes in each recorded structure from the CBT circuit are shown for an example experiment with 5-HT1A agonist treatment (Fig 12). Injection of 8-OH-DPAT abolished gamma oscillations in cortico-striatal structures completely (Fig. 12b), and concomitantly alleviated dyskinesia (Fig. 12a), and, furthermore, this effect was fully reversible by injection of 5-HT1A antagonist (WAY-100,635). However, the neurophysiological state during the drug treated non-dyskinetic period did not fully resemble the control state (parkinsonian or intact non-treated), for example, showing different patterns of relative LFP power content in low frequencies (delta/theta and beta) in some structures. Additionally, even though dyskinesia was also abolished after 5-HT1A antagonist injection, the animal showed certain other behavioral abnormalities (flat body posture and reoccurring forepaw movements, similar to those reported after excessive serotonergic stimulation, Jacobs 1974). It was clear that each of these conditions displayed specific neurophysiological states that were significantly different from each other.

In these experiments injection of L-DOPA preceded any treatment with experimental antidyskinetic drugs, and each drug produced its own set of neurophysiological patterns. It was therefore very complex and challenging to analyze changes in the single specific frequency bands that were previously observed. To overcome this, we decided to describe the changes in CBT circuits caused by the drugs on a more global level. For this, a specific computational method was applied which enabled us to visualize the data in two or three dimensional space (described in detail in the paper III). LFPs and unit activity, both went through same computational procedure, where they were sampled with 8s binning throughout the length of the different described conditions (control, parkinsonian, dyskinetic, and drug) which were also separated for each animal. All the recorded structures of CBT loop was then assembled into a single vector for each 8s sample point which was then possible to project down to a distinct coordinate in a two/three dimensional coordinate system. When representing each 8s bin for all structures as a dot in the chosen coordinate system, we noticed that dots belonging to the same condition form a very distinct cluster, thereby

establishing a way for us to identify specific neurophysiological state in each animal for a number of specific conditions.

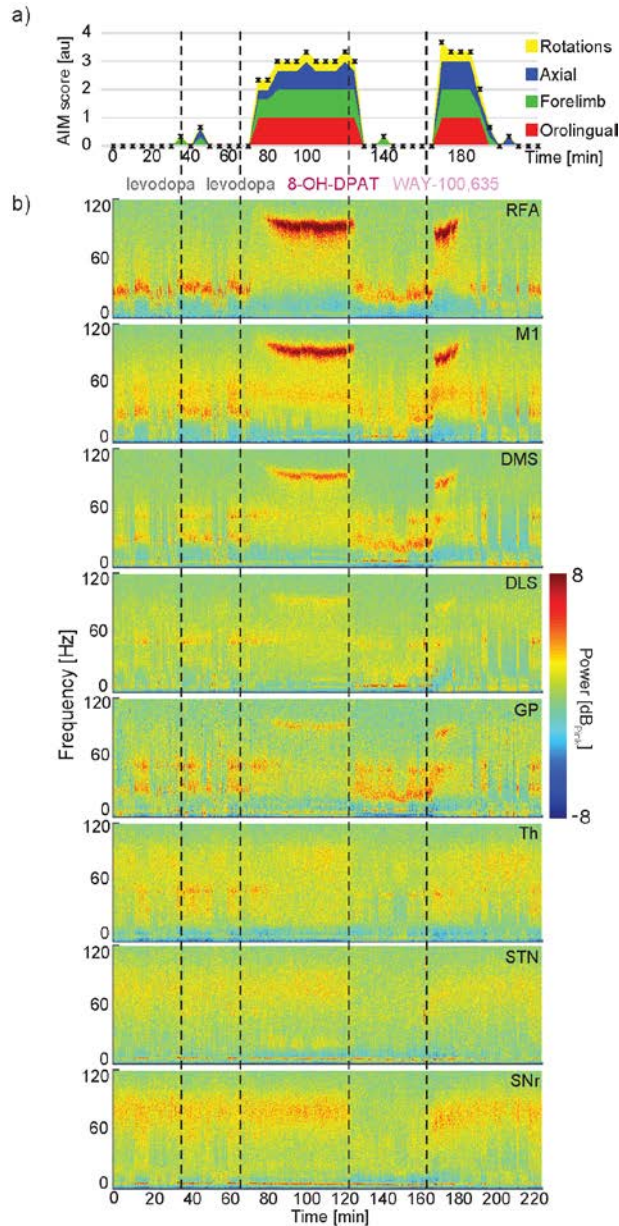


Figure 12. Systemic treatment with a 5-HT_{1A} receptor agonist (8-OH-DPAT) alleviates dyskinesia and alters the neurophysiological state and is, subsequently, reversed with WAY-100,635 (recordings in lesioned hemisphere shown). **a)** severity of dyskinesia scored during 1-min periods once every 5 min (marked by crosses).

Dashed lines indicate times of drug injections (levodopa was administered twice in this experiment to induce the dyskinetic state, represented by the first two lines. AIM, abnormal involuntary movement; au, arbitrary units.**b)** Spectrogram showing relative change in LFP spectral contents from 8 CBT structures throughout an example experiment.

Each of the animals displayed specific neurophysiological states represented in a common coordinate space caused by each unique drug, whereas the other states (control, parkinsonism and dyskinesia) were positioned in this space in a very similar way across animals.

To get an overview of the data from all recordings we represented LFP data from 8 different conditions in a three dimensional space based on the first three principal components (PCs) containing the most of the data (Fig. 13b ;principal component analysis (PCA) is a method to statistically re-express a multivariate data set, where first PC contains most of the total variance in the dataset, then each subsequent PC, which are orthogonal to the previous ones captures gradually less of the total variance of the dataset). Furthermore, we could also compare the behavioral assessments of AIMs for 7 experiments with these 4 different drugs in one animal (Fig. 13a).These experiments revealed that most of the drugs decreased dyskinesia but with variable effect both between different drugs and sometimes between two different experiments with the same drug.

We can clearly see a partial separation with some overlap between clusters based on different experimental conditions, thus confirming how this method could be used to evaluate best treatments for LID symptoms. In order to facilitate comparison between different neurophysiological states we plotted them in two dimensional space, where the x- axis represents the difference vector between parkinsonian and control, and y- axis represents the difference vector between dyskinetic and control state in the direction orthogonal to the x-axis (Fig. 13c). Even though some experimental drugs formed different and separate clusters then dyskinesia cluster, and thus indicated that they reduced dyskinesia (which was also evident in Fig. 13a from behavioral assessments), they, however, often seemed to return the neurophysiological state of CBT circuits to a state more similar to the initial parkinsonian state. Overall these results clearly suggest that this method, which distinguishes systems-level neurophysiological states, could be extremely beneficial for searching new therapies.

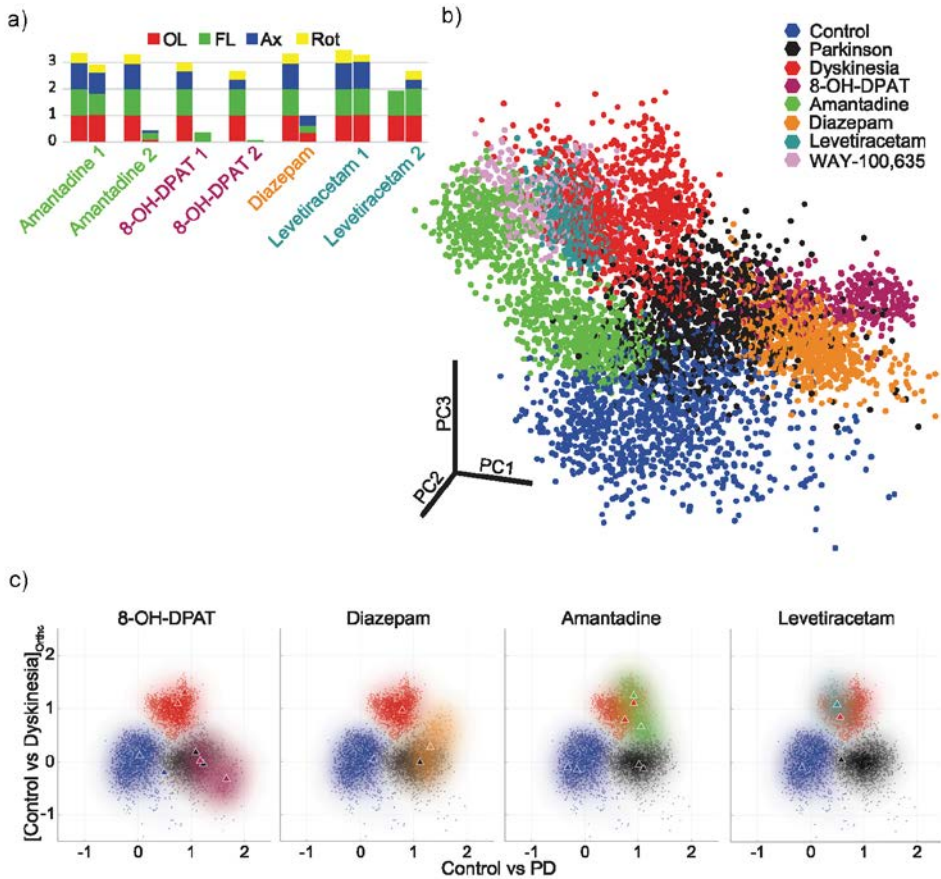


Figure 13. Systems-level characterizations of pharmacological interventions alleviating dyskinesia. **a)** treatment with 4 different drugs in 7 separate recordings in one rat show different reduction in normalized dyskinesia scores compared to the dyskinetic state (orolingual dyskinesia (OL), forelimb dyskinesia (FL), axial dyskinesia (Ax), Rotation (Rot). **b)** overview of the systems-level neurophysiological states induced by the different pharmacological interventions based on the spectral contents of recorded LFPs. **c)** representation of the systems-level state induced by each of the drugs in 2D space with axes defined by the main spectral differences between control state vs. PD and (control vs. dyskinesia).

When monitoring characteristic neurophysiological patterns occurring under different conditions such as parkinsonism and dyskinesia, it is hard not to think of the possible long-term changes in CBT circuits that might play a role. After dopaminergic cell loss, it is very likely that re-organizing of synapses occurs in CBT circuits, as well as in the process where L-DOPA loses its therapeutic window, and lesioned animal become dyskinetic and at the same time start exhibiting specific neurophysiological patterns (Dupre et al. 2016). In that case, how plastic are different parts of the circuit? We chose to address the question of how changeable

different parts of the CBT circuit is by searching for changes that could occur due to extensive motor training.

As mentioned in Introduction, in order to be able to control and guide voluntary movements we need to be able to modify them based on sensory information. This information could perhaps also be found in neurons of motor cortex (Rosén and Asanuma 1972). Basically, in this study the authors showed how M1 neurons that were activating certain muscles which moved skin on the volar side of the monkey's hand were displaying receptive fields (RFs) with spatial distributions proportional to the change in pressure that was inflicted on the skin by contraction of a given muscle when activated (a receptive field is the area of sensory space that upon stimulation changes a neuron's firing rate). We hypothesized that there might be some kind of experience-dependent plasticity that would occur due to extensive training needed to acquire a novel motor skill. Thus, experiments, that would help us elucidate this question, were performed before and after extensive training of animals in a skilled forelimb reaching and grasping task. Experiments consisted of tactile stimulation on the glabrous skin of the forepaws under light isoflurane anesthesia, and was performed two to three times before as well as after the training. As only one of the forepaws underwent training, the other forepaw was used as a control. Once again, the same type of custom made multi-channel electrode was used, which was able to record both LFPs and unit activity from 16 structures in the CBT circuit. First we looked at evoked changes in LFP signals based on the event of the tactile stimulation, called evoked potentials (EPs) which show evoked activity of a population of cells upon stimulation (median values were used for representations). Averages of EPs from all experiments in all animals were constructed and split for responses from ipsilateral and contralateral hemisphere to stimulation of the forepaws (Fig. 14a, Table 1). It was evident that the contralateral hemisphere displayed significantly larger responses in all structures recorded compared to ipsilateral hemisphere. Responses from ipsilateral and contralateral hemisphere resulted in different temporal patterns except for SNr that showed rather similar responses between hemispheres. In Table 1. EP amplitude and peak latency for each structure is displayed. It is noticeable that contralateral M1 and striatum show fast responses (around 23 ms) to the tactile stimuli, whereas contralateral RFA, GP, Thal, STN and ipsilateral hemisphere show slower response (around 55 ms) to the same stimuli. Though, overall, all recorded structures seems to receive somatosensory input, the shorter latency of cortico-striatal structures contralateral to the stimulated forepaw might indicate a more direct and unprocessed representation of the tactile input than the rest of the structures.

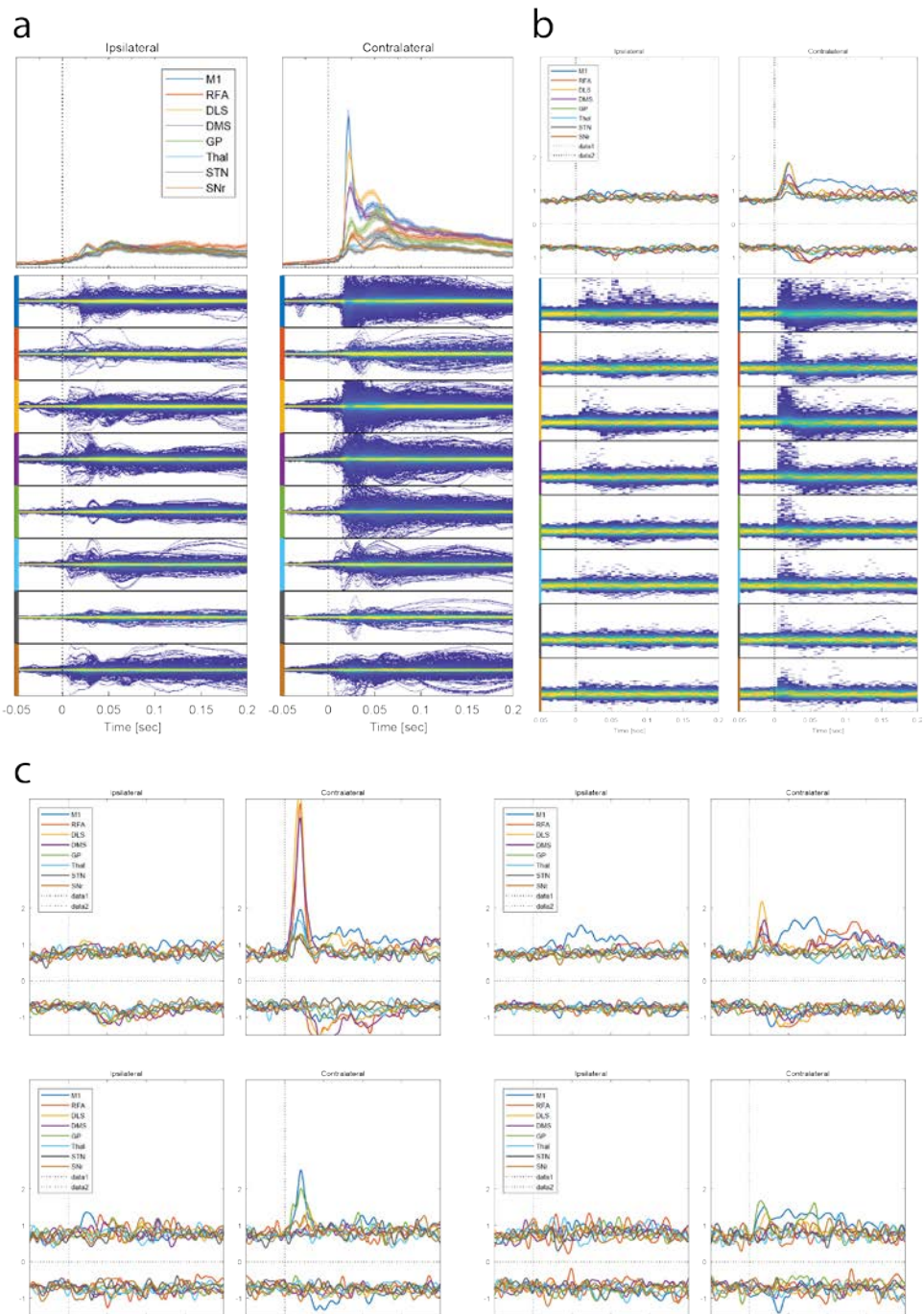


Figure 14. Overview of evoked LFP and spiking activity divided by brain structure. a) Grand average of evoked potentials (EPs) for the hemisphere ipsilateral and contralateral to the stimulated paw. **b)** Grand average of peri-stimulus time histograms (PSTHs) of the unit activity recorded for the hemisphere ipsilateral and contralateral to the

stimulated paw. Increases and decreases in firing rate has been split into two separate panels to illustrate the relatively later onset of inhibition. **c)** Average PSTHs for the trained (top) and untrained paw (bottom), recorded before (left) and after (right) a few weeks of extensive reaching training.

Table 1. Summary of evoked potential (EP) characteristics.

Median values for area under curve (AUC), peak values and latency to peak for EPs recorded between all electrodes located in the respective structures of the cortico-basal ganglia-thalamic loop (n=45 recordings in 11 rats).

	Ipsilateral			Contralateral		
	AUC (uVs)	Peak (uV)	Peak latency (s)	AUC (uVs)	Peak (uV)	Peak latency (s)
M1	12.50	81.02	0.054	28.29	502.58	0.023
RFA	12.87	82.12	0.133	18.51	129.62	0.065
DLS	11.48	87.19	0.054	27.65	383.41	0.023
DMS	11.24	85.34	0.052	24.56	268.99	0.024
GP	11.37	86.21	0.054	19.16	187.59	0.055
Thal	9.74	71.66	0.055	13.50	111.80	0.051
STN	9.67	66.75	0.067	12.04	103.84	0.058
SNr	10.90	77.91	0.029	12.37	108.66	0.026

Next, we looked into evoked unit activity which, in contrast to EPs, may be closer related to the output of the recorded structures, if the majority of recorded cells are projecting cells. Peri-stimulus time histograms (PSTHs) of the spiking activity were created to analyze average response patterns. Even though the fraction of modulated cells did not directly relate to the size of EPs, latencies to the bin with the highest firing rate roughly corresponded to the latencies seen in EPs (around 20-30 ms). However, we noticed biphasic response, where after this initial rise in firing rate inhibition followed (around 70-150 ms after the stimulation) (Fig. 14b). When looking at the averages of these increased/decreased firing rate responses in all the structures separately, it is noticeable that M1 differs from other structures, where sub-population of cells show prolonged increase in firing. To further investigate these particular responses, we separated data for untrained and trained forepaw before and after the training for neurons that are ipsilateral or contralateral to the stimulated paw (Fig. 14c). It became evident that this particular prolonged increase in firing rate in contralateral M1 (Fig. 14b) mainly happens after stimulating the trained paw (Fig. 14c), which implies that the cause of it could be extensive training. More specifically, we saw that when stimulating the trained paw, both ipsilateral and contralateral M1 (50-125 ms) exhibited this prolonged increase in activity, as well as contralateral RFA (100- 175ms). Interestingly, we also observed statistically significant similar prolonged increase in activity of M1 in both hemispheres upon stimulating untrained paw (50-125 ms), though much less pronounced. This could

be similar to the transfer of performance after unilateral tactile learning happening in primates (Harris et al. 2001). Additionally, when re-analyzing EPs divided into before and after training, we found that RFA shows clearly this increased late phase response. Altogether, based on these results it is likely that due to extensive training, representation of somatosensory input of the glabrous skin of forepaw is changed, mostly evident for M1.

Further, we wanted to create RFs that would spatially represent the organization of somatosensory input to the recorded motor areas, but as we already noticed biphasic responses, this representation would be incomplete if we just summed all the time-bins together.

Thus, for the illustration purposes, we divided this biphasic response to fast phase response (0-35 ms) and to slower phase response (35-100 ms), which displayed excitatory and inhibitory response, respectively. In Fig 15. an example representative contralateral M1 neuron is shown in this manner (averaged for each phase separately) displaying RF of this neuron based on standardized (z-scores) evoked activity.

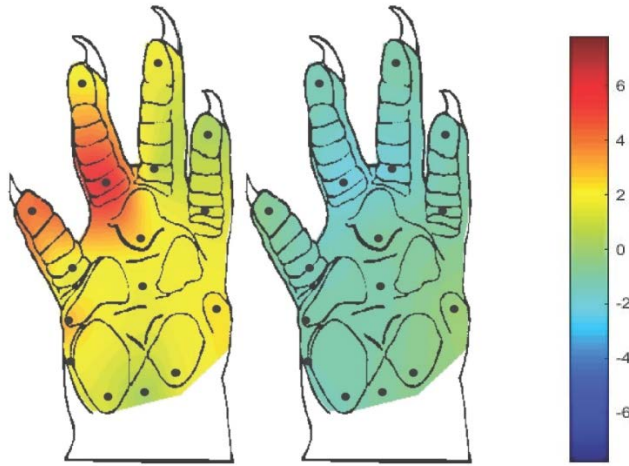


Figure 15. Receptive fields constructed from the PSTHs upon stimulation of the contralateral paw. The left representation is based on the timespan 0-35 ms after applying the stimulus, while the paw to the right is based on the timespan 35-100 ms after the tactile stimulation. Green represents a z-score = 0, i.e. an unchanged firing rate compared to baseline (100 ms before the tactile stimuli), while red/blue indicate z-scores larger than zero or smaller than zero, i.e., increased and decreased firing compared to baseline, respectively.

Furthermore, in order to observe any changes that happened in RF properties due to extensive reaching training, we analyzed temporal patterns (10-100ms post stimulus) of all responses from all 16 stimulated sites on the plantar skin of the

forepaw. Considering that a single neuron is hard to track over longer time span in recordings occurring over several weeks, the RFs were created for all cells that could be found within the same recording channel. These RFs were compared pairwise between individual recordings before-before and after-after, as well as the comparisons between before-after training. We then tested for the null-hypothesis that before-after pairs of RFs were not significantly different from before-before and after-after. Interestingly, we could only discard this hypothesis for M1 when the trained paw was stimulated. Thus, it is very likely that this change of the spatiotemporal representation of the trained paw in contralateral M1 has changed due to extensive reaching training. These changes were not observed in the other structures, or when stimulating the untrained paw (data not shown).

General discussion and concluding remarks

It has for thousands of years been a great mystery to human kind how our own brain functions. Once we roughly know which brain regions are involved in which functions, we would like to understand fully what processes are the ones where a particular function is enabled and controlled by certain brain region. The topic of my thesis was investigating structures in the brain that are known to be involved in the control of voluntary movement. Looking deeper into the functioning and activity of cortico-basal ganglia-thalamic circuits proved to be quite a complex task. Based on a lack of technology to observe this network simultaneously in all structures there was a need for improvement. We accomplished this by developing a custom made multi-channel electrode which was adequate to monitor systems level processes in freely moving animals under different experimental conditions. This enabled us to record a unique set of electrophysiological data previously not available to the scientific community. It also allowed for specific analyses that were not possible to perform in the same way before, such as measuring coherence between a large number of different structures. Another important aspect is that, due to this electrode being able to record in freely behaving animals during different neurophysiological states, it was possible to look at changes in the activity of single neurons and LFPs throughout extended experimental procedures, thus including the timeline of many physiological changes occurring. With this, we also created a template for other scientific groups to study brain circuits in rodents throughout different behaviors, in particular disease states where one can monitor activity while symptoms occur. It is in certain parts a novel technique in rodents, which are often used in research in neurophysiology, and considering that it allows for adjustments of electrodes numbers and coordinates, it is a research tool of potential importance for the whole field of neurophysiology.

When investigating how acute pharmacological dopamine manipulations affect the CBT circuit, a particularly relevant finding of this thesis is that even though the changes we see in firing rates in different structures of the CBT circuit are rather balanced in their net output, those complex changes are the ones likely giving rise to typical motor symptoms that one can see after pharmacological dopamine manipulation (akinesia). As this was previously hard to examine, this thesis is the first study which investigated activity from all structures of the CBT circuit, in

parallel. We could not explain our findings based on the Albin-DeLong model (Albin et al. 1989; DeLong 1990, see in Introduction) as most of the structures following treatment with any of the drugs used to manipulate dopamine or its receptors did not change net firing rate as this model would predict. This was in line with some previous studies where cortex and striatum showed mixed results of firing rate activity after dopamine depletion (Costa et al. 2006; Burkhardt et al. 2009). This is perhaps not so surprising considering that, in the last decades, studies have revealed more anatomical and functional connections in the CBT circuit. Additionally, we now know that all structures are under partial influence of dopamine as dopamine receptors are distributed at surface of most of the cells in all CBT structures, not only striatum as proposed in the original model. Based on this, we could consider a somewhat modified CBT circuit model (Fig. 16) as a more realistic description. Looking at Fig. 16, we can see complex interaction between separate structures that are additionally heavily dependent on homeostasis of dopamine concentration in order to function normally. Any disturbance in dopamine levels could easily disturb the activity of each and every structure, and subsequently this structure would also influence the activity of others.

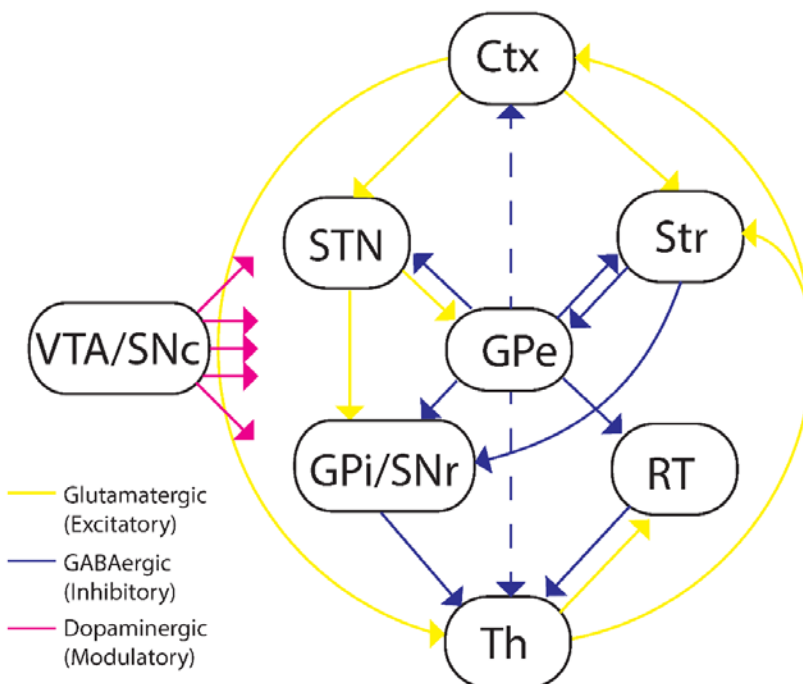


Figure 16. Modified model of cortico-basal ganglia-thalamic circuit showing more interconnected structures than originally postulated in Albin-DeLong model. All structures are under influence of dopamine. Ctx- cortex, Str- striatum, GPe- Globus pallidus externa (in rats Globus pallidus), GPi- globus pallidus interna (in rats entopeduncular nucleus), STN- subthalamic nucleus, SNr- substantia nigra pars reticulata, SNc- substantia nigra pars compacta, VTA- ventral tegmental area, Th- thalamus, RT- reticulate nucleus of thalamus. Image adapted from Tante M. PhD thesis.

Even though in our study we have separated subgroups of cells that were deemed to be interneurons and principal cells, in cortex and striatum, we have still not been able to consistently say which group of cells is changing more, as they both seemed to change their firing rates up and down. Thus, it was not clear how their specific firing rate change contribute to the changes in neural signaling that ultimately leads to motor symptoms. As such, there is a need to analyze more in depth which cell groups are the ones that change their firing rate and contribute to the pathophysiology of disease. This could be done, for example, by optogenetics where specifically designed genetic manipulations in specific populations of neurons, that could be activated or/and deactivated, could help us elucidating these populations of cells. Currently, the most abundant genetically modified rodents are mice, for which our custom made electrode is adjustable too, however, there are already some rat models and possibly in the future a range of genetic manipulations will be available in rats also. Finding which specific groups of cells that induce the symptoms of dopamine depletion will possibly enable us to modify their activity in order to avoid motor symptoms. It would be very challenging to adjust for any further changes that would occur after and this is perhaps achievable in future. At this moment we will most likely still rely on dopamine replacement therapy or similar alternatives that involve dopamine agonists, monoamine oxidase B (MAO-B) inhibitors (an enzyme that breaks dopamine), DBS and possibly combinations of levodopa with serotonergic agonists. As mentioned previously in Results, levodopa loses some of its therapeutic benefits after a couple of years in most of PD patients, and serotonergic neurons are proposed to play role in it due to their ability to transform levodopa to dopamine, however, in uncontrolled manner. It was proposed that possibly serotonin agonists could work in alleviating dyskinesia as they could regulate release in serotonergic cells (in rats and primates Bibbiani et al. 2001, in PD patients Bara-Jimenez et al. 2005). It is speculated that serotonergic agonists that preferentially bind to presynaptic autoreceptors compared to ones that only bind to postsynaptic serotonergic heteroreceptors should be enough in reducing dyskinesia symptoms. However, it was suggested in a recent study that serotonergic receptor agonists that are binding to both presynaptic and postsynaptic receptors (5HT1A) are possibly the most beneficial to alleviate dyskinesia and possibly act on different mechanisms than the ones only related to dyskinesia (Brys et al. 2018).

Further, yet another interesting finding of this thesis is that appearance of, specifically, increase in low beta oscillation in the CBT circuit in dopamine manipulated or depleted animals might arise from decreased dopamine binding to D2R type as we did not find the same when dopamine could freely bind to the D2R type. Similarly to this, the chronic PD rat model has been shown to display preferential entrainment of subpopulation of striatal projection neurons involved in indirect pathway (activated by D2R type), over the ones involved in direct pathway (activated by D1R type), to exaggerated low beta oscillation in cortex (Sharott et al. 2017). The authors proposed that specific population of cells involved in the indirect

pathway could drive pathological low beta oscillation (15-30 Hz). In our study, beta oscillation appears regularly when drugs that obstruct dopamine binding for D2R type are used, however, it is rather a secondary effect that might influence diseased state and not the cause of the negative motor symptoms, as motor symptoms appear even when D2R type binds dopamine. Furthermore, it is interesting that chronic hemiparkinsonian rat model exhibits low beta oscillation similar to acute pharmacologically manipulated animals when immobile, while they exhibit high beta oscillation when mobile or attentive (Sharott et al. 2005; Avila et al. 2010; Brazhnik et al. 2012). In our study, all acute pharmacologically treated animals were almost completely immobile due to effects of drugs (akinesia) and all analyses were done on immobile states.

We also created a new method where we can observe different global neurophysiological states which can be visualized in a functionally meaningful coordinate space. This method for monitoring parkinsonian versus dyskinetic states and comparing any experimental treatments to reduce dyskinesia can potentially greatly speed up the development of new therapies aimed at alleviating negative symptoms of PD therapy. This method could also be used to test experimental therapies for other central nervous system (CNS) diseases, for example schizophrenia or epilepsy, where we can observe neurophysiological states which have an effect on symptoms, in particular, we can have an insight of the more global neurophysiological state at different experimental conditions. Additionally, the previously mentioned high-frequency phenomenon that appears in dyskinesia should be further investigated. It would be interesting to know if this happens due to serotonergic cells uncontrollably releasing dopamine, which groups of cells are the most sensitive to it, if this oscillation starts from activity of cells from e.g. motor cortex and then possibly propagates, or if activity of another region such as STN or thalamus disturbs activity of motor cortex which then exhibits this high-frequency oscillation. Research shows that animals can become dyskinetic also by amphetamine (alpha methylphenethylamine) which targets catecholamine neurotransmitters norepinephrine and dopamine, and by grafting striatum in 6-OHDA lesioned animals from healthy mesencephalic fetal tissue that contains mostly dopaminergic cells and some serotonergic cells, called graft-induced dyskinesia (GID) (Smith et al. 2012). Even though it was suggested that serotonergic cells in these grafts solely contribute to GID, this is likely not the case (Lane et al. 2009). Grafts with only serotonergic cells, however, contribute to LID, whereas grafts with mixture of serotonergic with dopaminergic cells show that LID can be reduced and avoided (Carlsson et al. 2007). The particular relative density of dopaminergic and serotonergic cells in the graft has been suggested to determine success of the graft itself and whether LID will appear or not (Carlsson et al. 2008). However, this needs to be investigated further considering that these grafts are mainly located in parts of striatum and the questions is what happens to all the other structures in this situation. For future directions, we should investigate whether

those animals that develop different kinds of dyskinesia, than LID, also exhibit the same 80 Hz oscillation and if not what differs there.

In addition reorganization of the network connectivity is likely also an important component of disease progression (Bevan et al. 2006; Fan et al. 2012). Another type of plastic changes, can be observed in the last study where we are providing new information on somatosensory input to CBT circuits. Although this study is not yet finalized, we have already at this point observed changes in evoked potentials and RFs of neurons in the CBT circuit as a consequence of prolonged skilled reaching and grasping training. Interestingly, motor cortex appears to be the one structure going through changes due to learning of a novel skill, while other structures do not. Considering that plasticity of neural circuits and reorganizing of synaptic connections possibly happens at every change in most brain structures and even spontaneously, it is very important to find how experiences influence shaping of our neuronal networks. Showing that motor cortex (the forelimb representation) is plastic after skilled training of the forelimb, is perhaps not so surprising. However, it definitely proves that neurons directly responsible for certain actions are influenced by the same actions they are controlling. This again sounds logical considering that learning a novel skill requires also learning and adjusting of neurons itself to the new experience. Still, this study is one of the first studies in rats to show that feedback from the somatosensory system influences motor systems, additionally, recorded in multiple motor brain regions. We have laid a template for future studies where simultaneously we could observe how animal learns to interact with the environment that surrounds it. It would be interesting to uncover how the nervous system experiences the world and learns from it. Thus, in this way sensory input that shapes plasticity of specific brain regions can help us creating models of various learning mechanism. This could even possibly help us better understanding how to create better self-learning strategies in artificial intelligence (AI). Even though there are AIs that obviously learn from experience, it is still of importance to understand how biological creatures learn to be able to implement sensorimotor transformations more accurately than current AIs. Lastly, understanding how we learn from our experiences would require understanding how plasticity occurs. It is a long standing notion that “neurons that fire together wire together” (Hebb 1950), but are these neurons just randomly firing, or do they actually have some hierarchical organization, or are they simply responsible for that action and that is why they tend to form stronger synapses (similarly as the suggested mechanism of learning of correction of erroneous movement in cerebellum by the climbing fiber, (Marr 1969; Albus 1971).

This thesis has provided certain mechanistic insights based on some new findings, both in healthy and abnormal/diseased CBT circuit states, but there is still a lot to learn and modify for future studies. For example, our electrodes contain numerous recording and reference channels (up to 144 wires) and they are still somewhat big in size (33 μm), thus they occupy quite a lot of brain volume. It could be that

substantial number of cells die along the passage of electrode through the brain structures, though some studies show very limited neuronal cell death around wire electrodes (Freire et al. 2015). Still, implanting a vast number of recording wires inevitably has impact on the circuits we record from, and most likely all other nearby structures where the electrode has passed. Beyond this, each electrode causes a certain degree of inflammation, where microglia come at the electrode site and encapsulate electrodes and thus creating a scar tissue. Not only that this process causes diminishing of recording capability by reducing the strength of measuring available electrical signal due to encapsulation of electrode, thus increasing impedance, but also it could possibly cause some abnormal or different firing activities of nearby neurons. One can speculate that, this also could contribute to a reorganization of synapses between neurons around implantation sites and plasticity. However, considering that implantation in our case takes place in both hemispheres, and experiments took place before and after some experimental event, it is at least possible that the effects from implantation could have taken place already before the experimental procedures started and that the activity from both hemispheres is comparable. In all cases, it is proposed that it would be beneficial to coat electrodes with gelatin based matrix for smoother implantation and reducing lesions during implantation, while simultaneously using more flexible electrodes that can be anchored and in this way can move together with micro-motions of the brain but still stay in place (Agorelius et al. 2015). Furthermore, due to mentioned scarring, electrodes are usable only for a couple of months, after which it is relatively hard to record any neuronal signals, beyond LFPs. Also, animals can lose implant during the course of experiments, or implanted electrode will simply not give enough of yield in signals from cells and thus the same procedure of making new electrode and implanting it in new rat has to be performed, which will take a longer time. As such, this type of experiments are already challenging and tedious and require relatively long time to collect the data. Next, due to electrodes being usable only for few months, it makes it sometimes hard to perform all needed experimental procedures in one animal, thus possibly creating only partial collection of data from some animals. While certain number of animals are needed for statistics, this will mean that more animals need to be implanted and experimented on. Also, in our case, experiments start on implanted animal before we know if the electrode has definitely ended up at correct coordinates. Thus, it can happen that few of the structures will have to be discarded in the analyses after histology check, which will cause longer time to collect sufficient amount of data. Lastly, it is still extremely hard to follow the same unit from the start of experiments to end, and even so between consecutive recordings. This makes some specific research at this moment impossible, such as in Paper IV where we want to investigate receptive field changes of a single neuron through time. Furthermore, it is also challenging to analyze data where some cells appear multiple times and are added as separate individual cells in analyses. As this type of recordings typically bring huge dataset, it would be

beneficial to apply a more automated search for single units and multi units versus noise, and search for the same cell present in different recordings.

For PD scientific community, monitoring acute dopamine pharmacological manipulation from healthy animal is beneficial to understand the possible first steps that could occur due to lack of dopamine. Observing all CBT structures in parallel during these different dopamine manipulations, also revealed what is the first change that occurs and how symptoms are related to it. However, there is a big difference between these experimental conditions and the chronic hemiparkinsonian rat model, and dopaminergic manipulations cannot be regarded as a PD model, but simply as help in elucidating the role of dopamine and its receptors in CBT structures. Our findings, indicate that future studies should be aimed at clarifying what populations of cells in the different structures that change their firing rates significantly and in which direction, and what role this has on motor symptoms and any other neurophysiological symptoms e.g. beta oscillation.

Lastly, creating computational method that can help us visualize global neurophysiological states from different behavioral conditions is a novel approach that in future should be further used, however, it still requires polishing of pulling together results from different animals to visualize it in the same coordinate system. This could happen due to non-consistent behavioral changes for the same drugs between animals and experiments in the same animal. Fundamentally, every individual animal has a unique brain, thus a certain number of reference neurophysiological states must be used to align the data.

Altogether, the CBT circuits display a number of changes in circumstances of dopamine dysregulation. These changes are found in all structures and this is likely due to dopamine receptors that are expressed all over the CBT loop (Smith and Villalba 2008) and due to the high degree of interconnectivity of almost all CBT structures. Furthermore, considering that dopamine dysregulation affects the whole circuit, it is only reasonable and appropriate to observe the circuit in parallel as a whole. Only this way we can come closer to finding what happens and how to help it.

For future studies we should experiment with local manipulations of CBT structures separately instead of applying drugs systematically in order to elucidate changes that one structure has and how this affects the others. Thus, further anatomical and physiological characterization of the CBT circuit is needed to display in detail how the structures of this circuit influence each structure's activity, and more knowledge is needed before we can fully understand what happens. Hence, new models should incorporate intrinsic processing within basal ganglia and their individual circuits (DeLong and Wichmann 2009).

At the same time it is needed to collect information about the even bigger picture, for example how cerebellum is involved. Cerebellum is another relevant motor structure that was not investigated in this thesis that should be recorded in parallel with other CBT structures. Initially, this was the goal, however, it was rather

challenging to experimentally accomplish implantations and subsequently good recordings due to the anatomy of rats and occasional internal bleeding in the cerebellum. Still, electrophysiological recordings of cerebellum in parallel to CBT circuit remains a challenging question to investigate in future. Considering that cerebellum corrects errors in motor movements, enables smooth execution and correct timing of muscle activation and reaction in order to perform movement, it is definitely one of the relevant structures to investigate. It would be especially interesting to see what kind of learning based on experience we could see in cerebellum and would it show plastic changes similarly to motor cortex as in our study presented here.

Ultimately, developing better techniques to monitor multiple brain structures in parallel was the key for the projects in this thesis, and beyond, to be realized. Moreover, investigating CBT structures in normally behaving animals and under acute dopamine pharmacological manipulation has provided new insights in how complex changes in firing rates are likely attributing to development of motor symptoms (akinesia). New method which was designed to help us finding new treatments against LID is going to speed up the screening process of potential candidate drugs. Lastly, providing information on how a motor system in the brain change based on sensory input will elucidate how we are all shaped by our experiences.

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Arriving to Sweden several years ago was somewhat of a cultural shock. However, in time I realized that Swedish people are just as welcoming as anywhere else in the world, although sometimes they need a bit of beer.

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