UPPSALA
UNIVERSITET

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 1958

The subthalamic nucleus in motor and affective functions

An optogenetic in vivo-investigation

ADRIANE GUILLAUMIN

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2020

ISSN 1651-6214
ISBN 978-91-513-0993-4
urn:nbn:se:uu:diva-417746

Dissertation presented at Uppsala University to be publicly examined in Friessalen, Evolutionsbiologiskt centrum, EBC, Norbyvägen 18, Uppsala, Monday, 12 October 2020 at 09:15 for the degree of Doctor of Philosophy. The examination will be conducted in English. Faculty examiner: Associate Professor Konstantinos Meletis (Karolinska Institutet, Department of Neuroscience).

Abstract
Guillaumin, A. 2020. The subthalamic nucleus in motor and affective functions. An optogenetic *in vivo*-investigation. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 1958. 88 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0993-4.

The basal ganglia form a group of subcortical interconnected nuclei involved in motor, limbic and cognitive functions. According to the classical model of the basal ganglia, two main pathways exert opposing control over movement, one facilitating movement and the other suppressing movement. The subthalamic nucleus (STN) plays a critical role in this function, and has also been implicated in reward processing. Despite ample knowledge of the role of the STN in motor dysfunctions in relation to Parkinson's disease, less is known about STN's natural role in healthy subjects.

The studies described in this thesis aimed to address the functional role of the STN in its natural neurocircuitry by using a transgenic mouse line which expresses Cre recombinase under the *Pitx2* promoter. The *Pitx2* gene is restricted to the STN and the use of Pitx2-Cre mice thereby allows selective manipulation of STN neurons by using optogenetics. By expressing Channelrhodopsin (ChR2) or Archaerhodopsin (Arch) in Pitx2-Cre neurons, we could optogenetically excite or inhibit STN Pitx2-Cre neurons and investigate the role of the STN in motor and affective functions. We showed that optogenetic inhibition and excitation of the STN induce opposite effects on motor activity. STN excitation reduced locomotion while STN inhibition enhanced locomotion, thereby providing experimental evidence to classical motor models postulating this role. We also showed that optogenetic excitation of the STN induces potent place avoidance, a behaviour relevant to aversion. Projections from the STN to the ventral pallidum (VP) exist that when excited induced the same behaviour. The VP projects to the lateral habenula (LHb), a structure known for its role in aversion. A glutamatergic multi-synaptic connection between the STN and the LHb was confirmed.

Aversive behaviour is also mediated by the hypothalamic-mesencephalic area. The *Trpv1* gene is expressed within the posterior hypothalamus. By applying optogenetics in a Trpv1-Cre mouse line, projection patterns to limbic brain areas were identified, and optogenetic excitation of Trpv1-Cre neurons was found to induce place avoidance.

The STN and posterior hypothalamus are thereby demonstrated as new players in the aversion neurocircuitry, while the long-assumed role of the STN in motor behaviour is confirmed. To enable future analyses of how STN manipulation might rescue motor and affective deficiency relevant to human disorders, a neuronal degeneration mouse model was generated.

To conclude, the results presented in this thesis contribute to enhanced neurobiological understanding of the role played by the STN in motor and affective functions.

Keywords: Subthalamic nucleus, optogenetics, basal ganglia, locomotion, Parkinson's disease, aversion

Adriane Guillaumin, Department of Organismal Biology, Comparative Physiology, Norbyv 18 A, Uppsala University, SE-75236 Uppsala, Sweden.

ISSN 1651-6214
ISBN 978-91-513-0993-4
urn:nbn:se:uu:diva-417746 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-417746)

“There is a single light of science, and to brighten it anywhere is to brighten it everywhere.”
— Isaac Asimov

À ma famille,

List of Papers

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

I Guillaumin, A., Serra, G.P., Georges, F., Wallén-Mackenzie, Å. (2020): *Optogenetic investigation into the role of the subthalamic nucleus in motor control.*
BioRxiv. doi: 10.1101/2020.07.08.193359.
Submitted to journal.

II Serra, G.P., Guillaumin, A., Baufreton, J., Georges F., Wallén-Mackenzie Å. (2020): *Aversion encoded in the subthalamic nucleus.*
BioRxiv. doi: 10.1101/2020.07.09.195610.
Submitted to journal.

III Guillaumin A., Vlcek B., Dumas S., Serra G.P., Wallén-Mackenzie Å. (2020): *Anatomical-functional analysis of the spatially restricted Transient receptor vanilloid-1 (Trpv1)-positive domain within the medial hypothalamic-mesencephalic area.*
Manuscript.

IV Guillaumin A. & Wallén-Mackenzie Å. (2020): *Optimization protocol for the 6-OHDA model of Parkinson´s disease in wild-type mice*
Manuscript.

Contents

Abbreviations

6-OHDA	6-hydroxydopamine
µg	microgram
µl	microliter
AAV	adeno-associated virus
Aldh1a1	Aldehyde dehydrogenase 1 family, member A1
AP	antero-posterior
Arch	Archaerhodopsin
BNST	bed nucleus of the stria terminalis
Calb1	Calbindin 1
ChR2	Channelrhodopsin 2
Cre	Cre recombinase
EP	entopeduncular nucleus
eYFP	enhanced yellow fluorescent protein
FA	formaldehyde
f/f	flanked by floxed sites
D1R	dopamine receptor subtype 1
D2R	dopamine receptor subtype 2
DA	dopamine
DBS	deep brain stimulation
DIG	digoxigenin
DV	dorso-ventral
EF1a	human elongation factor 1alpha promoter
EP	entopeduncular nucleus
GABA	gamma-aminobutyric acid
GP	globus pallidus
GPi	globus pallidus *interna*
Grp	Gastrin-releasing peptide
HFS	high-frequency stimulation
IF	interfascicular nucleus
IMAO-B	inhibitor of the monoamine oxidase B
IPF	interpeduncular fossa
LFS	low-frequency stimulation
LHA	lateral hypothalamic area
LHb	lateral habenula
MAB	maleate buffer
mAcbSh	medial part of the nucleus accumbens shell
MFB	median forebrain bundle

mg	milligram
ml	medial lemniscus
ML	medio-lateral
mm	millimeter
MM	mammillary bodies
MnM	medial mammillary nucleus, median part
mPFC	medial prefrontal cortex
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSN	medium spiny neurons
NAc	nucleus accumbens
nl	nanoliter
nm	nanometer
PAG	periaqueductal gray matter
PBP	parabrachial pigmented nucleus
PBS	phosphate buffer saline
PCR	polymerase chain reaction
PD	Parkinson's disease
PFA	paraformaldehyde
PIF	parainterfascicular nucleus
Pitx2	Paired-like homeodomain 2 transcription factor
PH	posterior hypothalamus
PHA	posterior hypothalamic area
PN	paranigral nucleus
pSTN	parasubthalamic nucleus
RLi	rostral linear nucleus
RM	retromammillary nucleus
RMM	retromammillary nucleus, medial part
RML	retromammillary nucleus, lateral part
RMTg	rostromedial tegmental nucleus
SNr	substantia nigra *pars reticulata*
SNc	substantia nigra *pars compacta*
SSC	saline-sodium citrate buffer
STN	subthalamic nucleus
SuM	supramammillary nucleus
tg	transgenic allele
Trpv1	Transient receptor potential cation channel subfamily V member 1 or vanilloid receptor 1
Vglut2	Vesicular glutamate transporter 2
Vglut3	Vesicular glutamate transporter 3
VP	ventral pallidum
VTA	ventral tegmental area
wt	wild-type allele
ZI	zona incerta

Introduction

All along our existence, we as human beings, but also any living animal, make decisions that will shape and orientate the course of our life. Those decisions are the final results of complex processes related to positive and negative emotions, motivation and memories, and will be implemented by following an adequate selection of actions to reach a goal. For example, our behaviour will change and adapt depending on sensory stimuli we perceive in different contexts: It is -20°C outside, drinking a warm tea by a fireplace would sound very pleasant and a series of actions will be implemented to reach this goal. However, if it was 35°C outside, our behaviour will adapt in a very different manner and we would probably end up in a swimming pool instead. Those behaviours, which include affective, cognitive and motor (executive) functions, are rendered possible by an assembly of subcortical nuclei called the basal ganglia that are connected to the cerebral cortex to form top-down control loops. Among those interconnected nuclei, the subthalamic nucleus (STN) plays an important and central role as an excitatory input to the GABA nuclei of the basal ganglia. In this thesis, studies have been performed in order to identify and analyse the role of the STN in two of the main functions of the basal ganglia: Motor and affective functions.

The basal ganglia

The basal ganglia are a group of interconnected subcortical nuclei: The striatum (caudate and putamen), pallidum, substantia nigra (SN) and the STN. The major input to the basal ganglia is the cerebral cortex which projects to the striatum and STN, while the internal part of the pallidum and the substantia nigra *pars reticulata* (SNr) are considered the basal ganglia output structures and project to the thalamus. The basal ganglia consists of three parallel loops corresponding to their role in affective, associative and motor functions (Alexander, Crutcher and DeLong, 1990). The motor loop is the best known and most well studied loop of the three while fewer studies have investigated the affective (or limbic) and associative loops. Accordingly, the

basal ganglia are mostly known for their role in movement-related behaviours, and their disorders.

The motor loop

The motor loop is necessary for executing movements. The cortex sends glutamatergic projections to the medium spiny neurons (MSNs) of the dorsal striatum. The dorsal striatum is primarily a GABAergic structure which, in addition to excitatory cortical input, is modulated by dopamine (DA) projections from the substantia nigra *pars compacta* (SNc). Via the striatum, two pathways function in parallel with opposite effects on movement: 1) *The direct pathway* which starts from MSNs in the striatum expressing the post-synaptic DA receptor D1 (D1R) that project directly to the output structures of the basal ganglia, the SNr and the globus pallidus *interna* (GPi, or EP for entopeduncular nucleus in rodents). This GABAergic pathway inhibits the SNr/EP which leads to disinhibition of the ventro-lateral thalamus, and consequently, to promotion of movement; and, 2) *The indirect pathway* which is initiated from MSNs expressing the post-synaptic DA D2 receptor (D2R) and projecting to the globus pallidus *externa* (GPe, or GP in rodents). The GP is a GABAergic nucleus which sends strong inhibitory projections to the

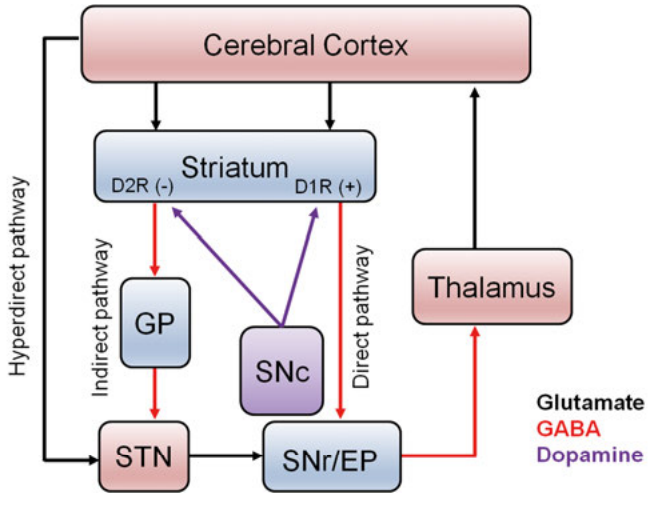

***Figure 1**:* Simplified representation of the basal ganglia circuitry with the direct, indirect and hyperdirect pathways. Excitatory structures are in red, inhibitory structures in blue and modulatory structures in purple. GP: globus pallidus; STN: subthalamic nucleus; SNc: substantia nigra *pars compacta*; SNr: substantia nigra *pars reticulata*; EP: entopeduncular nucleus; D1R: dopamine receptor D1; D2R: dopamine receptor D2.

STN. MSNs of the indirect pathway inhibit the GP which leads to the disinhibition of the STN, an excitatory nucleus. The STN excites the output structures of the basal ganglia (SNr/EP) which in turn inhibit the ventro-lateral thalamus and consequently suppresses unwanted movements. In addition to the direct and indirect pathways, a third pathway, *The hyperdirect pathway*, by-passes the striatum and serves as a fast stopping pathway. In this pathway, the STN receives direct projections from the cortex.

The direct, indirect and hyperdirect pathways regulate executive functions according to the classical basal ganglia model (Figure 1). The direct pathway facilitates movement while the indirect and hyperdirect pathways act as a "brake" and suppress movement. The normal functioning of the basal ganglia is therefore necessary to perform adequate movements, like walking or reaching for a glass of water.

The limbic loop

The limbic loop engages the same brain structures as the motor loop, but different areas within these. Instead of the dorsal striatum, the limbic loop engages the ventral striatum, known as the nucleus accumbens (NAc). The NAc receives DA projections from the ventral tegmental area (VTA) instead of the SNc, and GABAergic projections from the ventral pallidum (VP) instead of the GP. The limbic loop conveys affective functions such as reward-related behaviours. The cortex sends excitatory input to the NAc, which contains MSNs, just as the dorsal striatum of the motor loop. Direct, indirect and hyperdirect pathways are also similar to as in the motor loop but here target the medial and ventral aspects of the pallidal and subthalamic structures: The ventral pallidum (VP) and the medial tip of the STN, also known as the limbic tip. In the limbic loop, the SNr/EP send projections to the medio-dorsal nucleus of the thalamus.

The cognitive loop

The cognitive loop, also called the associative loop, is the third parallel loop of the basal ganglia which slightly differs anatomically. Indeed, this loop involves the dorso-lateral part of the prefrontal cortex which sends projections to the anterior caudate (anterior striatum in rodents). GABAergic neurons from the anterior caudate innervate the GPi and SNr which in turn inhibit the medio-dorsal and ventral-anterior nuclei of the thalamus. The involvement of the basal ganglia in cognitive and associative functions is well

known (Brown, Schneider and Lidsky, 1997). Several studies have shown its role in goal-directed behaviours, decision-making, action selection and attention (Middleton and Strick, 2000; Rogers *et al.*, 2001; Stocco, 2018; Rusu and Pennartz, 2020). Impairment of the cognitive loop leads to cognitive symptoms observed in many brain disorders implicating the basal ganglia, like PD and obsessive compulsive disorder (OCD), further discussed below (Benzina *et al.*, 2016; O'Callaghan and Lewis, 2017).

Basal ganglia-related disorders

Given the importance of the basal ganglia in motor, cognitive and affective functions, dysregulation of basal ganglia pathways is strongly associated with disorders and diseases, including Parkinson´s disease, hemiballismus, chorea, Huntington´s disease and Obsessive compulsive disorder. Below follows a short description of Parkinson´s disease and Obsessive compulsive disorder, both of which are considered in the studies of this thesis.

Parkinson's disease

Parkinson's disease (PD) is the second most common progressive neurodegenerative disease after Alzheimer disease with a prevalence of 1 to 2 per 1000 (Tysnes and Storstein, 2017). There are two main forms of PD: *Familial PD* and *idiopathic PD*. The onset of familial PD starts before the age of 50 and is often the consequence of a mutation. The idiopathic form of PD appears later in life, above 60 years, and is the consequence of neurodegeneration of the dopaminergic neurons in the SNc. The death of SNc DA neurons leads to decreased DA release in the dorsal striatum, which in turn affects basal ganglia function (Obeso *et al.*, 2017; Tysnes and Storstein, 2017; Khan *et al.*, 2019).

The cause of DA cell degeneration is unclear but has been proposed to depend on a mixture of genetic background and environmental factors (Marras, Canning and Goldman, 2019). DA cell degeneration causes dysregulation of the basal ganglia with over-activation of the indirect pathway over the direct pathway. Changes in firing activity of basal ganglia nuclei has been observed, in particular in the STN where the firing pattern becomes irregular (Bergman *et al.*, 1994; Benazzouz *et al.*, 2002). This dysfunction leads to progressive motor symptoms such as bradykinesia (slowness of movements), akinesia (failure to make a movement, freezing), tremor, difficulty to initiate

movement, and rigidity. Non-motor symptoms are also present in PD, sometimes before the appearance of motor symptoms: Olfactory dysfunction, apathy, mood disorders, sleep disorders, cognitive changes and autonomous-related functions like constipation and urination (Khoo *et al.*, 2013; Obeso *et al.*, 2017).

The main histological feature of PD is the presence of protein aggregates of abnormally folded alpha-synuclein. Alpha-synuclein is a naturally occurring protein in the brain, however, in PD, misfolded alpha-synuclein proteins aggregate into large complexes, forming so called Lewy bodies. These accumulate progressively in cerebral structures, including the DA neurons of the SNc (Braak *et al.*, 2003). Already in the early 2000's, Braak and colleagues hypothesized that alpha-synuclein could travel via the vagus nerve from the gut to the brain, and several studies have recently provided evidence supporting this hypothesis (Liddle, 2018; Kim *et al.*, 2019; Elfil *et al.*, 2020).

Current treatments for PD focus mostly on replacing the loss of DA by dopaminergic agonists or levodopa, a precursor to DA. These treatments are often combined with inhibitors of the monoamine oxidase B (IMAO-B) to prevent the degradation of DA. Another treatment used for patients suffering an advanced-stage PD is deep brain stimulation (DBS) of the STN which aims to stabilize aberrant STN activity by applying high-frequency electrical stimulation (will be discussed more below). Finally, another type of strategy to treat PD consists of transplanting mesencephalic DA neurons derived from human pluripotent stem cells, also called hPSC-derived mesDA neurons. This method aims to replace the loss of mesencephalic neurons in the SNc of PD patients by transplanting hPSC-derived mesDA neurons directly into the putamen (striatum). Pre-clinical studies have shown interesting results in animal models of PD. DA release from the transplanted hPSC-derived mesDA neurons was observed as well as improved motor symptoms (Grealish *et al.*, 2014; Chen *et al.*, 2016). The translation of this method to PD patients seems promising with one clinical study recently initiated (Cyranoski, 2018; Parmar, Grealish and Henchcliffe, 2020).

Intensive research is still on-going to find better treatments for PD. To do so, researchers use various methods to generate animal parkinsonian models. Among them, neurotoxin-based models are the most common. For example, application of either of the toxins 1-methyl-4-phenyl-1,2,3,6-

tetrahydropyridine (MPTP) or 6-hydroxydopamine (6-OHDA) is commonly used to generate experimental PD models in non-human primates and rodents, primarily mice and rats. 6-OHDA is a neurotoxin commonly injected into the median forebrain bundle (MFB), the dorsal striatum, or the SNc with the objective to induce degeneration of the nigrostriatal pathway and mimic the loss of DA neurons in PD. 6-OHDA injections lead to degeneration of SNc DA neurons while VTA DA neurons are substantially less affected, for reasons not entirely known. Unilateral injection of 6-OHDA leads to unilateral degeneration of SNc DA neurons, and, consequently, the experimental animals display strong motor impairments with ipsilateral rotations (Boix, Padel and Paul, 2015; Park *et al.*, 2015).

Obsessive compulsive disorder

Obsessive compulsive disorder (OCD) is a common and chronic disorder characterized by excessive and uncontrollable thoughts (obsessions) and/or behaviours (compulsions). Since these obsessions and compulsions can go on for several hours a day, OCD has a large negative impact on everyday life.

The symptoms of OCD often appear in early adulthood with an earlier onset in boys than girls. The cause of OCD is unknown but seems multifactorial: Genetic background, environmental factors like childhood trauma, and even infections (Williams and Swedo, 2015; Robbins, Vaghi and Banca, 2019; Stein *et al.*, 2019). Imaging studies have shown that affected brain areas include the orbital frontal cortex and the basal ganglia, in particular the caudate nucleus (Baxter, 1987; Pauls *et al.*, 2014; Haber, 2016). Two current hypotheses aim to explain the neurobiological basis of OCD: "*The cognitive hypothesis"* which suggests a dysfunction in the valence attribution to a goal-directed behaviour and its outcome; and "*the habit hypothesis"* which postulates that OCD symptoms are due to a shift from goal-directed behaviours to excessive habit formation, with the compulsive action preceding the obsessive thoughts (Gillan and Robbins, 2014). Besides impairment in cognitive control and goal-directed and habit imbalance, emotional vulnerability including anxiety has long been considered an important factor in the etiology of OCD (Robbins, Vaghi and Banca, 2019). In addition to OCD, compulsive behaviour is also included in the symptom domain of substance abuse disorder and pathological gambling (Figee *et al.*, 2016).

Deep brain stimulation

Early in the 1960's, a study from Albe Fessard and colleagues reported that high-frequency stimulation (HFS), also called Deep brain stimulation (DBS) in humans, of the ventro-intermediate thalamic nucleus was efficient for treating tremor in PD patients (Benabid *et al.*, 1991). The results were similar to those obtained by surgical lesioning of the area, but more efficient than a thalamotomy and with the important advantage of being reversible. The DBS method used consisted of implanting electrodes in the desired cerebral structure and sending short pulses (60-100 μs) at a frequency ranging from 100 to 185 Hz. DBS was later on improved by Benabid and colleagues in the late 1980's, and applied in the 1990's in non-human primate models and subsequently in PD patients for the treatment of motor symptoms (Benabid *et al.*, 1994). Among different brain areas used as implantation sites for the stimulating electrodes, including thalamic nuclei, the GP and the STN, the STN was found to be the most efficient area for improving motor symptoms in PD, in particular when associated with levodopa treatment (Vizcarra *et al.*, 2019). While motor improvement in PD is more efficient when applying DBS in the STN (STN-DBS) compared to GPi (GPi-STN) (Odekerken *et al.*, 2016), GPi-STN has the advantage that patients need not adjust their medication to avoid treatment-induced dyskinesia (Vitek, 2002). Today, DBS treatment is a recommended clinical approach to alleviate motor symptoms in PD at the advanced-stage when DA neurons have substantially degenerated, and DA-based treatments no longer are efficient.

Theories of DBS mechanisms

The mechanisms underlying the beneficial effects of DBS remain to fully resolve, and are therefore still debated. Because STN-DBS induces similar clinical outcome as a lesion or a STN blockade (Bergman, Wichmann and DeLong, 1990; Luo, 2002), the main theory is that DBS inhibits neuronal activity. Neuronal inhibition induced by STN-DBS was first observed in rats and subsequently in non-human primates and humans in the 1990's. Several studies have provided evidence supporting the hypothesis that STN-DBS induces an inhibition of STN neurons and STN output structures. Studies in humans, monkeys and rodents have shown a decrease in the firing rate of STN neurons and STN output structures upon HFS (Tai *et al.*, 2003; Filali *et al.*, 2004; Meissner *et al.*, 2005). The mechanisms are unclear but several hypotheses exist: 1) Activation of the presynaptic inhibitory fibres innervating the STN (Boraud *et al.*, 1996; Deniau *et al.*, 2010; Chiken and Nambu,

2013); 2) block depolarization (Bikson *et al.*, 2001); and 3) inactivation of voltage-gated currents (Beurrier *et al.*, 2001; Shin *et al.*, 2007).

Despite strong evidence supporting the "inhibition theory", other studies have shown the opposite, that STN-DBS is, in fact, excitatory. First, studies have shown that HFS of the STN causes release of glutamate in some of the output structures of the STN (Lee *et al.*, 2004) as well as an increase in firing rate and c-fos levels in some of the target structures of the STN, namely the GP, EP and SNr (Hashimoto *et al.*, 2003; Galati *et al.*, 2006; Reese *et al.*, 2011; Shehab *et al.*, 2014). Second, STN-DBS triggers antidromic activation of cortical areas and induces changes in oscillatory activities and synchronicities between the basal ganglia nuclei (Li *et al.*, 2007; Moran *et al.*, 2011; Degos *et al.*, 2013). Finally, it has been proposed that the beneficial effects on movement observed in PD patients could come from the disruption of the abnormal activity in the indirect and hyperdirect pathways during PD, which would allow regulation of the aberrant hyperactivity displayed by the STN (Chiken and Nambu, 2016).

Side-effects upon DBS treatment

Despite giving rise to great improvements in motor control, observations have shown that STN-DBS can cause adverse side-effects, primarily in limbic and cognitive functions (Kim, Jeon and Paek, 2015; Serranová *et al.*, 2019). DBS is a non-selective electrical stimulation method through which all neural structures that come in contact with the HFS will be affected. Because of this lack of specificity, not only STN neurons, but also cerebral structures surrounding the STN as well as passing fibres will be reached by the electrical stimulation. In addition, as discussed further below, the STN is likely composed of several internal domains, or territories, that are differentially involved in motor, cognitive and affective functions. Thus, depending on the precise position of the DBS electrodes in the subthalamic area, the treatment can cause both alleviation of motor symptoms and unwanted side-effects (Petry-Schmelzer *et al.*, 2019).

The reasons for the variability in success rate and the appearance of side-effects are not entirely known. It has been suggested that the clinical outcome of STN-DBS in PD patients depends on the stimulation of different domains within the STN, the electrical parameters of the stimulation, damages along the trajectory of the electrodes, changes in the medication and/or

the progressive nature of the disease. For example, it has been shown that electrodes placed in different positions within the STN motor domain induce different clinical outcomes depending on the antero-posterior location (Serranová *et al.*, 2013). Several studies have shown mixed results in post-surgery depression and apathy with either improvements or deteriorations (Czernecki, 2005; Kalteis *et al.*, 2006; Le Jeune *et al.*, 2009; Pariwatcharakul *et al.*, 2013; Pinsker *et al.*, 2013; Robert *et al.*, 2014; Accolla and Pollo, 2019). Non-motor side-effects that are regularly observed are depression, apathy, weight gain, mood changes, worsened verbal fluency, hyper/hypomania and impulse control disorder (Bronstein *et al.*, 2011; Witt, Daniels and Volkmann, 2012; Nassery *et al.*, 2016).

The subthalamic nucleus, STN

The STN is a bilateral, small and dense nucleus located between the zona incerta dorsally and the cerebral peduncle ventrally, posteriorly to the EP and anteriorly to the midbrain. In humans, it is considered as a "closed" nucleus, meaning that STN dendrites are mainly restricted within the nucleus itself, except on its medial aspect where it opens on the lateral hypothalamic area (LHA) (Figure 2). The STN primarily contains glutamatergic projection neurons expressing the *Vglut2/Slc17a6* gene encoding the Vesicular glutamate transporter 2 (VGLUT2). VGLUT2, together with VGLUT1 and VGLUT3, form a family of proteins in which members enable packaging of glutamate into presynaptic vesicles for neurotransmitter release. The three VGLUTs show different distribution patterns in the brain. The *Vglut2* gene is predominantly expressed in neurons of the STN, thalamus, hypothalamus,

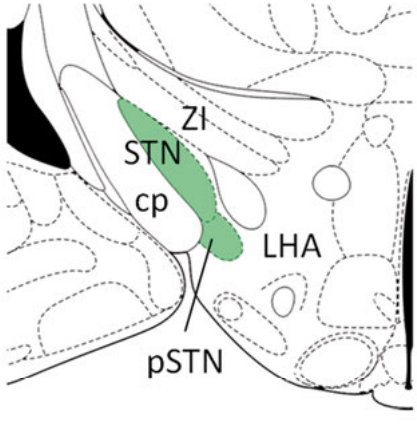

***Figure 2**:* Schematic representation of the subthalamic nucleus (STN) and para-STN nucleus (pSTN) in green on a coronal plan at -2.06 mm from the bregma. ZI: zona incerta; cp: cerebral peduncle; LHA: lateral hypothalamic area. Adapted from the Franklin & Paxinos atlas.

brainstem and some forebrain structures; the VGLUT2 protein localizes to synaptic vesicles in the presynaptic terminals of these neurons (Herzog *et al.*, 2001; Kaneko and Fujiyama, 2002; Oliveira *et al.*, 2003; El Mestikawy *et al.*, 2011).

While VGLUTs show broad distribution patterns and cover the vast extent of glutamatergic neurons in the brain, the *Pitx2* gene encoding the Paired-like homeodomain 2 (PITX2) transcription factor shows high selectivity for the STN. The expression of the *Pitx2* gene in the STN was identified already in the early 2000´s in studies also showing that, in the mouse, *Pitx2* gene expression is required for the primary neuronal migration from the hypothalamus to form the STN, and consequently necessary for its development (Martin *et al.*, 2004a; Skidmore *et al.*, 2008a). In the adult rodent, Pitx2 mRNA can also be found in the adjacently located para-STN (pSTN) and in some hypothalamic and mammillary neurons, but at substantially lower levels than in the STN (Wallén-Mackenzie 2020). Pitx2 mRNA overlaps to near-100% with Vglut2 mRNA, further identifying the glutamatergic nature of the Pitx2-positive STN neurons (Schweizer *et al.*, 2016).

The primate STN is often divided in three internal anatomical-functional domains, or territories, corresponding to the three loops of the basal ganglia: A dorso-lateral domain involved in motor functions, a ventral domain involved in associative/cognitive functions and the medial tip, the limbic tip, for limbic functions (Figure 3). This tripartite macro-architecture, the so called *tripartite model*, was first characterized by anatomical tracing studies followed by clinical results obtained in STN-DBS treatment of PD, and the improvement of imaging technologies (Lambert *et al.*, 2012; Alkemade, Schnitzler and Forstmann, 2015). Indeed, the location of DBS electrodes in the dorso-lateral domain of the STN is crucial to alleviate motor symptoms in PD patients while limiting side-effects. However, a strict tripartite organization of the STN in humans and non-human primates is debated (Alkemade, Schnitzler and Forstmann, 2015).

Several studies have suggested that the macro-architecture of the STN and many other cerebral structures is more intermingled in rodents than in humans and non-human primates with the overlap of different neuronal subpopulations (Figure 3) (Mallet *et al.*, 2007; Alkemade, Schnitzler and Forstmann, 2015; Janssen *et al.*, 2017). Here, the identification of intermingled cellular organization, rather than domain-type organization, in other basal ganglia structures such as the GP (Hegeman *et al.*, 2016) and in midbrain structures like the VTA (Viereckel *et al.*, 2016a; Poulin *et al.*, 2018), does not lend support to the tripartite model. Furthermore, studies in humans and monkeys investigating the distribution of various proteins and mRNAs in the STN have found contradicting results with both clear expression in one of the STN domain for some mRNA/proteins like parvalbumin and calretinin (Parent *et al.*, 1996; Augood *et al.*, 1999) and homogeneous expression in the whole STN for others, like tyrosine hydroxylase (TH), prepro-Enkephalin B, $GABA_B$ and $GABA_A$ receptors (Kultas-Ilinsky, Leontiev and Whiting, 1998; Charara *et al.*, 1999; Hedreen, 1999; Aubert *et al.*, 2007).

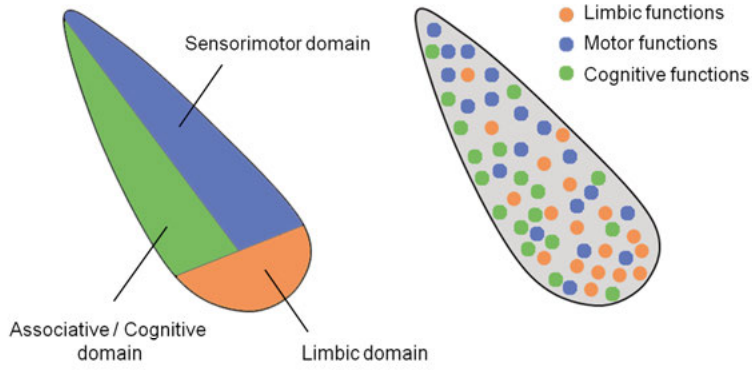

***Figure 3**:* Schematic representation of the internal organization of the STN according to the tripartite model (left) and the intermingled hypothesis (right). Left: Blue, the sensorimotor domain (dorso-lateral); green, the associative/cognitive domain (ventro-medial); orange, the limbic domain (medial, also known as the limbic tip). Right: An overlap of intermingled subpopulations of neurons across the entire STN but the preservation of some topographic organization.

Afferent projections to the STN

The inputs and outputs of the STN were mostly investigated in the 1980's and 1990's by using neuronal tracers like the retrograde cholera toxin subunit B, CTB, and the anterograde protein from *Phaseolus vulgaris*, PHA-L.

Studies using neuronal tracing methods showed that the STN receives excitatory input from various cortical areas and the centro-median parafascicular complex of the thalamus, and inhibitory input from the GP and VP (Figure 4). Monoaminergic projections from the dorsal raphe nucleus (serotonin) and SNc (DA) were also identified in several publications (Parent and Hazrati, 1995).

Efferent projections from the subthalamic nucleus

The STN sends glutamatergic projections to both basal ganglia and non-basal ganglia structures. Substantial projections reach the SNr and EP, and the STN also communicates reciprocally with the GP. To a lesser extent, STN neurons innervate the SNc, VP and the pedunculopontine nucleus (PPN) (Figure 4) (Schweizer *et al.*, 2016; Fife *et al.*, 2017). In addition to these well-known target structures, tracing studies have shown projections from the STN to the striatum, the thalamus, and the VTA (Nauta and Cole, 1978; Kita and Kitai, 1987; Groenewegen and Berendse, 1990).

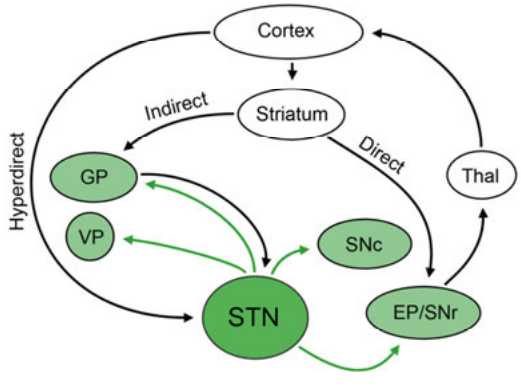

***Figure 4**:* Illustration of the STN circuitry within the basal ganglia. The STN projects to the globus pallidus (GP), substantia nigra *pars compacta* (SNc) and *pars reticulata* (SNr), the entopeduncular nucleus (EP) and the ventral pallidum (VP).

Motor functions of the STN

"Despite the current interest, still very little is known about the STN's normal function in relation to movement"

(Alkemade, Schnitzler, and Forstmann 2015).

The role of the STN in motor functions has long been investigated in relation to PD. However, as stated in the quote above, knowledge of its natural role in movement is surprisingly limited (Alkemade, Schnitzler and Forstmann, 2015). Instead, most knowledge is derived from various disease models, and through clinical observations. For example, Whittier and Mettler showed already in 1949 that a lesion of the STN induces hemichorea/ballism in monkeys (Whittier and Mettler, 1949; Carpenter, Whittier and Mettler, 1950). Later on, studies confirmed these findings with more selective lesions. Crossman and colleagues showed that injection of bicuculline, a $GABA_A$ receptor antagonist, into the STN, induced uncontrollable and irregular limb movements, also called hemiballism (Crossman, Sambrook and Jackson, 1984). This finding was confirmed by Beurrier and colleagues by lesioning the STN in healthy monkeys (Beurrier *et al.*, 1997). Several studies in rodents and monkeys have used neurotoxin-based models to induce Parkinsonism. It has been shown that subthalamotomy in MPTP-lesioned or 6-OHDA-lesioned rodents and monkeys abolishes the toxicity-induced motor symptoms (Bergman, Wichmann and DeLong, 1990; Aziz *et al.*, 1991; Beurrier *et al.*, 1997; Chang *et al.*, 2003; Darbaky *et al.*, 2003; Marin *et al.*, 2013). Similar results were observed in PD patients after subthalamotomy or pallidotomy (Laitinen, Bergenheim and Hariz, 1992; Lozano *et al.*, 1995; Heywood and Gill, 1997). According to the classical basal ganglia model, this alleviation of motor symptoms in PD is derived from the removal of STN over-activation which lowers the neuronal activity of GPi/EP neurons, and consequently disinhibits the thalamus and promotes movement.

Beyond STN-DBS in PD and STN-HFS studies in animal PD models, only few studies have investigated the normal function of the STN. Instead, most studies have used STN-HFS in non-human primates or rodents to better understand the mechanisms of STN-DBS in PD. However, some STN-HFS studies have included healthy rodents as control groups and showed that unilateral STN-HFS induces contralateral rotations and dyskinesia (Bergmann *et al.*, 2004; Boulet *et al.*, 2006).

In addition to electrical stimulation models, another method to experimentally study the function of a brain structure is conditional mouse genetics. Mice in which glutamate packaging and release has been reduced in STN neurons, by the combination of Pitx2-Cre mice (Martin *et al.*, 2004a) with the floxed allele of *Vglut2* (Wallen-Mackenzie, 2006), resulted in hyperlocomotion characterized by increased horizontal and vertical (rearing) activities without

affecting gait and fine motor coordination (Schweizer 2014, 2016, Pupe, Schweizer, Mackenzie, 2014). These studies thereby provided indirect support for the role of STN glutamatergic neurotransmission in locomotor regulation, in accordance with the basal ganglia model of movement regulation.

Over the past decade, optogenetics has been implemented to study the STN. However, in contrast to other cerebral structures, the behavioural effect of optogenetic excitation or inhibition of the STN has only rarely been investigated in normal, healthy animals. Instead, the vast majority of optogenetic studies of the STN have been performed in 6-OHDA lesioned rodents. For example, it has been shown that optogenetic inhibition of the STN in 6-OHDA lesioned rats improves forelimb akinesia and levodopa-induced dyskinesia (Yoon *et al.*, 2014, 2016). Also in 6-OHDA lesioned rodents, activation of cortico-subthalamic afferent projections with HFS above 100 Hz reverses the induced motor symptoms while low frequency stimulation (LFS) worsen the motor symptoms, or has no effect (Gradinaru *et al.*, 2009; Sanders and Jaeger, 2016). In healthy mice, Tian and colleagues observed hyperkinesia, decreased exploratory activity and stereotyped movements (grooming) upon activation of GABAergic GP neurons with 20 Hz optogenetic stimulation. The same study also showed that activation of subthalamic glutamatergic terminals in the GP induces hyperkinesia and stereotyped movements such as grooming and dystonia-like behaviours (Tian *et al.*, 2018). A hypothesis aiming to explain the opposite behavioural outcome between STN-HFS and STN-LFS in PD animal models postulates that spontaneous activity is still present during STN-LFS while STN-HFS suppresses spontaneous activity to replace it with a different and regular pattern (Garcia *et al.*, 2005).

Finally, only one study has investigated the effect of direct optogenetic excitation of the STN in healthy mice, and could demonstrate that brief activation of the STN was sufficient to interrupt an on-going licking behaviour while inhibition of the STN could suppress the interruptive effect of surprise (Fife *et al.*, 2017). This study was important as it directly pin-pointed the role of the STN in pausing/stopping movements that have already been initiated.

Affective and associative functions of the STN

Since the discovery of STN-DBS in the 90's, most studies have focused on motor functions of the STN. However, increasing research is now directed towards the role of the STN in affective functions, not least due to the concern for non-motor symptoms in PD. In addition, there is increasing interest in the potential role played by the STN in addiction and OCD (Bari and Robbins, 2013; Pelloux and Baunez, 2013; Creed, 2018; Pelloux *et al.*, 2018; Rappel *et al.*, 2018). The role of the STN in limbic functions is not as well understood as for motor functions. Several studies have shown that the non-motor side effects induced by STN-DBS could come from the stimulation of the limbic and/or associative domains of the STN itself, and not from surrounding regions or fibres (Temel *et al.*, 2005, 2006; Mallet *et al.*, 2007; Haegelen *et al.*, 2009; Baláž *et al.*, 2011). This is an interesting observation which points towards the importance of increasing the understanding of precisely how the STN is involved in regulation of the various functions it has been associated with, i.e. motor, cognitive/associative and limbic/affective functions. Improved knowledge of the anatomical-functional organization of the STN is clearly of essence.

In the context of affective functions, the STN is intrinsically connected to limbic brain structures. The STN receives its cortical projections from the prefrontal cortex area, well known for its role in cognitive and limbic functions (Haynes and Haber, 2013), and from the VP, a central structure of the reward system which projects directly to the VTA and the lateral habenula, LHb (Root *et al.*, 2015; Wulff *et al.*, 2019). In turn, the STN sends direct projections to limbic structures such as the VP, or via relay structures such as the EP, to the LHb, a pathway involved in evaluating action outcomes (Stephenson-Jones *et al.*, 2016). The LHb itself is known for its role in regulating negatively motivated behaviours and for its contribution to psychiatric diseases like major depression disorder and addiction (Lecca *et al.*, 2017; Hu, Cui and Yang, 2020). Additional structures of the limbic system are affected by STN-HFS with an increase of DA release in the NAc shell (NAcbSh) and NAc core (NAcbC), and a decrease of GABA in the VTA (Winter, Lemke, *et al.*, 2008). Further, mice lacking Vglut2 in Pitx2-Cre STN neurons also displayed an increase in dopamine transporter (DAT) capacity in the NAcbSh (Schweizer *et al.*, 2016).

Similar to the majority of studies investigating the role of the STN in motor function, most studies interested in the role of the STN in limbic and cogni-

tive functions have used STN-HFS, primarily in PD models. Several studies in rodents have demonstrated a role of the STN in reward processing: Electrophysiological recordings in the STN showed that neurons responded differently to positive and/or negative stimuli as well as to reward omission. In addition, changes in the activity of such STN subpopulations predicted the availability of reward, pointing towards a role of STN neurons in reward prediction error (Breysse, Pelloux and Baunez, 2015). Further, it has been shown that STN-HFS decreases the motivation for cocaine (Baunez *et al.*, 2005; Rouaud *et al.*, 2010) and prevents the re-escalation of heroin intake in rats (Wade *et al.*, 2017), confirming the importance of the STN in addictive behaviours. Another study showed that lesioning of the STN affects the processing for positive and negative reinforcers like saccharine and lithium chloride (Pelloux *et al.*, 2014). These findings are comparable to results obtained by Schweizer and colleagues in which mice with reduced *Vglut2* levels selectively in Pitx2-Cre neurons showed decreased sugar consumption compared to controls, but without reduced reward-related learning, memory, motivation or ability for task-switching (Schweizer *et al.*, 2016).

Besides affecting reward-related behaviours, several lines of evidence suggest that the STN is a key structure in regulating compulsive behaviours. In rodents, compulsive behaviours are characterized by stereotyped movements defined as motor responses that are repetitive, invariant, and seemingly without purpose or goal, for example excessive self-grooming, licking or self-gnawing (Kelley, 1998; Kalueff *et al.*, 2016). Studies using STN-HFS or STN lesioning have shown a decrease in compulsive behaviours in different OCD and autism animal models (Baup *et al.*, 2008; Winter, Mundt, *et al.*, 2008; Klavir *et al.*, 2009; Chang *et al.*, 2016). In contrast to STN inhibition or inactivation, disinhibition of the STN induces strong self-grooming (Tian *et al.*, 2018). Several animal studies have demonstrated that compulsive behaviours can be induced by activating different pathways within, or connected to, the basal ganglia: Overactivation of the ventral thalamus via injections of bicuculline in monkeys triggers repetitive and time-consuming motor acts (Rotge *et al.*, 2012); activation of the anterior part of the GPe via bicuculline injections also gave rise to compulsive behaviours in monkeys (Grabli, 2004); HFS, but not LFS, of the GP and the EP, reduced excessive self-grooming in an OCD rat model (Klavir, Winter and Joel, 2011); and lesion of the VP and GP impaired grooming without affecting its sequential organization (Cromwell and Berridge, 1996). The conclusions of these studies demonstrate the role of basal ganglia-related structures in compulsive behav-

iours, often characterized in rodent models of human disorders by excessive self-grooming. However, the involvement of many cerebral structures suggests that compulsive behaviours rely on complex circuitries which might process different aspects of compulsivity.

The ventral pallidum, VP

The VP, also referred as substantia innominata, is a structure located in the basal forebrain. The VP is about 2 mm long in mice, and extends along the antero-posterior axis from below the NAc to the anterior commissure, the bed nucleus of the stria terminalis (BNST) and the GP in its most posterior aspect. The VP was first described as an extension of the GPe but the presence of strong substance-P GABAergic fibres from the NAc distinguishes the VP from the dorsal aspect of the pallidum (Haber *et al.*, 1985; Zahm, 1989). Another difference between the VP and GP is the cellular heterogeneity of the VP with a majority of GABA neurons, but also glutamatergic and cholinergic neurons. GABAergic and glutamatergic VP neurons project reciprocally to the NAc and STN, and to the VTA and the LHb, while cholinergic VP neurons target preferentially the prefrontal cortex and the basolateral amygdala (Jones, 2004; Unal, Pare and Zaborszky, 2015). GABAergic and glutamatergic VP neurons seem to project to the same target structures, with some exceptions, and regulate the value of a stimulus in an opposite manner (Faget *et al.*, 2018). Indeed, as a part of the limbic loop of the basal ganglia, the VP plays an important role in reward- and aversive-related behaviours and motivated behaviours and also, to a lesser extent, in motor and cognitive behaviours (Root *et al.*, 2015; Saga *et al.*, 2017). The VP is well known for its role in addiction to drugs of abuse and has recently been proposed as a possible target for DBS as a new treatment strategy (McGovern and Root, 2019).

The lateral habenula, LHb

The habenula is a pair of medially positioned nuclei located dorsally of the thalamus. It is divided into two parts: the medial habenula and the lateral habenula, LHb. Neurons of the LHb are mainly glutamatergic and express the *Vglut2* or *Vglut3* genes, but some studies have also shown the presence of inhibitory interneurons expressing the *Glutamic acid decarboxylase 2 (GAD2)* gene and the *GABA transporter 1 (GAT1)* gene that trigger inhibito-

ry responses when stimulated optogenetically (Zhang *et al.*, 2018; Flanigan *et al.*, 2020; Webster *et al.*, 2020). However, the presence of functional GABA neurons in the LHb was recently questioned by Wallace and colleagues who confirmed a low expression of *GAD2* and *GAT1* but also showed the absence of expression of the *SlcSlc32a1* and *Slc18a2* genes, encoding mRNAs of the Vesicular inhibitory amino acid transporter, Viaat, and Vesicular monoamine transporter 2,Vmat2, respectively (Wallace *et al.*, 2020).

The LHb receives input from many forebrain structures including the medial prefrontal cortex (mPFC), lateral hypothalamic area (LHA), VP and EP. LHb neurons project to midbrain and brainstem structures including the SNc, VTA, rostromedial tegmental nucleus, dorsal raphe nucleus and locus coeruleus (Hu, Cui and Yang, 2020). The LHb gained substantial attention when Hikosaka and colleagues discovered the role of the LHb in negative reward processing (Hikosaka, 2010). Indeed, with its connections to both the dopaminergic and serotoninergic system, the LHb regulates many essential functions including reward-seeking behaviours, avoidance-like behaviours, sleep, anxiety, stress and pain. Dysfunction of the LHb is consequently involved in several severe neuropsychiatric disorders, including both addiction and major depression disorder (Matsumoto and Hikosaka, 2009; Hikosaka, 2010; Baker *et al.*, 2016; Lecca *et al.*, 2017; Shabel *et al.*, 2019; Hu, Cui and Yang, 2020).

A couple of studies have shown that STN-HFS can either activate or inhibit LHb neurons and that it induces c-fos expression in the LHb (Tan *et al.*, 2011; Hartung *et al.*, 2016). Curiously, however, no direct connection has ever been demonstrated between the STN and the LHb. Some studies have shown that the VP and/or the EP could be relay structures between the STN and the LHb. Indeed, it was recently shown that a subpopulation of VP neurons expresses *Vglut2* and innervates the LHb. Furthermore, while general optogenetic stimulation of the VP structure induced place preference, selective activation of the glutamatergic subpopulation within the VP instead triggered place avoidance (Faget *et al.*, 2018; Tooley *et al.*, 2018). Even though a STN→VP→LHb pathway thus seems likely, the only projections actually demonstrated are the glutamatergic projections from the STN to the VP, and from the VP to the LHb. However, no study has shown that glutamatergic VP neurons projecting to the LHb receive glutamatergic input from the STN. Another possible pathway explaining the activation of the

LHb via STN stimulation is through the EP. The EP is mostly a GABAergic structure but also contains a subset of neurons releasing only glutamate or both glutamate and GABA (Stephenson-Jones *et al.*, 2016). It was recently shown that the limbic tip of the STN projects to EP neurons that synthesize glutamate, and that in turn send projections to the LHb (Stephenson-Jones *et al.*, 2016; Wallace *et al.*, 2017, 2017; Li, Pullmann and Jhou, 2019). To summarize, STN stimulation may lead to responses in the LHb via indirect projections.

The medial hypothalamic-mesencephalic area

The ventral tegmental area, VTA

The VTA is a midline structure located ventrally of the third ventricle in the midbrain, and it is flanked bilaterally by the SNc and SNr. The VTA is composed of several subnuclei: The interfascicular nucleus (IF) medially, the parabrachial pigmented nucleus (PBP) laterally, the paranigral nucleus (PN) and the parainterfascicular nucleus (PIF) ventral to the PBP, and more rostrally, the ventral area rostral nucleus (VTAr) (from Franklin & Paxinos atlas). Some studies group the IF subnucleus with the caudal aspect of the hypothalamus (Cavanaugh *et al.*, 2011), but most commonly, the IF is considered a VTA subnucleus. In addition, the rostral and caudal linear nuclei are close to the VTA, and sometimes considered VTA subnuclei, especially the rostral linear nucleus (Figure 5).

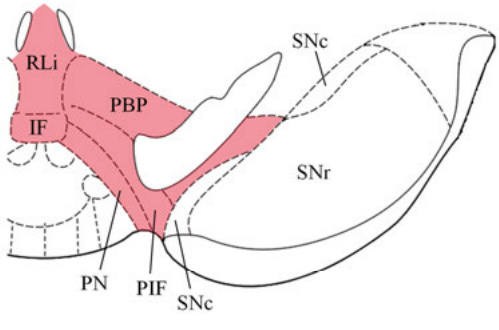

***Figure 5**:* Schematic representation of the ventral tegmental area at -3.40 mm from Bregma. The VTA consists of an assembly of several nuclei in the medial aspect of the ventral midbrain (red area). RLi: rostral linear nucleus; IF: interfascicular nucleus; PBP: parabrachial pigmented nucleus; PN: paranigral nucleus; PIF: parainterfascicular nucleus; SNc: substantia nigra *pars compacta*; SNr: substantia nigra *pars reticulata*.

While long believed to be composed of DA neurons only, the VTA has recently gained attention as a strongly heterogeneous structure in which glutamatergic and GABAergic neurons are intermixed with the DA neurons, and also with neurons able to co-release these neurotransmitters (Trudeau *et al.*, 2014). The distribution of these different types of neurons varies across subnuclei. The density of DA neurons increases from the midline to the lateral part of the VTA, with the exception of the IF, while glutamatergic neurons are highly concentrated in the medial VTA and become gradually more sparse in the lateral VTA (Morales and Root, 2014; Morales and Margolis, 2017). DA neurons express the *Th* gene encoding Tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis, which is commonly used to identify these neurons, and VTA glutamatergic neurons are characterized by *Vglut2* gene expression. Th/Vglut2 mRNA co-labeling has often been used to distinguish DA-glutamate co-releasing neurons, most common in the medial VTA.

The VTA is well known for its role in motivated behaviour, reward and aversion through projections to various forebrain structures: NAc, olfactory tubercule, PFC, amygdala, hippocampus, BNST, VP, LHb, locus coeruleus and periaqueductal gray matter (Morales and Margolis, 2017). While these pathways have been carefully mapped for VTA DA neurons over several decades, the more recently discovered VTA glutamatergic neurons have been shown to have a similar projection pattern (Hnasko *et al.*, 2012). VTA GABA neurons are mostly interneurons that regulate DA neuron activity (Tan *et al.*, 2012; Creed, Ntamati and Tan, 2014). Through substantial projections to the NAc, VTA DA neurons are involved in reward processing. Indeed, the firing rate of VTA DA neurons increases upon delivery of an unexpected reward but also in expectation of a reward, and this induces DA release in the NAc. Moreover, when a reward is systematically paired with a neutral stimulus, the increased response of DA neurons shifts in time from the delivery of the reward to the reward-predicting stimulus. This mechanism forms the basis of reward-prediction and error evaluation (Schultz, Dayan and Montague, 1997).

In addition, VTA neurons co-releasing DA and glutamate in the mAcbSh modulate a behaviour switch depending on positive or negative reinforcers (Mingote *et al.*, 2019). Several studies over the past decade have shown that DA-glutamate co-releasing neurons are important for reinforcement processing of both natural rewards, such as sugar, and addictive drugs, such as cocaine (Birgner *et al.*, 2010; Alsio *et al.*, 2011; Fortin *et al.*, 2012; Papathanou *et al.*, 2018). VTA glutamatergic neurons projecting to the LHb

have been shown to mediate aversion (Root *et al.*, 2014; Lammel *et al.*, 2015) while VTA GABA neurons have been proposed to locally inhibit DA neurons (Tan *et al.*, 2012).

The hypothalamic-mesencephalic area

Glutamatergic neurons of the midbrain are not restricted to the VTA but form a rostro-caudal continuum reaching the posterior hypothalamic area (PHA). The hypothalamus develops from the ventral diencephalon and is composed of three main regions: the rostral thalamus with preoptic areas, the tuberal hypothalamus containing the lateral hypothalamic area (LHA) and the infundibulum, and a posterior region which consists of the mammillary bodies (MM), tuberomammillary, retromammillary nucleus (RMM), supra-mammillary (SuM), and posterior hypothalamic nucleus (PH) (Figure 6) (Saper and Lowell, 2014).

The hypothalamus contains both GABAergic and glutamatergic neurons, involved in autonomic brain functions such as sleep/wake cycle (Sapin *et al.*, 2010; Luppi and Fort, 2019), thermoregulation (Contreras *et al.*, 2016; Ishi-wata and Greenwood, 2018), food intake (Schwartz *et al.*, 2000), nociception (Akerman, Holland and Goadsby, 2011), aversion (Lazaridis *et al.*, 2019) and more. These various functions arise from the many diverse subnuclei of the hypothalamus and recent studies have identified neuronal subpopulations expressing different molecular patterns (Chen *et al.*, 2017). Furthermore, a study identified a neuronal population expressing the *Trpv1* gene encoding the Transient receptor potential cation channel subfamily V member 1 (TRPV1) located in the ventral hypothalamic-mesencephalic area (Viereckel *et al.*, 2016b). In mice, *Trpv1* is highly expressed in the mes-di-encephalon between E14 and P3 but is only weakly expressed in the adult (Viereckel *et al.*, 2016b; Dumas and Wallén-Mackenzie, 2019). In the adult mouse, *Trpv1* expression is restricted to a rostro-caudal band from the PH and SuM to the medial nuclei of the VTA (IF, PN, PIF and RLi) and is mainly glutamatergic (Figure 6) (Cavanaugh *et al.*, 2011; Viereckel *et al.*, 2016b).

It is unclear if the neuronal subpopulation expressing the *Trpv1* gene plays a distinct role in any of the known functions of the hypothalamus and no study has looked at the projections of this subpopulation. Only one anatomical study using PHA-L showed that the SuM strongly projects to the hippocam-pus, the septum, the dorsal raphe nucleus, the dorsomedial hypothalamic

area, the thalamus, preoptic areas, BNST, VP, VDB, DEn and cortical areas (Vertes, 1992).

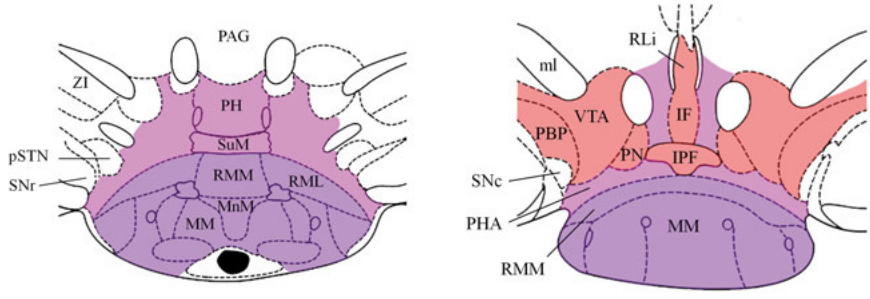

***Figure 6**:* Schematic representation of the hypothalamic-mesencephalic area at two different bregma levels, -2.70 mm on the left and -3.08 mm on the right. The posterior hypothalamic area corresponds to the pink area, the mammillary bodies to the purple area and the VTA to the red area. ZI: zona incerta, pSTN: parasubthalamic nucleus; SNr: substantia nigra *pars reticulata*; PAG: periaqueductal gray matter; PH: posterior hypothalamus; SuM: supramammillary nucleus; RMM: retromammillary nucleus; MnM: median part of the mammillary nucleus; MM: medial mammillary nucleus; RML: lateral mammillary nucleus; RLi: rostral linear nucleus; VTA: ventral tegmental area; ml: lemniscus; PBP: parabrachial pigmented nucleus; SNc: substantia nigra *pars compacta*; PHA: posterior hypothalamic area; PN: paranigral nucleus of the VTA; IF: interfascicular nucleus; IPF: interpeduncular fossa.

Overall aim

It has long been established, primarily via clinical conditions and animal models of disease, that the STN is involved in motor, associative and limbic functions. But how does the STN, with its modest size, engage in such different functions? Further, which functions can really be pin-pointed as regulated by the STN during normal, non-pathological conditions? And, does the STN actually engage in so many different functions, or are some of these mediated by immediately surrounding brain structures or even passing fibres? For example, when stimulating DBS electrodes are placed in or even near the STN, which of the observed effects are due to stimulation of STN neurons? To understand this, the role of the STN under baseline conditions must be better understood than it is today. Further, each of these functions, motor, associative and limbic, contain many different aspects – which ones can actually be ascribed to the STN? For example, which particular aspects of motor control and movement involve the STN? The STN has also been implicated in reward processing but considering recent revelations that some components within the STN circuitry, as well as structures adjacent to the STN itself, engage in aversion, does the STN also play a similar role? Many questions remain to answer before the STN and its role in neurocircuitry and behavioural regulation can be fully decoded.

The overall aim of this thesis work has been to reveal neurobiological underpinnings of the STN and its network by experimentally addressing critical questions regarding the neurocircuitry of the STN, and its role in behaviour. Many aspects have been assumed as solved, such as the role of STN in motor control. However, experimental evidence has been largely lacking. In this thesis, some of the crucial STN queries have been addressed experimentally, with both expected and surprising findings as the result. While many issues remain to fully solve, the work presented brings new knowledge about the mouse STN that should help towards decoding this elusive but clinically important brain structure and its natural brain habitat.

Material and Methods

	Study I	Study II	Study III	Study IV
Viral injections	x	x	x	NA
Cannula implantations	x	x	x	NA
In vivo electrophysiology	x	x	x	NA
Behavioural experiments	x	x	x	x
6-OHDA injections	NA	NA	NA	x
In situ hybridization	NA	NA	SD	x
Immunohistochemistry	x	x	x	x
Genotyping of mice	x	x	x	NA

***Table 1**:* Overview of my contribution (X) to the different methods performed in the four studies described in this thesis. Behavioural experiments were also performed by additional co-authors. NA: not applicable. SD: performed by other co-author.

Transgenic mice

The technology enabling gene mutations in mice, also called transgenic mouse technology, is now widely used in many fields of biology. It allows the manipulations of the genome in order to delete, insert, translocate or inverse a specific sequence of DNA. A commonly used method using transgenic mouse lines is the Cre-Lox technology developed in the 1990's by Tsien and colleagues (Tsien, 2016). By introducing the gene encoding the enzyme Cre recombinase downstream of a promoter of interest, specific manipulations of a cellular population in which the promoter is actively transcribed can be achieved. The Cre recombinase, commonly abbreviated as Cre, has the capacity to recombine short DNA sequences called Lox sites. Depending on the orientation of the Lox sequences, any DNA sequence which is flanked by Lox sites will be excised, inverted or translocated.

Transgenic mice expressing Cre recombinase are usually named after the promoter which controls expression of Cre expression, and they are referred to as strains or lines of mice. In the current studies, we have used two transgenic mouse lines: Pitx2-Cre (Study I and II) and Trpv1-Cre (Study III).

The Pitx2-Cre mouse line was created by Dr Martin and colleagues by crossing a pre-existing *Pitx2*$^{creneo/+}$ line with a FLPe recombinase-expressing transgenic mouse line to remove neomycin sequences that might interfere with the normal expression of *Pitx2* gene (Liu, 2003; Martin *et al.*, 2004b; Skidmore *et al.*, 2008b). Martin and colleagues maintained Pitx2-Cre mice on a C57NL/6J background. Upon import to our laboratory, the line has been bred in-house by mating Pitx2-Cre$^{tg/wt}$ males with C57BL/6NTac female mice.

The Trpv1-Cre mouse line (also called B6.129-Trpv1tm1(cre)Bbm/J) was developed by Dr Basbaum's laboratory (Cavanaugh *et al.*, 2011) and is available for purchase at The Jackson Laboratory. The mice contain a myc-tagged IRES-cre sequence inserted downstream of the *Trpv1* stop codon. This method ensures that the endogenous *Trpv1* coding sequence is not disrupted. Trpv1-Cre mice were bred in-house. The line was maintained by mating Trpv1-Cre$^{tg/wt}$ or Trpv1-Cre$^{tg/tg}$ males with C57BL/6NTac female mice.

The genotype of Pitx2-Cre and Trpv1-Cre mice were confirmed by PCR analyses using DNA extracted from ear biopsies and Cre-directed primers (Forward primer: 5'-CACGACCAAGTGACAGCAAT-3' and reverse primer: 5'-AGAGACGGAAATCCATCGCT-3'). Both female and male mice were used in the experiments.

Optogenetics

Optogenetics is a technique combining genetic and optical methods which was developed to allow controlled manipulation of neuronal activity by applying light (Gradinaru *et al.*, 2007; Deisseroth, 2011). It became possible with the discovery of light sensitive proteins in microorganisms such as channelrhodopsins, bacteriorhodopsins and halorhodopsins. Light of a specific wavelength can activate each of these light-sensitive proteins and trigger an exchange of ions to, depending on the opsin, hyperpolarize or depolarize the neuron expressing the opsin. A commonly used channelrhodopsin, which has

been implemented in the current studies I-III, is the Channelrhodospin 2 (ChR2), a light-gated ion channel from the algae *Chlamydomonas reinhardtii.* ChR2 is a seven transmembrane protein containing a nonspecific cation channel which opens when receiving light at 473 nm. The flow of ions induces the depolarization of the neuron leading to the generation of action potentials. Another common opsin, used in the current study I, is the Archeorhodopsin 3.0 (Arch). This opsin is a proton pump from *Halorubrum sodomense* activated by a 532 nm wavelength light which induces strong photocurrents leading to hyperpolarization and inhibition of the neurons expressing the opsin (Gradinaru *et al.*, 2010).

When applying optogenetics in rodents, selectivity is commonly achieved through the Cre-Lox system. Cre-transgenic mice are selected based on the promoter driving the expression of Cre. The DNA construct encoding the opsin contains Lox sites to allow recombination selectively in neurons expressing Cre (Figure 8). Through the Cre-Lox system, spatial selectivity can be achieved. This way, optogenetics allows a level of selectivity that is not possible by electrical stimulation. Even if light stimulation covers a broad area, only neurons expressing Cre and containing the floxed allele will be activated or inhibited when receiving the light (Figure 9).

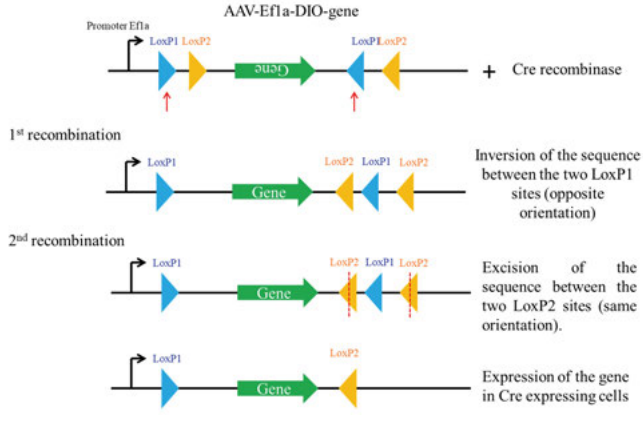

Figure 8: Illustration of the Cre-Lox system used with a double-floxed with inverted orientation (DIO) strategy.

In the current studies, Cre-dependent Adeno-associated viruses (AAVs) (Studies I, II, III: AAV2-DIO-Ef1a-(hChR2(H134R))-eYFP; Study I: AAV2-EF1a-DIO-(Arch3.0)-eYFP) were injected to specifically target neurons expressing the Cre recombinase under the *Pitx2* or the *Trpv1* promoter, as explained above. The AAV2 serotype is known for its high transfection efficiency in neuronal cells and allowed us to visualize Pitx2-Cre and Trpv1-Cre positive cell bodies, their dendrites, axons and terminals. Here, a double-floxed strategy with inverted orientation (DIO) was used. AAV viruses carried a construct containing the gene for an opsin, either ChR2 or Arch, and the gene encoding the fluorescent reporter eYFP. Mice were injected in either the STN (Pitx2-Cre) or the PHA (Trpv1-Cre) allowing expression of the opsin and reporter genes in these brain structures (Figure 8).

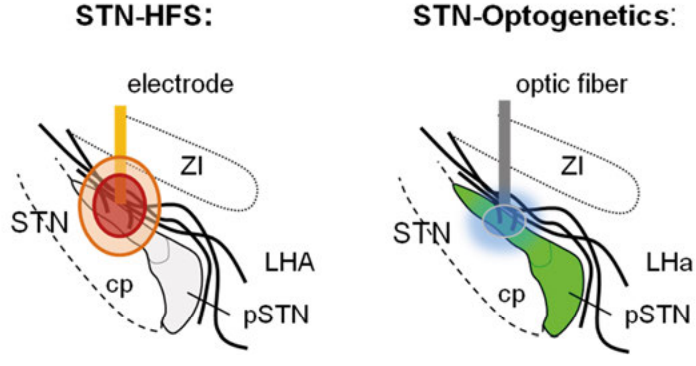

***Figure 9**:* Illustration of two stimulating methods of the STN: Left, High-frequency stimulation (HFS) which non-selectively affects all structures that are in the vicinity of the electrode, including the STN but also possibly surrounding areas and passing fibers; Right, Optogenetic stimulation in Pitx2-Cre mice injected into the STN with optogenetic virus which allows selective manipulation upon light-stimulation. STN: subthalamic nucleus; pSTN: parasubthalamic nucleus; ZI: zona incerta; cp: cerebral

Surgery and viral injections

All mice in Studies I, II and III received injections of a viral construct to express ChR2, Arch or only eYFP. Mice were anesthetized with a mixture of air/isoflurane (1.4-1.8 % isoflurane/air v/v) and placed in a stereotaxic apparatus. An anti-inflammatory solution (Carprofen, 5 mg/ml) was subcutaneously injected before starting the surgery. After applying a local anaesthetic (Marcain 1:3), the skin was incised to access the skull. Holes were then

drilled above the assigned stereotaxic coordinates. A virus, AAV2-EF1a-DIO-hChR2(H134R)-eYFP or AAV2-EF1a-DIO-Arch3.0-eYFP for experimental mice or AAV2-EF1a-DIO-eYFP for control mice, was injected at 100 nl.min-1 either in the STN (Studies I and II) or in the PHA (Study III). For behavioural experiments, optic cannulas were implanted to achieve intracranial light-stimulation, also referred to as photostimulation. Detailed protocols for each study are described below and in the manuscripts.

In Study I, AAV2-ChR2-eYFP, AAV2-Arch3-eYFP or AA2-eYFP was injected bilaterally in Pitx2-Cre mice in the STN. Mice are referred to as Pitx2/ChR2/eYFP mice, Pitx2/Arch/eYFP mice or Pitx2/eYFP-C (for ChR2 group) or Pitx2/eYFP-A (for Arch group) mice, respectively. Coordinates for the STN were: AP: -1.90 mm, ML: +/- 1.70 mm, DV: -4.65 and -4.25. A volume of two times 250 nl was injected at the two different depths. For behavioural experiments, optic cannulas were implanted above the STN and fixed with dental cement (Figure 10). Mice received another injection of Carprofen 20 to 24 hours after the surgery.

In Study II, AAV2-ChR2-eYFP or the control virus AAV2-eYFP was injected bilaterally in Pitx2-Cre mice in the STN. Coordinates for the STN were: AP: -1.90 mm, ML: +/- 1.70 mm, DV: -4.65 and -4.25 mm. A volume of two times 250 nl was injected at the two different depths. For behavioural experiments, optic cannulas were implanted above either the STN (Pitx2/ChR2/eYFP and Pitx2/eYFP mice) or the VP (Pitx2/ChR2/eYFP/VP and Pitx2/eYFP/VP mice) and fixed with dental cement (Figure 10). Coordinates for optic cannulas in the VP were AP: +0.45 mm, ML: +/- 1.55 mm, DV: -4.00 mm. Mice received another injection of Carprofen 20 to 24 hours after the surgery.

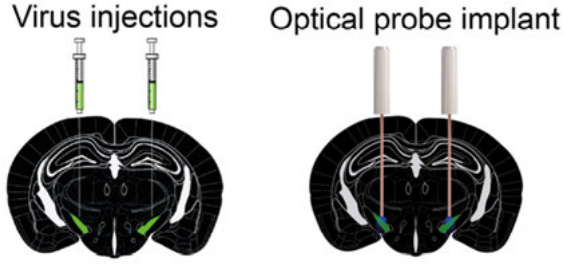

***Figure 10**:* Schematic representation of bilateral virus injections and optic canula implantations in the STN of Pitx2-Cre mice.

In Study III, AAV2-ChR2-eYFP or AAV2-eYFP was injected unilaterally in Trpv1-Cre mice in the PHA. Mice are referred to as Trpv1/ChR2 and Trpv1/eYFP mice, respectively. Coordinates for the PHA were: AP: -2.80 mm, ML: - 0.60 mm, DV: -4.40 mm, 8° angle in the frontal axis to avoid the sagittal vein and target the midline. A volume of 300 nl was injected. For behavioural experiments, an optic cannula was implanted unilaterally above the PHA and fixed with dental cement. Mice received another injection of Carprofen 20 to 24 hours after the surgery.

Behavioural experiments

The optogenetic technology for control of neuronal activity was here combined with behavioural testing in order to characterize: 1) The effect on Pitx2-Cre STN neurons upon optogenetic excitation and inhibition on motor (Study 1) and limbic functions, primarily place preference (Study II); 2) the effect on Trpv1-Cre PHA neurons upon optogenetic excitation on behavioural output, primarily place preference (Study III). Four weeks after viral injections and optic cannula implantations, mice were progressively habituated to the experimenter, the testing environment, and the connection to optic fibres. Validated behavioural paradigms were implemented in the context of optogenetic stimulations in the relevant brain area to assess if these had measurable effects on the behavioural display of the mice. The Ethovision software, connected to a camera, was used to record the behavioural output of the mice.

Intra-cranial light-stimulation protocols: For all ChR2-based experiments except study III: 20 Hz, 5 ms pulse duration, 5 mW stimulation. Study III: 40 Hz, 10 ms pulse duration, 10 mW stimulation. For Arch-based experiments: continuous stimulation of 10 mW. Lasers were controlled by an Arduino Uno card, connected to the computer through a TTL box.

In Study I, mice were divided in two groups: 1) The Pitx2/ChR2 group, mice injected with ChR2-eYFP or control (eYFP) virus; 2) the Pitx2/Arch group, mice injected with Arch-eYFP or control (eYFP) virus. Pitx2/ChR2 mice and corresponding controls (Pitx2/eYFP-C) were connected to a blue laser (473 nm, CNI Lasers, Changchun, China) while Pitx2/Arch and corresponding controls (Pitx2/eYFP-A) were connected to a green laser (532 nm, CNI Lasers, Changchun, China).

In Study II, Pitx2/ChR2/eYFP, Pitx2/ChR2/eYFP/VP mice and corresponding controls were connected to a blue laser (473 nm) (CNI Lasers, Changchun, China).

In Study III, Trpv1/ChR2/eYFP mice and corresponding controls were connected to a blue laser (473 nm) (CNI Lasers, Changchun, China).

Motor-related tests

Open field test

The open field test was performed in Study I on Pitx2/ChR2 and Pitx2/Arch mice and corresponding controls as well as in Study III on Trpv1/ChR2 and respective controls. This test is commonly used test to assess general locomotion, exploratory activity and anxiety and was here used to assess if mice change these behaviours upon optogenetic stimulation. The test arena consists of a square plexiglas transparent box (55x55x22 cm) with a white floor. Centre and borders were virtually delimited (centre corresponds to 25 % of the total arena). The test was divided into four consecutive periods of 5 minutes: OFF-ON-OFF-ON where OFF periods correspond to the absence of intracranial light-stimulation and ON periods correspond to the presence of intracranial light-stimulation. Mice were placed at the centre of the arena and allowed to freely explore for 5 plus 20 min, corresponding to a 5 min habitation time plus the four periods. Distance moved, speed, time spent in centre and frequency in crossing the centre were recorded automatically, while rearing (the mouse standing on the hind legs and raise its forelimbs to explore/investigate a stimulus), self-grooming (the mouse cleans its nose, body and tail with its front paws in a specific sequence of movements) and jumping were recorded manually. All these behaviours involve the motor system and also recruits the limbic system: Rearing is related to exploration; self-grooming is a stereotyped behaviour which can be a sign of impulsivity and compulsivity when too long and repetitive (Kalueff *et al.*, 2016); a jumping behaviour can be the sign of an escaping behaviour because of aversion, anxiety or pain.

Rotarod

The rotarod test used in Study I consists of a rotating rod on which mice can walk. The test is used to assess gait and motor coordination, and was here used to assess if optogenetic stimulation of the STN affects these behaviours. Different speeds of the rotating rod as well as the onset of intracranial light-

stimulation were set by the experimenter. The rotarod test was performed in study I on Pitx2/ChR2 mice and corresponding controls. Mice were trained in four sessions (four days) to walk on the rod at different fixed-speed: 6, 8, 12 and 16 rpm. One trial consisted in a maximum of three attempts and was considered as a success when the mouse could walk on the rod for 120 s, and a failure when the mouse fell prior to full time. No intracranial light-stimulation was applied during the training days. After the training days, only mice that succeeded in the Pre-test could go for the test on the next day. The test day consisted in 4 trials at fixed 16 rpm speed with two OFF periods (no light-stimulation) alternating with two ON periods (light-stimulation) (OFF-ON-OFF-ON). Intracranial light-stimulation started 5 seconds after the mouse was placed on the rod and stopped either when the mouse fell or when the full trial of 120 s had elapsed.

Beam walk test

The beam walk test used in Study I measures the ability of mice to walk on a round wooden beam and is used to detect subtle motor skills deficits in motor coordination and balance. This test was performed in study I to assess these parameters upon optogenetic stimulation in Pitx2/ChR2 mice and corresponding controls. The test consisted of several training days where mice learnt to walk on a wooden horizontal 80 cm long beam divided into three successive segments called “starting area” (SA), recording area (RA), and “Goal zone” GZ. Trials were performed during which onset of intracranial light-stimulation differed. During the test day, the latency to fall and hind foot slips were recorded and summarized in a Neuroscore rating scale.

Limbic-related tests

Elevated plus maze

The elevated plus maze (EPM) used in Study II is a standard test to measure anxiety and exploratory behaviours and was implemented here to assess if these behaviours are affected upon optogenetic stimulation of the STN. Pitx2/ChR2/eYFP mice and corresponding controls as well as Pitx2/ChR2/eYFP/VP mice and corresponding controls were assessed. The test arena consists of a 50 cm elevated plus shaped apparatus with two open arms (35 cm) and two closed arms (35 cm) that cross in the middle, forming a connecting centre area (Figure 11). In this test, mice choose between their natural preference for the protected closed arms and their curiosity to explore a new environment. Initially, each mouse was placed in the centre area, facing an open arm and was allowed to freely explore the apparatus for 10 minutes. Intracranial light-stimulation was applied during the whole duration of the test. The time spent and the number of entries in the closed arms, open arms and centre were recorded by the Ethovision software.

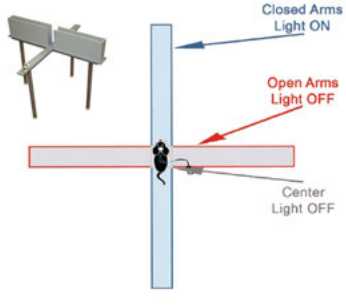

***Figure 11**:* Overview of the elevated plus maze experiment with the plus shape of the apparatus on the top left corner and the delimitations of the closed arms (blue), open arms (red) and centre (grey).

Elevated plus maze avoidance

The EPM avoidance test is an in-house created variant of the standard EPM described above and was used in Study II on Pitx2/ChR2/eYFP mice and Pitx2/ChR2/eYFP/VP mice and corresponding controls. EPM avoidance was specifically designed in order to test if an optogenetic light-stimulation which induced aversion-like behaviour was more or less potent than the natural aversion for the open arms. Here, light-stimulation was only applied upon entry in any of the closed arms and stopped when the mouse left it. Time spent in closed arms, open arms and centre as well as the number of entries were recorded by the software.

Real-time place preference

The real-time place preference (RT-PP) test is an optogenetic variant of the classical conditioned place preference (CPP) paradigm. It is used to measure the preference, or the avoidance, to an optogenetic stimulus in real-time. The RT-PP apparatus used in the current Studies II and III consisted of three connected compartments: Two main compartments with visual and textural differences that serve as cues, and a small, transparent and neutral compartment connecting the two main compartments. One of the main compartments is paired with the optogenetic light-stimulation (light-paired-compartment). That is, intracranial light-stimulation is turned on upon entry into this chamber. Mice were initially placed in the neutral compartment before opening the accesses to the two main compartments whereupon they were allowed to freely explore the arena. If optogenetic stimulation of the neuronal population in question is perceived as rewarding, then mice are expected to spend more time and/or have more entries into the light-paired-compartment, whereas if it is perceived as aversive, less time and/or less entries is expected to take place. The time spent and the number of entries in all compartments were recorded and analysed to detect place preference or place avoidance.

In Study II, Pitx2/ChR2/eYFP, Pitx2/ChR2/eYFP/VP mice and respective controls were used to assess if intracranial light-stimulation of the STN or the VP gives rise to a place preference or place avoidance behaviour. The RT-PP test (Figure 12) consisted of two days for habituation and Pre-test (15 min) without any light-stimulation for reference followed by two days of conditioning (Cond1 and Cond2, 30 min) in which intracranial light-stimulation was applied upon entry into one of the main compartments (the light-paired compartment) and stopped when the mouse left it. This was followed by two test days during which no light was applied but the preference of the mice was recorded. Next followed two reverse conditioning days (Cond3 and Cond4, 30 min) during which the previously unpaired compartment was now paired with intracranial light-stimulation upon entry. This allows a measure of cognitive flexibility. Finally, a last test day, during which no light-stimulation was applied, was performed and place preference assessed.

***Figure 12**:* Experimental timeline of the RT-PP test used on Pitx2/Chr2 mice and controls (Study II).

In study III, Trpv1/ChR2 and control mice were analysed in the RT-PP test using a protocol consisting of four conditioning days and no reverse conditioning days (Figure 13). In addition, a stronger intracranial light-stimulation was used in this test: 40 Hz, 10 ms pulse duration and 10 mW light-stimulation protocol.

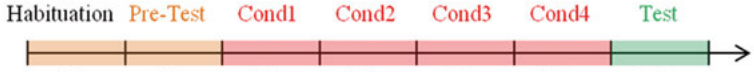

***Figure 13**:* Experimental timeline of the RT-PP test used for Trpv1/ChR2 mice and controls (Study III).

In vivo electrophysiology

Single-cell extracellular recordings were performed at least four weeks after Pitx2-Cre mice were injected with either AAV2-EF1a-DIO-hChR2(H134R)-eYFP or AAV2-EF1a-DIO-Arch3.0-eYFP (Studies I and II). Mice were anesthetized with a mixture of air/isoflurane and placed in a stereotaxic frame. After subcutaneous injection of a local anaesthetic (lidocaine), the skin was incised to reveal the skull. Recording electrodes consisted of glass micropipettes (tip diameter 1-2 µm, resistance 10 to 15 MΩ) filled either with pontamine sky blue 0.5 M sodium acetate or neurobiotine 2% in 0.5 M acetate sodium solution. Optic fibres (100 µm diameter, Thorlabs) were either placed directly above the cerebral structure or glued onto the recording pipette to make an optrode. The distance between the tip of the recording pipette and the tip of the optic fibre ranged from 650 to 950 nm. Optic fibres were connected to either a blue (473 nm) or a green (532 nm) laser (CNI Lasers, Changchun, China) for optogenetic excitation or inhibition of Pitx2-Cre STN neurons. For all *in vivo* experiments, the power of the light-stimulation was calibrated with a power meter (PM100D, Thorlabs) to 5-8 mW for excitation and 9-10 mW for inhibition. Extracellular action potentials were recorded and amplified with an Axoclamp-2B and filtered (300 Hz/0.5 kHz). Single extracellular spikes were collected online (CED 1401, SPIKE2; Cambridge Electronic Design).

After baseline recording for 100 seconds, two different light-stimulation protocols were applied to optogenetically excite the STN in Pitx2/ChR2 mice: a 0.5 Hz, 5 ms light pulse duration protocol performed to create a peri-

stimulus time histogram (PSTH) and a 20 Hz, 5 ms light pulse duration protocol, referred as "behavioural protocol" similar to the light-stimulation protocol used in behavioural experiments. Similarly, different light protocols were applied to optogenetically inhibit STN Pitx2-Cre neurons in Pitx2/Arch mice: 0.5 Hz with 10 ms light pulse duration, 100 ms light pulse duration with 200 ms intervals, 100 ms light pulse duration with 1 s interval, 2 s light pulse duration with 8 s intervals and continuous stimulation for 20-50 s.

STN optotagging

The objective of STN optotagging was to confirm the excitation and inhibition of STN neurons upon the same light-stimulation protocols that were used in the behavioural experiments. Custom-made optrodes were used to record the neuronal activity of STN Pitx2-Cre neurons in both Pitx2/ChR2 and Pitx2/Arch (Study I and II). Initial coordinates for the STN were: AP: -1.90 mm, ML:+/-1.70 mm and DV: from -4.10 to -4.70 mm. A deposit of sky blue pontamine was made at the last recording coordinates by iontophoresis (continuous current at -20 μA for 20 to 30 min) for histological analysis.

GP recordings

The GP is one of the main targets of the STN. It has been shown that optogenetic stimulation of STN neurons results in glutamate release in the GP (Viereckel, Konradsson-Geuken and Wallén-Mackenzie, 2018). The GP was therefore selected for electrophysiological confirmation of target area excitation upon STN optogenetic stimulation according to the protocols used in behavioural experiments (Study I). Coordinates for the optic fibre in the STN were: AP: -1.90 mm, ML: +/-1.70 mm and DV: -4.30 mm; Initial recording coordinates for the GP were: AP: -0.30 mm, ML: +/-1.50 mm and DV: from -3.00 to -4.30 mm. A deposit of sky blue pontamine was made by electrophoresis at the last recording coordinates for histological analysis.

LHb recording

The objective was to stimulate STN neurons in Pitx2/ChR2/eYFP mice and record LHb neurons to validate a glutamatergic polysynaptic connection between the STN and the LHb (Study II). An optic fibre was placed above the STN. Upon light-stimulation of the STN, LHb neurons were recorded using a recording glass micropipette filled with either pontamine sky blue or

neurobiotine. Coordinates for the STN were the same as described above and initial recording coordinates for the LHb were: AP: -1.60 mm, ML: +/-0.60, DV: from -2.00 to -3.00 mm.

6-OHDA lesions

C57BL/6NTac wild-type mice were used to generate a 6-OHDA parkinsonian mouse model (Study IV). Mice were deeply anesthetized with a mixture of air/isoflurane (v/v), placed in a stereotaxic apparatus and injected subcutaneously with an anti-inflammatory drug (5 mg/ml, Carprofen). After injection of a local anaesthetic (0.17 % Marcain), the skin was incised to expose the skull. A hole was drilled above the coordinates of the median forebrain bundle, MFB: AP: -1.20 mm, ML: -1.10 mm and DV: -4.75 mm. Mice were unilaterally injected with either 1.85 mg of 6-OHDA diluted in a 0.9% NaCl 0.02% ascorbic acid solution or just the 0.9% NaCl 0.02% ascorbic acid solution for control mice. The 6-OHDA solution was kept in darkness during the whole procedure. For post-surgery care, mice received another injection of the anti-inflammatory drug (Carprofen) 20-24 hours after the surgery plus daily subcutaneous injections of saline (1 mL) until sacrifice. Mice were sacrificed at two different time points post-injection: 1) Two weeks post-injection (N=4 6-OHDA injected mice; N=4 control mice) and 2) Three weeks post-injection (N=4 6-OHDA injected mice; N=3 control mice).

In addition to these 15 mice, four additional mice were injected unilaterally with 6-OHDA. Two were used for histological analysis and two for behavioural analysis to estimate the severity of the lesion. One mouse received an intraperitoneal injection of amphetamine (2.5 mg/Kg) and one other mouse received saline. For behavioural assessment, each mouse was placed in a round arena (50 cm diameter) and allowed to habituate to the environment for 10 minutes. Thereafter, the number of ipsilateral turns was counted during 10 minutes. The results were expressed as number of ipsilateral turns per minute. A high ratio corresponds to a high percentage of DA neurons degeneration. Two or three weeks after injections, the cohort of 15 injected mice were sacrificed by cervical dislocation, brains extracted and snapped-frozen in -25/-30°C isopentane. Brains were cut with a cryostat and mounted on glass slides. Sections were stored at -80°C until usage.

Histological analyses

Immunohistochemistry

Upon completion of behavioural experiments, Pitx2-Cre mice (Studies I and II) and Trpv1-Cre mice (Study III) were transcardially perfused with 1x phosphate buffer saline (PBS) followed by 4% formaldehyde. Brains were extracted, post-fixed and 60 µm-thick sections were cut at the vibratome. Immunohistochemistry using standard protocols was performed to enhance the eYFP signal (Studies I, II and III) and to detect c-fos (Study I), TH (Study III) and neurobiotine (Study II). Pontamine sky blue naturally emits in the red part of the spectrum, no enhancement was needed (Study I and II). Slices were stained with DAPI for detection of cell nuclei, mounted and scanned using a NanoZoomer 2-0-HT.0 (Hamamatsu) scanner. Results were visualized using the NDPView2 software (Hamamatsu).

In situ hybridization

In situ hybridization experiments were performed to detect mRNA using colorimetric (Study III, performed by other co-author) and fluorescent (Study III, IV) labelling.

In study IV, the selected mRNAs were standard markers for midbrain DA neurons: Th, Aldehyde dehydrogenase 1 (Aldh1a1), Calbindin1 (Calb1) and Gastrin-releasing peptide (Grp). *In situ* hybridization was performed on 16 µm-thin frozen sections. Sections were thawed and post-fixed in 4% para-formaldehyde (4% PFA) for 10 min at room temperature (RT). After washes in PBS, sections were incubated in triethanolamine pH 8 (TEA) for 5 min followed by acetylation. Riboprobes (DIG labelled riboprobe, 50-75 ng/100 µl and fluorescein labelled riboprobe, 75-100 ng/100 µl) were denatured in a solution of 50% hybridization buffer / 50% formamide at 85°C for 10 min and thereafter applied onto the sections for a 16-18 hours in a humidified chamber at 65°C. After hybridization, sections were washed in 65°C saline sodium citrate buffer (SSC) baths (5X SSC followed by 0.2X SSC solutions) and a last wash with a 0.2X SSC at RT. Sections were then washed in 1x maleic acid buffer containing TritonX-100 (MABT) to decrease non-specific probe bindings. The fluorescein riboprobe was revealed by first incubating the sections in a blocking solution containing a blocking reagent (Roche), FBS heat inactivated and 5xMAB. Then, sections were incubated with an anti-fluorescein horseradish peroxidase-conjugated (POD) antibody (Roche)

for one hour. An amplification step was performed by incubating the sections in TSA-biotin amplification buffer (Perkin Elmer) for 15 min. The fluorescein-TSA-biotin complex was revealed by adding Neutravidin-Oregon Green (Invitrogen) for 15 min. After rinsing the sections in TritonX-100 PBS (PBST), hydrogen peroxidase was inhibited by incubating the sections in 0.1M glycine pH 2.1 and in 3% hydrogen peroxide (H_2O_2). A similar procedure was performed to reveal the DIG riboprobe with an anti-DIG-POD antibody diluted in the blocking solution and TSA buffer plus Cy3 (Perkin Elmer). Sections were subsequently incubated for 10 min in 1/50000 DAPI solution and mounted with Fluoromount (Southern Biotech). Sections were scanned with NanoZoomer 2-0-HT.0 scanner using the NDP.scan 3.3 software (Hamamatsu) and visualized with the NDP.view2 software (Hamamatsu).

Statistical analysis

Behavioural experiments

For the open field test, EPM and RT-PP, statistical analysis was performed using a 2-way repeated measures (RM) ANOVA followed, when the p-value was below 0.05, by a Tukey or a Bonferroni Post-hoc analysis. In the open field test (Study I), a two-tailed Wilcoxon matched-pairs test was used to compare the number of jumps between Pitx2/ChR2 and control mice. Measured parameters were compared between days, compartments and the interaction between days and compartment for RT-PP experiments (Studies II and III). For EPM and EPM avoidance tests (Study II), the number of entries and the time spent in each arm were compared between groups (ChR2 *vs*. Controls), arms and the interaction between groups and arms. For the rotarod and the beam walk test (Study I), a Friedman test was used to analyse the data followed by Dunn's multiple comparison when needed.

All data is expressed on the plots as means ± SEM; when appropriate, data was averaged for the two ON and OFF trials.

In vivo electrophysiology experiments

For extracellular *in vivo* recordings in the GP (Study I) and the LHb (Study II), the non-parametric Friedman test was used to compare the firing rate

before, during and after optogenetic stimulation followed by a Dunn's multiple comparison.

Study I

Aim

The aim was to characterize the role of the STN in several aspects of movement by systematically applying optogenetic excitation and inhibition to the STN in Pitx2-Cre mice and analysing circuitry effects as well as impact on motor behaviour.

Results and discussion

The STN is a central excitatory nucleus within the indirect pathway of the basal ganglia. Despite knowledge of the role of the STN in movement in relation to PD, little is known on the natural role of the STN in the healthy subject. However, a few studies have shown that activation of the STN decreases locomotion, induces stereotyped movements and can interrupt an ongoing behaviour while its inactivation facilitates movement, induces contralateral rotations and suppresses the interruptive effect of surprise (Bergmann *et al.*, 2004; Schweizer *et al.*, 2014; Fife *et al.*, 2017; Tian *et al.*, 2018). None of the studies mentioned above investigated the effect of a direct optogenetic excitation or inhibition of the STN on general locomotion, coordination and fine motor movements.

In this study, we used optogenetics in Pitx2-Cre mice to ensure selectivity for the STN over surrounding structures. By expressing excitatory (ChR2) and inhibitory (Arch) opsins in the STN structure, the STN could be directly, selectively and reversibly excited or inhibited, respectively. Control mice lacked the opsin in their optogenetic constructs but where otherwise identical to the experimental mice.

First, we confirmed STN projection target areas by visualization of eYFP-reporter positive fibres. Strong eYFP signal was visible in the GP, SNr and EP and weaker signal was observed in the VP, SNc and PPN.

To validate our optogenetic approach in Pitx2/ChR2 mice, we analysed c-fos expression in the STN after light-stimulation in the STN itself. We observed the presence of c-fos-positive neurons in the STN, below the tip of the optic fibre in Pitx2/ChR2 mice. As expected, no c-fos expression was found in control mice. Furthermore, we confirmed the excitation of Pitx2-Cre STN neurons in Pitx2/ChR2 mice by using *in vivo* extracellular electrophysiology recordings in the STN and in the GP. By simultaneously stimulating and recording STN neurons (optotagging), we could observe light-evoked action potentials when applying a 0.5 Hz 5 ms 5-8 mW stimulation protocol. In the GP, one of main targets of the STN, we observed both light-evoked action potentials with a 0.5 Hz 5 ms 5-8 mW light-stimulation protocol and an increase in the firing rate when using the same light-stimulation protocol as the one used for behavioural experiments (20 Hz, 5 ms, 5-8 mW). The increase in frequency lasted the whole duration of the light stimulation (100 s) with a return to baseline as soon as the light was off. Electrophysiological recordings in Pitx2/Arch mice were performed but could not confirm the inhibition of STN Pitx2-Cre neurons due to their low spontaneous activity.

Next, we assessed the effect of STN excitation and inhibition in different behavioural tests measuring various aspects of motor function. Results from the open field test showed that optogenetic excitation and inhibition of the STN has opposite effects on horizontal and vertical locomotion. Pitx2/Arch mice displayed an increased locomotor activity with increased distance moved, speed, time spent moving and rearing. In contrast, Pitx2/ChR2 mice showed a decrease in distance moved, speed, time spent moving and rearing. Surprisingly, a strong face-grooming was observed in Pitx2/ChR2 mice only seconds after the intracranial light-stimulation was ON. In contrast, Pitx2/Arch mice showed a decrease in the natural levels of self-grooming compared to controls. The results confirmed the long-assumed role of the STN in movement. That is, STN excitation has a reducing effect on locomotion while STN inhibition has an elevating effect on locomotion. The excessive face-grooming observed upon light-stimulation in Pitx2/ChR2 mice was surprising but is in accordance with results obtained by Tian and colleagues who showed that activation of glutamatergic STN terminals in the GP induces grooming (Tian *et al.*, 2018).

To further investigate the reduced motor activity observed in Pitx2/ChR2 mice upon optogenetic excitation, we tested their general and fine motor coordination using the rotarod and beam walk tests. Upon light-stimulation,

Pitx2/ChR2 mice failed to walk on the rotating rod more than few seconds. Further, compared to control mice, Pitx2/ChR2 mice had difficulties in crossing the beam when light-stimulation was applied shortly prior to the test.

Finally, to further assess the impact of excitation *vs.* inhibition of the STN in basal ganglia function, we tested our hypothesis that unilateral light-stimulation of the STN in Pitx2/ChR2 and Pitx2/Arch mice would induce opposite rotations. According to previous studies and the classical basal ganglia model, activation of the STN would inhibit movement on the side of the stimulation and induce ipsilateral rotations, while inhibition of the STN would promote movement on the side of the stimulation and induce contralateral rotations. This was indeed what we observed: Unilateral optogenetic excitation or inhibition of the STN induced a strong rotational behaviour with ipsilateral rotations in Pitx2/ChR2 mice and contralateral rotations in Pitx2/Arch mice.

The results obtained in this study experimentally confirm the expected output upon STN excitation and inhibition as postulated by basal ganglia models. We show that optogenetic activation of the STN indeed reduces movement while its inhibition promotes movement. Beyond this finding, we also identify impaired motor coordination upon STN excitation, and, quite surprisingly, STN excitation directly and immediately induces face-grooming behaviour.

Study II

Aim

The primary aim of this study was to investigate the role of the STN in limbic functions related to reward and aversion. Upon identification of place avoidance induced by optogenetic STN excitation, the follow-up aim was to address putative neurocircuitry engaged in this unexpected behaviour.

Results and discussion

The STN has long been investigated in motor functions because of its role in PD. However, it has also been shown that the STN regulates reward-related behaviours and, more precisely, that it encodes positive or negative values (Baunez *et al.*, 2005; Rouaud *et al.*, 2010; Pelloux *et al.*, 2014; Breysse, Pelloux and Baunez, 2015; Schweizer *et al.*, 2016). Besides some evidence showing a role of the STN in encoding negative values (Breysse, Pelloux and Baunez, 2015), no study has ever shown a direct correlation between activation of the STN and aversive-like behaviours. Furthermore, based on the finding in Study I that STN excitation causes self-grooming behaviour, a putative correlate to compulsive behaviour in human OCD, it was of interest to assess if STN excitation caused aversion or anxiety or other types of affective or cognitive behaviours that could explain the stereotyped grooming.

We first applied optogenetics in the STN of Pitx2-Cre mice to excite this structure selectively. We validated our excitatory optogenetic approach with three complementary methods: We confirmed the presence of eYFP cell bodies in the STN and eYFP projections to the STN target areas; *in vivo* and *ex vivo* electrophysiological recordings in the STN (optotagging) were both implemented to confirm excitation of Pitx2/ChR2 STN neurons upon light-stimulation.

Next, we tested the effects of STN optogenetic excitation in the RT-PP paradigm. Depending on the effect of the stimulation (pleasant or unpleasant),

mice would spend more or less time in the light-paired compartment. We observed a decrease in the time spent in the light-paired compartment and consequently an increased time spent in the unpaired-compartment. These results showed for the first time that excitation of the STN induces place avoidance.

To determine if anxiety plays a role in this behaviour, we decided to test Pitx2/ChR2 mice in the elevated plus maze, EPM. STN-directed light-stimulation did not affect the time spent in either open or closed arms in Pitx2/ChR2 mice and controls. However, when applying the light-stimulation only in the closed arms (EPM avoidance), Pitx2/ChR2 mice avoided the open arms, naturally aversive for mice, and also the closed arms, where the light-stimulation was applied. The result was an increased time spent in the centre of the apparatus, in the light-unpaired cross between closed and open arms. These results confirmed that STN excitation induces strong avoidance behaviour.

Secondly, we hypothesized, based on previous studies, that this avoidance behaviour was mediated through a pathway reaching the LHb (Tan *et al.*, 2011; Hartung *et al.*, 2016; Stephenson-Jones *et al.*, 2016; Faget *et al.*, 2018; Tooley *et al.*, 2018). The STN projects directly to the VP and the EP which in turn project to the LHb. We decided to investigate the STN→VP→LHb pathway, first because it has never been shown that VP neurons receiving glutamatergic inputs from the STN project to the LHb, second because the VP is well known for its role in limbic functions, and third, because it was recently shown that activation of VP glutamatergic neurons projecting to the LHb induces place avoidance (Tooley *et al.*, 2018).

We first wanted to demonstrate if an indirect projection from the STN to the LHb exists at all. To do so, we used *in vivo* extracellular recordings with an optic fibre stimulating the STN and a recording pipette placed in the LHb. We observed evoked action potentials in the LHb in 50% of the recorded LHb neurons with 10.08 ms +/- 0.81 ms delay. Moreover, patch-clamp recordings showed excitation of VP neurons upon light stimulation, confirming STN glutamatergic projections on the VP.

Next, a new batch of Pitx2/ChR2 mice, in which ChR2 is expressed in the STN, were tested in the RT-PP and EPM paradigms, but now, the intracranial light-stimulation was directed to the VP instead of the STN. This would

allow us to selectively study the behavioural effect upon optogenetic excitation of the STN→VP terminals (Pitx2/ChR2/eYFP/VP mice). Also these new mice showed a decrease in the time spent in the light-paired compartment. Results in the EPM and EPM avoidance were also similar to the ones obtained in Pitx2/ChR2/eYFP mice, when the STN itself was stimulated. These results showed for the first time that the STN→VP pathway is involved in place avoidance.

Additional experiments analyzing this newly identified circuitry could be performed to confirm the role of this pathway in aversion and demonstrate the functional connection between the STN, the VP and the LHb. Also additional pathways between the STN and the LHb might contribute to the processing of aversive stimuli.

The main take-home message of this study is that excitation of the STN induces aversive behaviour, a finding which has potential clinical importance, not least in the STN-DBS context in which adverse side-effects include affective symptoms, including anxiety and depression.

Study III

Aim

The aim of this study was to characterize the neuronal population in the hypothalamic-mesencephalic area which expresses the *Trpv1* gene, its projection pattern and its functional role in limbic functions.

Results and discussion

Several evidence support the hypothesis that different populations within the hypothalamic and mesencephalic areas mediate aversion and avoidance-type behaviours. Indeed glutamatergic, GABAergic and dopaminergic subpopulations of the VTA and hypothalamus have been shown to be coding for aversive or reward-related behaviours (Pupe and Wallén-Mackenzie, 2015; Chen *et al.*, 2017; Morales and Margolis, 2017; Sharpe *et al.*, 2017; Bimpisidis *et al.*, 2019; de Jong *et al.*, 2019; Lazaridis *et al.*, 2019). The identification of such populations is of importance to improve our understanding of brain function in healthy and pathological conditions.

In this study, we describe the expression of a very restricted hypothalamic-mesencephalic neuronal population defined by its expression of the *Trpv1* gene from developmental stages. At E14.5, Trpv1 mRNA was found in Th-positive VTA neurons and in the PHA. Trpv1-positive neurons were mostly expressing *Vglut2* in the medial hypothalamic area and expressed *Th* in the VTA with some scattered neurons positive for Vglut2 mRNA. Trpv1 mRNA was not detected in any other brain regions. At P3, Trpv1 neurons were present along the midline from the PHA to the VTA. Trpv1 mRNA was mainly co-localized with Th mRNA in the medial nuclei of the VTA (IF, PN, PBP) and Trpv1/Vglut2 co-localization was also identified. In more rostral aspects of the VTA and the PHA, Trpv1 mRNA was mostly co-localized with Vglut2 mRNA. In the adult, Trpv1 mRNA was similarly distributed as in P3 but at a much lower level, making it nearly undetectable.

Next, we hypothesized that this Trpv1 subpopulation could play a role in aversion based on previous studies showing that glutamatergic neurons of both the midbrain and the hypothalamus (lateral hypothalamic area) are involved in aversion (Root *et al.*, 2014; Lammel *et al.*, 2015; Lazaridis *et al.*, 2019).

To selectively manipulate this neuronal population, we used Trpv1-Cre mice, originally imported from the Jackson Laboratory, and that we injected with optogenetic viruses, creating Trpv1/ChR2 mice. Firstly, by analysis of eYFP-positive projections throughout the brain, we identified the various brain areas innervated by Trpv1-Cre neurons. We observed strong eYFP-positive projections to the septal area, preoptic areas, CA1 and CA3 fields of the hippocampus, and the amygdalohippocampal nucleus (AHiAL). We also identified weaker but consistent projections to the endopiriform nucleus (DEn) and the fimbria of the hippocampus, as well as weaker and less consistent projections to the BNST, the dorsal tenia tecta (DTT), the mAcbSh and along the midline in the orbital and infralimbic cortices. Projections reaching forebrain structures were going through the median forebrain bundle, MFB, where transversally-sectioned eYFP fibres were visible in most injected Trpv1-Cre mice. Overall, the amount and density of projections was surprisingly high compared to the small amount of Trpv1-Cre cell bodies present in the hypothalamic-mesencephalic area, into which the opsin constructs were injected.

Secondly, we tested Trpv1/ChR2 mice in an RT-PP paradigm. According to the theory of this paradigm, if light-stimulation of Trpv1-Cre neurons of the hypothalamic-mesencephalic area is aversive, we should observe a decrease in the time spent in the light-paired compartment. Along the four consecutive conditioning days, Trpv1-Cre mice indeed decreased the time spent in the light-paired compartment, reaching significant difference on the fourth conditioning day. These results confirmed the involvement of Trpv1-Cre population in place avoidance. However, more studies will be required to fully understand this behaviour which was found progressive and which did not seem to result in significant learning.

Further experiments will also be needed to identify the pathways that support the observed place avoidance. In particular, it would be interesting to stimulate Trpv1-Cre terminals in septal or preoptic areas, two of the main target areas we observed for Trpv1-Cre neurons, and which are strongly involved

in limbic functions with projections to the LHb (Yetnikoff *et al.*, 2015; Barker *et al.*, 2017).

Study IV

Aim

The aim of this study was to generate a 6-OHDA mouse parkinsonian model to enable future investigations of optogenetic excitation *vs*. inhibition of the STN in a parkinsonian paradigm.

Results and discussion

The 6-OHDA parkinsonian mouse model is one of the most used methods to study PD. When injected intracerebrally into the MFB, the 6-OHDA neurotoxin induces a degeneration of DA neurons of the SNc and consequently of the nigrostriatal pathway. In accordance with the loss of SNc DA neurons, this parkinsonian model causes strong motor impairments. However, alpha-synuclein aggregates are not observed in the 6-OHDA model.

In study I, we showed that optogenetic inhibition of Pitx2/Arch mice increased locomotor activity. In this study, we tested protocols to create a 6-OHDA mouse model to be able to, in future experiments, test if selective optogenetic inhibition of the STN can improve toxin-induced motor impairments. The model is also useful to compare the effect of STN inhibition with STN excitation, in order to evaluate STN stimulation paradigms. These types of experiments would allow further study of how selective STN manipulations might be used to rescue parkinsonian motor deficiency. Indeed, one previous study showed that optogenetic inhibition of the STN in 6-OHDA lesioned rats improves contralateral forelimb akinesia and levodopa-induced dyskinesia (Yoon *et al.*, 2016). In addition, we aimed to optimize the method to increase the survival rate and well-being of the mice by implementing two different time-duration protocols associated with post-operative cares. Also the amount of toxin was reduced compared to most studies, in order to improve the well-being of the mice.

First, we observed that unilateral injection of 6-OHDA in the MFB rapidly induced strong motor impairments characterized by spontaneous ipsilateral rotations, akinesia and bradykinesia. Despite these strong motor symptoms, the survival rate reached 88% (N=15/17 mice). This was likely a direct consequence of daily post-operative cares which lasted until sacrifice. Sacrifice took place either two or three weeks post-surgery.

Second, we compared the degeneration of DA neurons between mice sacrificed two or three weeks post-lesion. For both groups, we showed a strong decrease of TH immunoreactivity in the SNc and dorsal striatum (near 100%). However, we also noticed a partial decrease of TH in the VTA with the ventro-lateral part affected by the lesion while the medial nuclei of the VTA remained intact. In accordance with the partial degeneration of VTA DA neurons, the ventral striatum was also partially affected. The mAcbSh was nearly intact while only weak TH labelling was observed in the lateral nucleus accumbens shell (LAcbSh) and the nucleus accumbens core (AcbC).

In situ hybridization experiments showed similar results with a strong decrease of Th mRNA in the SNc and the ventro-lateral part of the VTA. No obvious differences were visible in Th mRNA between mice sacrificed two or three weeks after 6-OHDA injection. Besides Th, we used additional molecular markers for midbrain DA neurons (Grp, Calb1 and Aldh1a1) to identify potentially spared subpopulations. These markers are more restricted to the VTA than the SNc. As expected, Aldh1a1 mRNA was nearly absent in the SNc on the lesioned side of 6-OHDA mice. In the VTA, we observed the same pattern as for Th mRNA with a lower density of Calb1, Grp and Aldh1a1 in the lateral part.

To conclude, a protocol for generation of the parkinsonian 6-OHDA model in mice was formulated. We found that sacrifice only two weeks post-lesion is sufficient to induce a strong degeneration of SNc DA neurons and partial degeneration of VTA DA neurons, associated with the expected motor symptoms, while maintaining a high survival rate and well-being.

Further experiments will be necessary to allow implementation of this parkinsonian model in Pitx2/Arch mice. Indeed, mice would have to go through a surgery combining 6-OHDA injection, AAV-Arch-YFP injection and optic cannula implantation.

Concluding remarks

This doctoral thesis aimed to investigate the role two neuronal populations in motor and limbic functions by using optogenetics, one expressing *Pitx2* and restricted to the STN and the other expressing *Trpv1* and restricted to the ventral hypothalamic-mesencephalic area.

First, we showed through *in vivo* and *ex vivo* electrophysiological experiments that optogenetic excitation induced excitation of STN neurons and its target structures in Pitx2-Cre mice. Next, we demonstrated that optogenetic excitation and inhibition of the STN induced opposite motor effects in a way that confirmed classical models of its role in the basal ganglia indirect and hyperdirect pathways. Surprisingly, selective STN excitation induced a robust self-grooming response and strong place avoidance. This is the first time that STN activation has been related with such an aversive-related behaviour. We hypothesized that this behaviour could be mediated through indirect connections to the LHb, a structure well-known for its role in aversion. Because the VP receives projections from the STN and, in turn, projects to the LHb, we hypothesized that an STN→VP→LHb pathway could be involved in the observed place-avoidance. In a follow-up study, we first confirmed, by using *in vivo* electrophysiology, an indirect excitatory connection between the STN and the LHb. Further, behavioural experiments indeed demonstrated place-avoidance behaviour when selectively exciting glutamatergic STN terminals in the VP. Together, these two studies are the first to experimentally provide direct evidence based on non-pathological conditions for the much-assumed role of the STN in motor regulation and to identify a direct role for the STN in inducing avoidance-related behaviour. These findings advance current knowledge of the STN and its neurocircuitry in behavioural regulation. In the context of PD, OCD and additional conditions in which the STN has been implicated, these revelations should help towards improving selectivity in current treatments based on electrical stimulations within the subthalamic area as well as provide increased knowledge as to the nature of aversive effects seen upon STN dysregulation and STN-based electrical treatment strategies.

Additional structures contribute to the aversion network in the brain, including the lateral aspect of the hypothalamus and the ventral midbrain, areas in which glutamatergic neurons defined by VGLUT2 reside. While the STN is anatomically close to the hypothalamus, a population of neurons expressing *Trpv1* is present in both the posterior aspect of hypothalamus and the ventral midbrain. This is a small population of heterogeneous neurotransmitter phenotype, primarily positive for VGLUT2. We could show that also this population, when stimulated with optogenetic excitation, induced place preference, albeit not as potent as when stimulation was aimed at the STN. The STN and the PHA are anatomically distinct but share their glutamatergic neurotransmitter phenotype. When comparing the projection target areas of the neurons expressing ChR2-eYFP upon stereotaxic injections in each respective area (STN and PHA), no joint target area was found, however, both structures projected to areas that in turn project to the LHb (Table 2).

Projection target areas/structures	Pitx2-Cre	Trpv1-Cre
Infralimbic and orbito-frontal cortices		x
mAcbSh		x
Septal areas		x
Preoptic areas		x
VP	x	
BNST		x
fimbria of the hippocampus (fi)		x
GP	x	
EP	x	
DEn		x
AHiAL		x
SNc	x	
SNr	x	
CA1 and CA3 fields of the hippocampus		x

Table 2: Summary of the structures/areas innervated by STN Pitx2-Cre neurons and Trpv1-Cre neurons of the hypothalamic-mesencephalic area as detected by histological analysis of eYFP reporter gene expression upon viral delivery of optogenetic constructs (Studies I-III). In dark purple, areas innervated by STN Pitx2-Cre neurons that project to the LHb. In light purple, areas innervated by Trpv1-Cre neurons that project to the LHb. mAcbSh: medial part of the nucleus accumbens shell; VP: ventral pallidum; BNST: bed nucleus of the stria terminalis; GP: globus pallidus; EP: entopeduncular nucleus; Den: endopirifom nucleus; SNr and SNc: substantia nigra *pars reticulata* and *pars compacta*; AHiAL: amydalo-hippocampal area.

Finally, to allow further study the effects of optogenetic inhibition of the STN in pathological conditions, a protocol for the 6-OHDA parkinsonian model was validated in mice. We hypothesise that inhibition of the STN would induce similar beneficial effects as STN-lesion by suppressing the overactivation of the indirect pathway and promoting movement.

To conclude, the results presented in this thesis add important insight to understand the role of the STN on motor control and limbic functions. In particular, we suggest that the STN might act as an important regulatory region upstream of the LHb in the neurocircuitry responding to aversive stimuli. A better comprehension of the basal ganglia and its role in executive and affective functions in healthy and pathological conditions is relevant for improving treatment prospects for PD and OCD.

Future perspectives

The studies presented in this thesis increase the current understanding of the role of the STN in motor and affective functions. However, further experiments will be needed to fully explain the functional organization of the STN underlying these diverse functions. Follow-up studies could, for example, use molecular and viral tracing to further investigate the connection between the STN and the LHb. Also, the combination of optogenetics and Designer Receptor Exclusively Activated by Designer Drugs (DREADDs) and/or pharmacological substances could allow us to confirm the role of the STN→VP→LHb pathway in aversion.

Finally, the internal organization of the STN remains to solve. This will be critical to understand the functional connection between the STN and behavioural regulation. Does the STN consist of the three different domains hypothesised in the STN tripartite model or are neurons intermingled into subpopulations? While the STN can be identified histologically by expression of *Pitx2*, *Vglut2* and additional genes, no molecularly distinct domains had for long been identified. Only this past summer was the first study published which could demonstrate the presence of distinct sets of gene expression patterns that anatomically identify domains within the STN (Wallén-Mackenzie *et al.*, 2020). Using Pitx2-Cre mice crossed with a floxed reporter and by implementing single-nuclei RNA sequencing, several clusters of genes were identified as differentially expressed. Upon histological analysis and anatomical mapping, it was clear that several genes show restricted expression within the STN structure, and by composite analysis, spatio-molecular maps

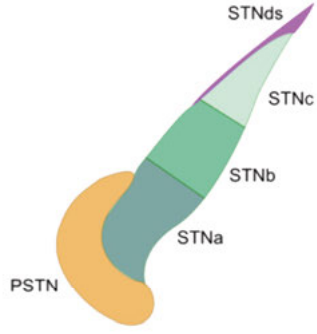

Figure 14: Schematic representation of the four internal domains recently identified within the mouse STN (*Wallén-Mackenzie* et al.*, 2020)*

could be created revealing four distinct domains (STNa, STNb, STNc, and STNds, (Figure 14). In addition to providing new information supporting the presence of distinct domains in the mouse STN, these patterns should allow exciting new possibilities of selective optogenetic targeting *within* the STN using the newly identified promoters as selectivity-drivers in future Cre-Lox-based research applications centred around the STN.

Acknowledgements

These past years as PhD student at Uppsala University have been a wonderful experience for me during which I could improve my technical and intellectual skills in research. I could not have achieved this thesis without my main supervisor, Professor **Åsa Mackenzie** who always pushed me to do my best, trusted in me and my choices. First, I would like to thank you for choosing me to be a PhD student in your lab. I think that doing my PhD in your lab will be a great advantage in my career. Also, your constant support and guidance allowed me to increase my self-confidence, to grow as a scientist and to achieve a successful thesis. You also created a dynamic and scientific environment in which we could have interesting and advanced discussions. Finally, thank you for proof-reading my thesis and for all the constructive comments.

Another person who helped me become the researcher I am today is **Gian Pietro Serra**, my co-supervisor. **GP**, thank you for everything you taught me. You are both a great scientist and a good friend and I always aimed to rise myself to your level. I will miss our long debates and great discussions. I also thank **Bianca Vlcek**, my PhD partner, for generously sharing your laboratory skills as well as for spreading a warm ray of sunshine over us all every single day. I think it is great to have another PhD student to support each other along the ups and downs we face in research.

Previous PhD students **Thomas Viereckel** and **Nadine Schweizer** who worked on the STN before me and finished only shortly after I joined the lab. Post-docs and researchers in the lab during these years, **Niclas König**, **Zisis Bimpisidis, Maria Papathanou**, **Åsa Konradsson-Geuken** and **Hanna Pettersson.** Thank you all. And **Hanna,** you deserve an extra thanks for advice about mice and Swedish administration when I first arrived.

Thank you to my colleagues at the University of Bordeaux, in particular to my co-supervisor **Francois Georges** who taught me how to perform surgeries and *in vivo* electrophysiology, and always supported me and believed in me. I would also like to thank **Jérôme Baufreton** who performed the patch-clamp experiments, as well as all the Dopamine and neuronal assemblies' team for their warm welcome during the different collaborations.

Thank you to **Sylvie Dumas** for teaching me *in situ* hybridization in the course we arranged in the lab, and for the *in situ* data you kindly and efficiently provided for the Trpv1 manuscript.

I also thank the heads of the **IOB department**, first **Irene Söderhäll** and now **Johan Ledin**, as well as the **administrative staff,** for making sure the department runs smoothly for us to work and study in, and the people at **IBG** who helped me with teaching matters. I also thank the colleagues in our **Unit of Comparative Physiology** headed by Åsa for interesting discussions during staff meetings throughout these years.

Special thanks to the **Foundation for Zoological Research** for financial support which has enabled my participation in international conferences and courses, in particular the Neuroscience of Addiction course in Cold Spring Harbor. These opportunities have greatly contributed to interactions with researchers around the globe and to my development as scientist.

My PhD period would not have been so enjoyable without all the members of the IOB department. Thank you to **Melanie**, **Dennis**, **Jake**, **Hannah**, **Cecile, Valeria**, **Laura**, **Francois**, **Sophie**, **Vincent**, **Virginia**, **Martin**, **Jordi**, **Daniel**, **Oscar**, **Beata**, **Mohammed**, **Matt**, **Imke**, **Chrysa**, **Therese**, **Manolis**, **Ehsan**, **Monica** and **Johan** for fun quiz and fika times. Extra special thanks to **Philipp** for being a great teaching partner in the Genes, Brain and Behavior course and for all the fun moments that came with it.

Finally, I would like to thank my **mum** and **dad**, my twin brother **William** and my twin sister **Anaëlle** and my step-dad **Thierry.** It is not always easy to be away from one's family but knowing that they believe in me and trust me in doing the best decisions for my life helped me to build self-confidence and achieve my goals successfully.

There is one more person I would like to thank. **Stefan**, I am so lucky that you entered in my life. These last years have been wonderful thanks to your constant support, trust and love. Thank you also to my new Swedish family, **Maggan**, **K-A** and **Lisa**.

Merci - Thanks - Tack!

References

Accolla, E. A. and Pollo, C. (2019) 'Mood Effects After Deep Brain Stimulation for Parkinson's Disease: An Update', *Frontiers in Neurology*, 10, p. 617. doi: 10.3389/fneur.2019.00617.

Akerman, S., Holland, P. R. and Goadsby, P. J. (2011) 'Diencephalic and brainstem mechanisms in migraine', *Nature Reviews Neuroscience*, 12(10), pp. 570–584. doi: 10.1038/nrn3057.

Alexander, G. E., Crutcher, M. D. and DeLong, M. R. (1990) 'Basal ganglia-thalamocortical circuits: parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions', *Progress in Brain Research*, 85, pp. 119–146.

Alkemade, A., Schnitzler, A. and Forstmann, B. U. (2015) 'Topographic organization of the human and non-human primate subthalamic nucleus', *Brain Structure and Function*, 220(6), pp. 3075–3086. doi: 10.1007/s00429-015-1047-2.

Alsio, J. *et al.* (2011) 'Enhanced Sucrose and Cocaine Self-Administration and Cue-Induced Drug Seeking after Loss of VGLUT2 in Midbrain Dopamine Neurons in Mice', *Journal of Neuroscience*, 31(35), pp. 12593–12603. doi: 10.1523/JNEUROSCI.2397-11.2011.

Aubert, I. *et al.* (2007) 'Enhanced Preproenkephalin-B–Derived Opioid Transmission in Striatum and Subthalamic Nucleus Converges Upon Globus Pallidus Internalis in L-3,4-dihydroxyphenylalanine–Induced Dyskinesia', *Biological Psychiatry*, 61(7), pp. 836–844. doi: 10.1016/j.biopsych.2006.06.038.

Augood, S. J. *et al.* (1999) 'Localization of calcium-binding proteins and GABA transporter (GAT-1) messenger RNA in the human subthalamic nucleus', *Neuroscience*, 88(2), pp. 521–534. doi: 10.1016/S0306-4522(98)00226-7.

Aziz, T. Z. *et al.* (1991) 'Lesion of the subthalamic nucleus for the alleviation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism in the primate', *Movement Disorders*, 6(4), pp. 288–292. doi: 10.1002/mds.870060404.

Baker, P. M. *et al.* (2016) 'The Lateral Habenula Circuitry: Reward Processing and Cognitive Control', *The Journal of Neuroscience*, 36(45), pp. 11482–11488. doi: 10.1523/JNEUROSCI.2350-16.2016.

Baláž, M. *et al.* (2011) 'Involvement of the subthalamic nucleus in cognitive functions — A concept', *Journal of the Neurological Sciences*, 310(1–2), pp. 96–99. doi: 10.1016/j.jns.2011.07.016.

Bari, A. and Robbins, T. W. (2013) 'Inhibition and impulsivity: Behavioral and neural basis of response control', *Progress in Neurobiology*, 108, pp. 44–79. doi: 10.1016/j.pneurobio.2013.06.005.

Barker, D. J. *et al.* (2017) 'Lateral Preoptic Control of the Lateral Habenula through Convergent Glutamate and GABA Transmission', *Cell Reports*, 21(7), pp. 1757–1769. doi: 10.1016/j.celrep.2017.10.066.

Baunez, C. *et al.* (2005) 'The subthalamic nucleus exerts opposite control on cocaine and "natural" rewards', *Nature Neuroscience*, 8(4), pp. 484–489. doi: 10.1038/nn1429.

Baup, N. *et al.* (2008) 'High-Frequency Stimulation of the Anterior Subthalamic Nucleus Reduces Stereotyped Behaviors in Primates', *Journal of Neuroscience*, 28(35), pp. 8785–8788. doi: 10.1523/JNEUROSCI.2384-08.2008.

Baxter, L. R. (1987) 'Local Cerebral Glucose Metabolic Rates in Obsessive-Compulsive Disorder: A Comparison With Rates in Unipolar Depression and in Normal Controls', *Archives of General Psychiatry*, 44(3), p. 211. doi: 10.1001/archpsyc.1987.01800150017003.

Benabid, A. L. *et al.* (1991) 'Long-term suppression of tremor by chronic stimulation of the ventral intermediate thalamic nucleus', *The Lancet*, 337(8738), pp. 403–406. doi: 10.1016/0140-6736(91)91175-T.

Benabid, A. L. *et al.* (1994) 'Acute and Long-Term Effects of Subthalamic Nucleus Stimulation in Parkinson's Disease', *Stereotactic and Functional Neurosurgery*, 62(1–4), pp. 76–84. doi: 10.1159/000098600.

Benazzouz, A. *et al.* (2002) 'Intraoperative microrecordings of the subthalamic nucleus in Parkinson's disease', *Movement Disorders*, 17(S3), pp. S145–S149. doi: 10.1002/mds.10156.

Benzina, N. *et al.* (2016) 'Cognitive Dysfunction in Obsessive-Compulsive Disorder', *Current Psychiatry Reports*, 18(9), p. 80. doi: 10.1007/s11920-016-0720-3.

Bergman, H. *et al.* (1994) 'The primate subthalamic nucleus. II. Neuronal activity in the MPTP model of parkinsonism', *Journal of Neurophysiology*, 72(2), pp. 507–520. doi: 10.1152/jn.1994.72.2.507.

Bergman, H., Wichmann, T. and DeLong, M. (1990) 'Reversal of experimental parkinsonism by lesions of the subthalamic nucleus', *Science*, 249(4975), pp. 1436–1438. doi: 10.1126/science.2402638.

Bergmann, O. *et al.* (2004) 'Subthalamic high frequency stimulation induced rotations are differentially mediated by D1 and D2 receptors', *Neuropharmacology*, 46(7), pp. 974–983. doi: 10.1016/j.neuropharm.2004.01.007.

Beurrier, C. *et al.* (1997) 'Subthalamic stimulation elicits hemiballismus in normal monkey':, *NeuroReport*, 8(7), pp. 1625–1629. doi: 10.1097/00001756-199705060-00014.

Beurrier, C. *et al.* (2001) 'High-Frequency Stimulation Produces a Transient Blockade of Voltage-Gated Currents in Subthalamic Neurons', *Journal of Neurophysiology*, 85(4), pp. 1351–1356. doi: 10.1152/jn.2001.85.4.1351.

Bikson, M. *et al.* (2001) 'Suppression of epileptiform activity by high frequency sinusoidal fields in rat hippocampal slices', *The Journal of Physiology*, 531(1), pp. 181–191. doi: 10.1111/j.1469-7793.2001.0181j.x.

Bimpisidis, Z. *et al.* (2019) 'The NeuroD6 Subtype of VTA Neurons Contributes to Psychostimulant Sensitization and Behavioral Reinforcement', *eneuro*, 6(3), p. ENEURO.0066-19.2019. doi: 10.1523/ENEURO.0066-19.2019.

Birgner, C. *et al.* (2010) 'VGLUT2 in dopamine neurons is required for psychostimulant-induced behavioral activation', *Proceedings of the National Academy of Sciences*, 107(1), pp. 389–394. doi: 10.1073/pnas.0910986107.

Boix, J., Padel, T. and Paul, G. (2015) 'A partial lesion model of Parkinson's disease in mice – Characterization of a 6-OHDA-induced medial forebrain bundle lesion', *Behavioural Brain Research*, 284, pp. 196–206. doi: 10.1016/j.bbr.2015.01.053.

Boraud, T. *et al.* (1996) 'High frequency stimulation of the internal Globus Pallidus (GPi) simultaneously improves parkinsonian symptoms and reduces the firing frequency of GPi neurons in the MPTP-treated monkey', *Neuroscience Letters*, 215(1), pp. 17–20. doi: 10.1016/S0304-3940(96)12943-8.

Boulet, S. *et al.* (2006) 'Subthalamic Stimulation-Induced Forelimb Dyskinesias Are Linked to an Increase in Glutamate Levels in the Substantia Nigra

Pars Reticulata', *Journal of Neuroscience*, 26(42), pp. 10768–10776. doi: 10.1523/JNEUROSCI.3065-06.2006.

Braak, H. *et al.* (2003) 'Staging of brain pathology related to sporadic Parkinson's disease', *Neurobiology of Aging*, 24(2), pp. 197–211. doi: 10.1016/S0197-4580(02)00065-9.

Breysse, E., Pelloux, Y. and Baunez, C. (2015) 'The Good and Bad Differentially Encoded within the Subthalamic Nucleus in Rats', *eNeuro*, 2(5), p. ENEURO.0014-15.2015. doi: 10.1523/ENEURO.0014-15.2015.

Bronstein, J. M. *et al.* (2011) 'Deep Brain Stimulation for Parkinson Disease: An Expert Consensus and Review of Key Issues', *Archives of Neurology*, 68(2). doi: 10.1001/archneurol.2010.260.

Brown, L. L., Schneider, J. S. and Lidsky, T. I. (1997) 'Sensory and cognitive functions of the basal ganglia', *Current Opinion in Neurobiology*, 7(2), pp. 157–163. doi: 10.1016/S0959-4388(97)80003-7.

Carpenter, M. B., Whittier, J. R. and Mettler, F. A. (1950) 'Analysis of choreoid hyperkinesia in the rhesus monkey. Surgical and pharmacological analysis of hyperkinesia resulting from lesions in the subthalamic nucleus ol luys', *The Journal of Comparative Neurology*, 92(3), pp. 293–331. doi: 10.1002/cne.900920303.

Cavanaugh, D. J. *et al.* (2011) 'Trpv1 Reporter Mice Reveal Highly Restricted Brain Distribution and Functional Expression in Arteriolar Smooth Muscle Cells', *Journal of Neuroscience*, 31(13), pp. 5067–5077. doi: 10.1523/JNEUROSCI.6451-10.2011.

Chang, A. D. *et al.* (2016) 'High-Frequency Stimulation at the Subthalamic Nucleus Suppresses Excessive Self-Grooming in Autism-Like Mouse Models', *Neuropsychopharmacology*, 41(7), pp. 1813–1821. doi: 10.1038/npp.2015.350.

Chang, J.-Y. *et al.* (2003) 'High frequency stimulation of the subthalamic nucleus improves treadmill locomotion in unilateral 6-hydroxydopamine lesioned rats', *Brain Research*, 983(1–2), pp. 174–184. doi: 10.1016/S0006-8993(03)03053-1.

Charara, A. *et al.* (1999) 'Pre- and postsynaptic localization of GABAB receptors in the basal ganglia in monkeys', *Neuroscience*, 95(1), pp. 127–140. doi: 10.1016/S0306-4522(99)00409-1.

Chen, R. *et al.* (2017) 'Single-Cell RNA-Seq Reveals Hypothalamic Cell Diversity', *Cell Reports*, 18(13), pp. 3227–3241. doi: 10.1016/j.celrep.2017.03.004.

Chen, Y. *et al.* (2016) 'Chemical Control of Grafted Human PSC-Derived Neurons in a Mouse Model of Parkinson's Disease', *Cell Stem Cell*, 18(6), pp. 817–826. doi: 10.1016/j.stem.2016.03.014.

Chiken, S. and Nambu, A. (2013) 'High-Frequency Pallidal Stimulation Disrupts Information Flow through the Pallidum by GABAergic Inhibition', *Journal of Neuroscience*, 33(6), pp. 2268–2280. doi: 10.1523/JNEUROSCI.4144-11.2013.

Chiken, S. and Nambu, A. (2016) 'Mechanism of Deep Brain Stimulation: Inhibition, Excitation, or Disruption?', *The Neuroscientist*, 22(3), pp. 313–322. doi: 10.1177/1073858415581986.

Contreras, C. *et al.* (2016) 'Hypothalamus and thermogenesis: Heating the BAT, browning the WAT', *Molecular and Cellular Endocrinology*, 438, pp. 107–115. doi: 10.1016/j.mce.2016.08.002.

Creed, M. (2018) 'Current and emerging neuromodulation therapies for addiction: insight from pre-clinical studies', *Current Opinion in Neurobiology*, 49, pp. 168–174. doi: 10.1016/j.conb.2018.02.015.

Creed, M. C., Ntamati, N. R. and Tan, K. R. (2014) 'VTA GABA neurons modulate specific learning behaviors through the control of dopamine and cholinergic systems', *Frontiers in Behavioral Neuroscience*, 8. doi: 10.3389/fnbeh.2014.00008.

Cromwell, H. C. and Berridge, K. C. (1996) 'Implementation of Action Sequences by a Neostriatal Site: A Lesion Mapping Study of Grooming Syntax', *The Journal of Neuroscience*, 16(10), pp. 3444–3458. doi: 10.1523/JNEUROSCI.16-10-03444.1996.

Crossman, A. R., Sambrook, M. A. and Jackson, A. (1984) 'EXPERIMENTAL HEMICHOREA/ HEMIBALLISMUS IN THE MONKEY: STUDIES ON THE INTRACEREBRAL SITE OF ACTION IN A DRUG-INDUCED DYSKINESIA', *Brain*, 107(2), pp. 579–596. doi: 10.1093/brain/107.2.579.

Cyranoski, D. (2018) '"Reprogrammed" stem cells implanted into patient with Parkinson's disease', *Nature*, pp. d41586-018-07407–9. doi: 10.1038/d41586-018-07407-9.

Czernecki, V. (2005) ‘Does bilateral stimulation of the subthalamic nucleus aggravate apathy in Parkinson’s disease?’, *Journal of Neurology, Neurosurgery & Psychiatry*, 76(6), pp. 775–779. doi: 10.1136/jnnp.2003.033258.

Darbaky, Y. *et al.* (2003) ‘High frequency stimulation of the subthalamic nucleus has beneficial antiparkinsonian effects on motor functions in rats, but less efficiency in a choice reaction time task’, *European Journal of Neuroscience*, 18(4), pp. 951–956. doi: 10.1046/j.1460-9568.2003.02803.x.

Degos, B. *et al.* (2013) ‘Subthalamic Nucleus High-Frequency Stimulation Restores Altered Electrophysiological Properties of Cortical Neurons in Parkinsonian Rat’, *PLoS ONE*. Edited by M. J. Chacron, 8(12), p. e83608. doi: 10.1371/journal.pone.0083608.

Deisseroth, K. (2011) ‘Optogenetics’, *Nature Methods*, 8(1), pp. 26–29. doi: 10.1038/nmeth.f.324.

Deniau, J.-M. *et al.* (2010) ‘Deep brain stimulation mechanisms: beyond the concept of local functional inhibition: Deep brain stimulation mechanisms’, *European Journal of Neuroscience*, 32(7), pp. 1080–1091. doi: 10.1111/j.1460-9568.2010.07413.x.

Dumas, S. and Wallén-Mackenzie, Å. (2019) ‘Developmental Co-expression of Vglut2 and Nurr1 in a Mes-Di-Encephalic Continuum Preceeds Dopamine and Glutamate Neuron Specification’, *Frontiers in Cell and Developmental Biology*, 7, p. 307. doi: 10.3389/fcell.2019.00307.

El Mestikawy, S. *et al.* (2011) ‘From glutamate co-release to vesicular synergy: vesicular glutamate transporters’, *Nature Reviews Neuroscience*, 12(4), pp. 204–216. doi: 10.1038/nrn2969.

Elfil, M. *et al.* (2020) ‘Implications of the Gut Microbiome in Parkinson’s Disease’, *Movement Disorders*, 35(6), pp. 921–933. doi: 10.1002/mds.28004.

Faget, L. *et al.* (2018) ‘Opponent control of behavioral reinforcement by inhibitory and excitatory projections from the ventral pallidum’, *Nature Communications*, 9(1), p. 849. doi: 10.1038/s41467-018-03125-y.

Fife, K. H. *et al.* (2017) ‘Causal role for the subthalamic nucleus in interrupting behavior’, *eLife*, 6. doi: 10.7554/eLife.27689.

Figee, M. *et al.* (2016) ‘Compulsivity in obsessive–compulsive disorder and addictions’, *European Neuropsychopharmacology*, 26(5), pp. 856–868. doi: 10.1016/j.euroneuro.2015.12.003.

Filali, M. *et al.* (2004) 'Stimulation-induced inhibition of neuronal firing in human subthalamic nucleus', *Experimental Brain Research*, 156(3), pp. 274–281. doi: 10.1007/s00221-003-1784-y.

Flanigan, M. E. *et al.* (2020) 'Orexin signaling in GABAergic lateral habenula neurons modulates aggressive behavior in male mice', *Nature Neuroscience*, 23(5), pp. 638–650. doi: 10.1038/s41593-020-0617-7.

Fortin, G. M. *et al.* (2012) 'Glutamate Corelease Promotes Growth and Survival of Midbrain Dopamine Neurons', *Journal of Neuroscience*, 32(48), pp. 17477–17491. doi: 10.1523/JNEUROSCI.1939-12.2012.

Galati, S. *et al.* (2006) 'Biochemical and electrophysiological changes of substantia nigra pars reticulata driven by subthalamic stimulation in patients with Parkinson's disease', *European Journal of Neuroscience*, 23(11), pp. 2923–2928. doi: 10.1111/j.1460-9568.2006.04816.x.

Garcia, L. *et al.* (2005) 'Impact of High-Frequency Stimulation Parameters on the Pattern of Discharge of Subthalamic Neurons', *Journal of Neurophysiology*, 94(6), pp. 3662–3669. doi: 10.1152/jn.00496.2005.

Gillan, C. M. and Robbins, T. W. (2014) 'Goal-directed learning and obsessive–compulsive disorder', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369(1655), p. 20130475. doi: 10.1098/rstb.2013.0475.

Grabli, D. (2004) 'Behavioural disorders induced by external globus pallidus dysfunction in primates: I. Behavioural study', *Brain*, 127(9), pp. 2039–2054. doi: 10.1093/brain/awh220.

Gradinaru, V. *et al.* (2007) 'Targeting and Readout Strategies for Fast Optical Neural Control In Vitro and In Vivo', *Journal of Neuroscience*, 27(52), pp. 14231–14238. doi: 10.1523/JNEUROSCI.3578-07.2007.

Gradinaru, V. *et al.* (2009) 'Optical Deconstruction of Parkinsonian Neural Circuitry', 324, p. 7.

Gradinaru, V. *et al.* (2010) 'Molecular and Cellular Approaches for Diversifying and Extending Optogenetics', *Cell*, 141(1), pp. 154–165. doi: 10.1016/j.cell.2010.02.037.

Grealish, S. *et al.* (2014) 'Human ESC-Derived Dopamine Neurons Show Similar Preclinical Efficacy and Potency to Fetal Neurons when Grafted in a Rat Model of Parkinson's Disease', *Cell Stem Cell*, 15(5), pp. 653–665. doi: 10.1016/j.stem.2014.09.017.

Groenewegen, H. J. and Berendse, H. W. (1990) 'Connections of the subthalamic nucleus with ventral striatopallidal parts of the basal ganglia in the rat', *The Journal of Comparative Neurology*, 294(4), pp. 607–622. doi: 10.1002/cne.902940408.

Haber, S. N. *et al.* (1985) 'Efferent connections of the ventral pallidum: Evidence of a dual striato pallidofugal pathway', *The Journal of Comparative Neurology*, 235(3), pp. 322–335. doi: 10.1002/cne.902350304.

Haber, S. N. (2016) 'Corticostriatal circuitry', *Dialogues in Clinical Neuroscience*, 18(1), pp. 7–21.

Haegelen, C. *et al.* (2009) 'The subthalamic nucleus is a key-structure of limbic basal ganglia functions', *Medical Hypotheses*, 72(4), pp. 421–426. doi: 10.1016/j.mehy.2008.07.065.

Hartung, H. *et al.* (2016) 'High-frequency stimulation of the subthalamic nucleus modulates neuronal activity in the lateral habenula nucleus', *European Journal of Neuroscience*. Edited by P. Bolam, 44(9), pp. 2698–2707. doi: 10.1111/ejn.13397.

Hashimoto, T. *et al.* (2003) 'Stimulation of the Subthalamic Nucleus Changes the Firing Pattern of Pallidal Neurons', *The Journal of Neuroscience*, 23(5), pp. 1916–1923. doi: 10.1523/JNEUROSCI.23-05-01916.2003.

Haynes, W. I. A. and Haber, S. N. (2013) 'The Organization of Prefrontal-Subthalamic Inputs in Primates Provides an Anatomical Substrate for Both Functional Specificity and Integration: Implications for Basal Ganglia Models and Deep Brain Stimulation', *Journal of Neuroscience*, 33(11), pp. 4804–4814. doi: 10.1523/JNEUROSCI.4674-12.2013.

Hedreen, J. C. (1999) 'Tyrosine hydroxylase-immunoreactive elements in the human globus pallidus and subthalamic nucleus', *The Journal of Comparative Neurology*, 409(3), pp. 400–410. doi: 10.1002/(sici)1096-9861(19990705)409:3<400::aid-cne5>3.0.co;2-4.

Hegeman, D. J. *et al.* (2016) 'The external globus pallidus: progress and perspectives', *European Journal of Neuroscience*. Edited by P. Bolam, 43(10), pp. 1239–1265. doi: 10.1111/ejn.13196.

Herzog, E. *et al.* (2001) 'The Existence of a Second Vesicular Glutamate Transporter Specifies Subpopulations of Glutamatergic Neurons', *The Journal of Neuroscience*, 21(22), pp. RC181–RC181. doi: 10.1523/JNEUROSCI.21-22-j0001.2001.

Heywood, P. and Gill, S. (1997) ‘Bilateral dorsolateral subthalamotomy for advanced Parkinson’s disease’, *The Lancet*, 350(9086), p. 1224. doi: 10.1016/S0140-6736(05)63455-1.

Hikosaka, O. (2010) ‘The habenula: from stress evasion to value-based decision-making’, *Nature Reviews Neuroscience*, 11(7), pp. 503–513. doi: 10.1038/nrn2866.

Hnasko, T. S. *et al.* (2012) ‘Ventral Tegmental Area Glutamate Neurons: Electrophysiological Properties and Projections’, *Journal of Neuroscience*, 32(43), pp. 15076–15085. doi: 10.1523/JNEUROSCI.3128-12.2012.

Hu, H., Cui, Y. and Yang, Y. (2020) ‘Circuits and functions of the lateral habenula in health and in disease’, *Nature Reviews Neuroscience*, 21(5), pp. 277–295. doi: 10.1038/s41583-020-0292-4.

Ishiwata, T. and Greenwood, B. N. (2018) ‘Changes in thermoregulation and monoamine release in freely moving rats during cold exposure and inhibition of the ventromedial, dorsomedial, or posterior hypothalamus’, *Journal of Comparative Physiology B*, 188(3), pp. 541–551. doi: 10.1007/s00360-017-1130-5.

Janssen, M. L. F. *et al.* (2017) ‘Cortico-subthalamic inputs from the motor, limbic, and associative areas in normal and dopamine-depleted rats are not fully segregated’, *Brain Structure and Function*, 222(6), pp. 2473–2485. doi: 10.1007/s00429-016-1351-5.

Jones, B. E. (2004) ‘Activity, modulation and role of basal forebrain cholinergic neurons innervating the cerebral cortex’, in *Progress in Brain Research*. Elsevier, pp. 157–169. doi: 10.1016/S0079-6123(03)45011-5.

de Jong, J. W. *et al.* (2019) ‘A Neural Circuit Mechanism for Encoding Aversive Stimuli in the Mesolimbic Dopamine System’, *Neuron*, 101(1), pp. 133-151.e7. doi: 10.1016/j.neuron.2018.11.005.

Kalteis, K. *et al.* (2006) ‘Influence of bilateral Stn-stimulation on psychiatric symptoms and psychosocial functioning in patients with Parkinson’s disease’, *Journal of Neural Transmission*, 113(9), pp. 1191–1206. doi: 10.1007/s00702-005-0399-9.

Kalueff, A. V. *et al.* (2016) ‘Neurobiology of rodent self-grooming and its value for translational neuroscience’, *Nature Reviews Neuroscience*, 17(1), pp. 45–59. doi: 10.1038/nrn.2015.8.

Kaneko, T. and Fujiyama, F. (2002) ‘Complementary distribution of vesicular glutamate transporters in the central nervous system’, *Neuroscience Research*, 42(4), pp. 243–250. doi: 10.1016/S0168-0102(02)00009-3.

Kelley, A. E. (1998) ‘Measurement of Rodent Stereotyped Behavior’, *Current Protocols in Neuroscience*, 4(1), p. 8.8.1-8.8.13. doi: 10.1002/0471142301.ns0808s04.

Khan, A. U. *et al.* (2019) ‘Awareness and current knowledge of Parkinson’s disease: a neurodegenerative disorder’, *International Journal of Neuroscience*, 129(1), pp. 55–93. doi: 10.1080/00207454.2018.1486837.

Khoo, T. K. *et al.* (2013) ‘The spectrum of nonmotor symptoms in early Parkinson disease’, *Neurology*, 80(3), pp. 276–281. doi: 10.1212/WNL.0b013e31827deb74.

Kim, H.-J., Jeon, B. S. and Paek, S. H. (2015) ‘Nonmotor Symptoms and Subthalamic Deep Brain Stimulation in Parkinson’s Disease’, *Journal of Movement Disorders*, 8(2), pp. 83–91. doi: 10.14802/jmd.15010.

Kim, S. *et al.* (2019) ‘Transneuronal Propagation of Pathologic α-Synuclein from the Gut to the Brain Models Parkinson’s Disease’, *Neuron*, 103(4), pp. 627-641.e7. doi: 10.1016/j.neuron.2019.05.035.

Kita, H. and Kitai, S. T. (1987) ‘Efferent projections of the subthalamic nucleus in the rat: Light and electron microscopic analysis with the PHA-L method’, *The Journal of Comparative Neurology*, 260(3), pp. 435–452. doi: 10.1002/cne.902600309.

Klavir, O. *et al.* (2009) ‘High frequency stimulation and pharmacological inactivation of the subthalamic nucleus reduces “compulsive” lever-pressing in rats’, *Experimental Neurology*, 215(1), pp. 101–109. doi: 10.1016/j.expneurol.2008.09.017.

Klavir, O., Winter, C. and Joel, D. (2011) ‘High but not low frequency stimulation of both the globus pallidus and the entopeduncular nucleus reduces “compulsive” lever-pressing in rats’, *Behavioural Brain Research*, 216(1), pp. 84–93. doi: 10.1016/j.bbr.2010.07.018.

Kultas-Ilinsky, K., Leontiev, V. and Whiting, P. J. (1998) ‘Expression of 10 GABAA receptor subunit messenger RNAs in the motor-related thalamic nuclei and basal ganglia of Macaca mulatta studied with in situ hybridization histochemistry’, *Neuroscience*, 85(1), pp. 179–204. doi: 10.1016/S0306-4522(97)00634-9.

Laitinen, L. V., Bergenheim, A. T. and Hariz, M. I. (1992) 'Ventroposterolateral Pallidotomy Can Abolish All Parkinsonian Symptoms', *Stereotactic and Functional Neurosurgery*, 58(1–4), pp. 14–21. doi: 10.1159/000098965.

Lambert, C. *et al.* (2012) 'Confirmation of functional zones within the human subthalamic nucleus: Patterns of connectivity and sub-parcellation using diffusion weighted imaging', *Neuroimage*, 60(1), pp. 83–94. doi: 10.1016/j.neuroimage.2011.11.082.

Lammel, S. *et al.* (2015) 'Diversity of Transgenic Mouse Models for Selective Targeting of Midbrain Dopamine Neurons', *Neuron*, 85(2), pp. 429–438. doi: 10.1016/j.neuron.2014.12.036.

Lazaridis, I. *et al.* (2019) 'A hypothalamus-habenula circuit controls aversion', *Molecular Psychiatry*, 24(9), pp. 1351–1368. doi: 10.1038/s41380-019-0369-5.

Le Jeune, F. *et al.* (2009) 'Subthalamic nucleus stimulation in Parkinson disease induces apathy: A PET study', *Neurology*, 73(21), pp. 1746–1751. doi: 10.1212/WNL.0b013e3181c34b34.

Lecca, S. *et al.* (2017) 'Aversive stimuli drive hypothalamus-to-habenula excitation to promote escape behavior', *eLife*, 6, p. e30697. doi: 10.7554/eLife.30697.

Lee, K. H. *et al.* (2004) 'Neurotransmitter release from high-frequency stimulation of the subthalamic nucleus', *Journal of Neurosurgery*, 101(3), pp. 511–517. doi: 10.3171/jns.2004.101.3.0511.

Li, H., Pullmann, D. and Jhou, T. C. (2019) 'Valence-encoding in the lateral habenula arises from the entopeduncular region', *eLife*, 8, p. e41223. doi: 10.7554/eLife.41223.

Li, S. *et al.* (2007) 'Resonant Antidromic Cortical Circuit Activation as a Consequence of High-Frequency Subthalamic Deep-Brain Stimulation', *Journal of Neurophysiology*, 98(6), pp. 3525–3537. doi: 10.1152/jn.00808.2007.

Liddle, R. A. (2018) 'Parkinson's disease from the gut', *Brain Research*, 1693, pp. 201–206. doi: 10.1016/j.brainres.2018.01.010.

Liu, W. (2003) 'Genetic dissection of Pitx2 in craniofacial development uncovers new functions in branchial arch morphogenesis, late aspects of tooth morphogenesis and cell migration', *Development*, 130(25), pp. 6375–6385. doi: 10.1242/dev.00849.

Lozano, A. M. *et al.* (1995) 'Effect of GPi pallidotomy on motor function in Parkinson's disease', *The Lancet*, 346(8987), pp. 1383–1387. doi: 10.1016/S0140-6736(95)92404-3.

Luo, J. (2002) 'Subthalamic GAD Gene Therapy in a Parkinson's Disease Rat Model', *Science*, 298(5592), pp. 425–429. doi: 10.1126/science.1074549.

Luppi, P.-H. and Fort, P. (2019) 'Sleep–wake physiology', in *Handbook of Clinical Neurology*. Elsevier, pp. 359–370. doi: 10.1016/B978-0-444-64032-1.00023-0.

Mallet, L. *et al.* (2007) 'Stimulation of subterritories of the subthalamic nucleus reveals its role in the integration of the emotional and motor aspects of behavior', *Proceedings of the National Academy of Sciences*, 104(25), pp. 10661–10666. doi: 10.1073/pnas.0610849104.

Marin, C. *et al.* (2013) 'Subthalamic 6-OHDA-induced lesion attenuates levodopa-induced dyskinesias in the rat model of Parkinson's disease', *Experimental Neurology*, 250, pp. 304–312. doi: 10.1016/j.expneurol.2013.10.006.

Marras, C., Canning, C. G. and Goldman, S. M. (2019) 'Environment, lifestyle, and Parkinson's disease: Implications for prevention in the next decade', *Movement Disorders*, 34(6), pp. 801–811. doi: 10.1002/mds.27720.

Martin, D. M. *et al.* (2004a) 'PITX2 is required for normal development of neurons in the mouse subthalamic nucleus and midbrain', *Developmental Biology*, 267(1), pp. 93–108. doi: 10.1016/j.ydbio.2003.10.035.

Martin, D. M. *et al.* (2004b) 'PITX2 is required for normal development of neurons in the mouse subthalamic nucleus and midbrain', *Developmental Biology*, 267(1), pp. 93–108. doi: 10.1016/j.ydbio.2003.10.035.

Matsumoto, M. and Hikosaka, O. (2009) 'Representation of negative motivational value in the primate lateral habenula', *Nature Neuroscience*, 12(1), pp. 77–84. doi: 10.1038/nn.2233.

McGovern, D. J. and Root, D. H. (2019) 'Ventral pallidum: a promising target for addiction intervention', *Neuropsychopharmacology*, 44(13), pp. 2151–2152. doi: 10.1038/s41386-019-0528-z.

Meissner, W. *et al.* (2005) 'Subthalamic high frequency stimulation resets subthalamic firing and reduces abnormal oscillations', *Brain*, 128(10), pp. 2372–2382. doi: 10.1093/brain/awh616.

Middleton, F. A. and Strick, P. L. (2000) 'Basal Ganglia Output and Cognition: Evidence from Anatomical, Behavioral, and Clinical Studies', *Brain and Cognition*, 42(2), pp. 183–200. doi: 10.1006/brcg.1999.1099.

Mingote, S. *et al.* (2019) 'Dopamine-glutamate neuron projections to the nucleus accumbens medial shell and behavioral switching', *Neurochemistry International*, 129, p. 104482. doi: 10.1016/j.neuint.2019.104482.

Morales, M. and Margolis, E. B. (2017) 'Ventral tegmental area: cellular heterogeneity, connectivity and behaviour', *Nature Reviews Neuroscience*, 18(2), pp. 73–85. doi: 10.1038/nrn.2016.165.

Morales, M. and Root, D. H. (2014) 'Glutamate neurons within the midbrain dopamine regions', *Neuroscience*, 282, pp. 60–68. doi: 10.1016/j.neuroscience.2014.05.032.

Moran, A. *et al.* (2011) 'Dynamic Stereotypic Responses of Basal Ganglia Neurons to Subthalamic Nucleus High-Frequency Stimulation in the Parkinsonian Primate', *Frontiers in Systems Neuroscience*, 5. doi: 10.3389/fnsys.2011.00021.

Nassery, A. *et al.* (2016) 'Psychiatric and Cognitive Effects of Deep Brain Stimulation for Parkinson's Disease', *Current Neurology and Neuroscience Reports*, 16(10), p. 87. doi: 10.1007/s11910-016-0690-1.

Nauta, H. J. W. and Cole, M. (1978) 'Efferent projections of the subthalamic nucleus: An autoradiographic study in monkey and cat', *The Journal of Comparative Neurology*, 180(1), pp. 1–16. doi: 10.1002/cne.901800102.

Obeso, J. A. *et al.* (2017) 'Past, present, and future of Parkinson's disease: A special essay on the 200th Anniversary of the Shaking Palsy: The Shaking Palsy: Past, Present and Future', *Movement Disorders*, 32(9), pp. 1264–1310. doi: 10.1002/mds.27115.

O'Callaghan, C. and Lewis, S. J. G. (2017) 'Cognition in Parkinson's Disease', in *International Review of Neurobiology*. Elsevier, pp. 557–583. doi: 10.1016/bs.irn.2017.05.002.

Odekerken, V. J. J. *et al.* (2016) 'GPi vs STN deep brain stimulation for Parkinson disease: Three-year follow-up', *Neurology*, 86(8), pp. 755–761. doi: 10.1212/WNL.0000000000002401.

Oliveira, A. L. R. *et al.* (2003) 'Cellular localization of three vesicular glutamate transporter mRNAs and proteins in rat spinal cord and dorsal root

ganglia: VGLUT1-3 in Spinal Cord Ventral Horn', *Synapse*, 50(2), pp. 117–129. doi: 10.1002/syn.10249.

Papathanou, M. *et al.* (2018) 'Targeting VGLUT2 in Mature Dopamine Neurons Decreases Mesoaccumbal Glutamatergic Transmission and Identifies a Role for Glutamate Co-release in Synaptic Plasticity by Increasing Baseline AMPA/NMDA Ratio', *Frontiers in Neural Circuits*, 12, p. 64. doi: 10.3389/fncir.2018.00064.

Parent, A. *et al.* (1996) 'Calcium-binding proteins in primate basal ganglia', *Neuroscience Research*, 25(4), pp. 309–334. doi: 10.1016/0168-0102(96)01065-6.

Parent, A. and Hazrati, L.-N. (1995) 'Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop', *Brain Research Reviews*, 20(1), pp. 91–127. doi: 10.1016/0165-0173(94)00007-C.

Pariwatcharakul, P. *et al.* (2013) 'Pathological crying after subthalamic nucleus stimulation: Pathological Crying After Subthalamic Nucleus Stimulation', *Movement Disorders*, 28(10), pp. 1348–1349. doi: 10.1002/mds.25517.

Park, S. E. *et al.* (2015) 'A time-course study of behavioral and electrophysiological characteristics in a mouse model of different stages of Parkinson's disease using 6-hydroxydopamine', *Behavioural Brain Research*, 284, pp. 153–157. doi: 10.1016/j.bbr.2015.02.019.

Parmar, M., Grealish, S. and Henchcliffe, C. (2020) 'The future of stem cell therapies for Parkinson disease', *Nature Reviews Neuroscience*, 21(2), pp. 103–115. doi: 10.1038/s41583-019-0257-7.

Pauls, D. L. *et al.* (2014) 'Obsessive–compulsive disorder: an integrative genetic and neurobiological perspective', *Nature Reviews Neuroscience*, 15(6), pp. 410–424. doi: 10.1038/nrn3746.

Pelloux, Y. *et al.* (2014) 'The subthalamic nucleus keeps you high on emotion: behavioral consequences of its inactivation', *Frontiers in Behavioral Neuroscience*, 8. doi: 10.3389/fnbeh.2014.00414.

Pelloux, Y. *et al.* (2018) 'Subthalamic nucleus high frequency stimulation prevents and reverses escalated cocaine use', *Molecular Psychiatry*, 23(12), pp. 2266–2276. doi: 10.1038/s41380-018-0080-y.

Pelloux, Y. and Baunez, C. (2013) ‘Deep brain stimulation for addiction: why the subthalamic nucleus should be favored’, *Current Opinion in Neurobiology*, 23(4), pp. 713–720. doi: 10.1016/j.conb.2013.02.016.

Petry-Schmelzer, J. N. *et al.* (2019) ‘Non-motor outcomes depend on location of neurostimulation in Parkinson’s disease’, *Brain*, 142(11), pp. 3592–3604. doi: 10.1093/brain/awz285.

Pinsker, M. *et al.* (2013) ‘Psychiatric Side-Effects of Bilateral Deep Brain Stimulation for Movement Disorders’, in Nikkhah, G. and Pinsker, M. (eds) *Stereotactic and Functional Neurosurgery*. Vienna: Springer Vienna, pp. 47–51. doi: 10.1007/978-3-7091-1482-7_8.

Poulin, J.-F. *et al.* (2018) ‘Mapping projections of molecularly defined dopamine neuron subtypes using intersectional genetic approaches’, *Nature Neuroscience*, 21(9), pp. 1260–1271. doi: 10.1038/s41593-018-0203-4.

Pupe, S. and Wallén-Mackenzie, Å. (2015) ‘Cre-driven optogenetics in the heterogeneous genetic panorama of the VTA’, *Trends in Neurosciences*, 38(6), pp. 375–386. doi: 10.1016/j.tins.2015.04.005.

Rappel, P. *et al.* (2018) ‘Subthalamic theta activity: a novel human subcortical biomarker for obsessive compulsive disorder’, *Translational Psychiatry*, 8(1), p. 118. doi: 10.1038/s41398-018-0165-z.

Reese, R. *et al.* (2011) ‘Subthalamic deep brain stimulation increases pallidal firing rate and regularity’, *Experimental Neurology*, 229(2), pp. 517–521. doi: 10.1016/j.expneurol.2011.01.020.

Robbins, T. W., Vaghi, M. M. and Banca, P. (2019) ‘Obsessive-Compulsive Disorder: Puzzles and Prospects’, *Neuron*, 102(1), pp. 27–47. doi: 10.1016/j.neuron.2019.01.046.

Robert, G. H. *et al.* (2014) ‘Preoperative factors of apathy in subthalamic stimulated Parkinson disease: A PET study’, *Neurology*, 83(18), pp. 1620–1626. doi: 10.1212/WNL.0000000000000941.

Rogers, R. D. *et al.* (2001) ‘Lesions of the medial and lateral striatum in the rat produce differential deficits in attentional performance.’, *Behavioral Neuroscience*, 115(4), pp. 799–811. doi: 10.1037/0735-7044.115.4.799.

Root, D. H. *et al.* (2014) ‘Single rodent mesohabenular axons release glutamate and GABA’, *Nature Neuroscience*, 17(11), pp. 1543–1551. doi: 10.1038/nn.3823.

Root, D. H. *et al.* (2015) 'The ventral pallidum: Subregion-specific functional anatomy and roles in motivated behaviors', *Progress in neurobiology*, 130, pp. 29–70. doi: 10.1016/j.pneurobio.2015.03.005.

Rotge, J. Y. *et al.* (2012) 'The associative and limbic thalamus in the pathophysiology of obsessive-compulsive disorder: an experimental study in the monkey', *Translational Psychiatry*, 2(9), pp. e161–e161. doi: 10.1038/tp.2012.88.

Rouaud, T. *et al.* (2010) 'Reducing the desire for cocaine with subthalamic nucleus deep brain stimulation', *Proceedings of the National Academy of Sciences*, 107(3), pp. 1196–1200. doi: 10.1073/pnas.0908189107.

Rusu, S. I. and Pennartz, C. M. A. (2020) 'Learning, memory and consolidation mechanisms for behavioral control in hierarchically organized cortico basal ganglia systems', *Hippocampus*, 30(1), pp. 73–98. doi: 10.1002/hipo.23167.

Saga, Y. *et al.* (2017) 'Ventral Pallidum Encodes Contextual Information and Controls Aversive Behaviors', *Cerebral Cortex*, 27(4), pp. 2528–2543. doi: 10.1093/cercor/bhw107.

Sanders, T. H. and Jaeger, D. (2016) 'Optogenetic stimulation of cortico-subthalamic projections is sufficient to ameliorate bradykinesia in 6-ohda lesioned mice', *Neurobiology of Disease*, 95, pp. 225–237. doi: 10.1016/j.nbd.2016.07.021.

Saper, C. B. and Lowell, B. B. (2014) 'The hypothalamus', *Current Biology*, 24(23), pp. R1111–R1116. doi: 10.1016/j.cub.2014.10.023.

Sapin, E. *et al.* (2010) 'A Very Large Number of GABAergic Neurons Are Activated in the Tuberal Hypothalamus during Paradoxical (REM) Sleep Hypersomnia', *PLoS ONE*. Edited by P. A. Bartell, 5(7), p. e11766. doi: 10.1371/journal.pone.0011766.

Schultz, W., Dayan, P. and Montague, P. R. (1997) 'A Neural Substrate of Prediction and Reward', *Science*, 275(5306), pp. 1593–1599. doi: 10.1126/science.275.5306.1593.

Schwartz, M. W. *et al.* (2000) 'Central nervous system control of food intake', *Nature*, 404(6778), pp. 661–671. doi: 10.1038/35007534.

Schweizer, N. *et al.* (2014) 'Limiting glutamate transmission in a Vglut2-expressing subpopulation of the subthalamic nucleus is sufficient to cause

hyperlocomotion', *Proceedings of the National Academy of Sciences*, 111(21), pp. 7837–7842. doi: 10.1073/pnas.1323499111.

Schweizer, N. *et al.* (2016) 'Reduced Vglut2/Slc17a6 Gene Expression Levels throughout the Mouse Subthalamic Nucleus Cause Cell Loss and Structural Disorganization Followed by Increased Motor Activity and Decreased Sugar Consumption', *eNeuro*, 3(5). doi: 10.1523/ENEURO.0264-16.2016.

Serranová, T. *et al.* (2013) 'Sex, Food and Threat: Startling Changes after Subthalamic Stimulation in Parkinson's Disease', *Brain Stimulation*, 6(5), pp. 740–745. doi: 10.1016/j.brs.2013.03.009.

Serranová, T. *et al.* (2019) 'Topography of emotional valence and arousal within the motor part of the subthalamic nucleus in Parkinson's disease', *Scientific Reports*, 9(1), p. 19924. doi: 10.1038/s41598-019-56260-x.

Shabel, S. J. *et al.* (2019) 'Stress transforms lateral habenula reward responses into punishment signals', *Proceedings of the National Academy of Sciences*, 116(25), pp. 12488–12493. doi: 10.1073/pnas.1903334116.

Sharpe, M. J. *et al.* (2017) 'Lateral Hypothalamic GABAergic Neurons Encode Reward Predictions that Are Relayed to the Ventral Tegmental Area to Regulate Learning', *Current Biology*, 27(14), pp. 2089-2100.e5. doi: 10.1016/j.cub.2017.06.024.

Shehab, S. *et al.* (2014) 'High-frequency electrical stimulation of the subthalamic nucleus excites target structures in a model using c-fos immunohistochemistry', *Neuroscience*, 270, pp. 212–225. doi: 10.1016/j.neuroscience.2014.04.016.

Shin, D. S. *et al.* (2007) 'High frequency stimulation or elevated K+ depresses neuronal activity in the rat entopeduncular nucleus', *Neuroscience*, 149(1), pp. 68–86. doi: 10.1016/j.neuroscience.2007.06.055.

Skidmore, J. M. *et al.* (2008a) 'Cre fate mapping reveals lineage specific defects in neuronal migration with loss of Pitx2 function in the developing mouse hypothalamus and subthalamic nucleus', *Molecular and Cellular Neuroscience*, 37(4), pp. 696–707. doi: 10.1016/j.mcn.2007.12.015.

Skidmore, J. M. *et al.* (2008b) 'Cre fate mapping reveals lineage specific defects in neuronal migration with loss of Pitx2 function in the developing mouse hypothalamus and subthalamic nucleus', *Molecular and Cellular Neuroscience*, 37(4), pp. 696–707. doi: 10.1016/j.mcn.2007.12.015.

Stein, D. J. *et al.* (2019) 'Obsessive–compulsive disorder', *Nature Reviews Disease Primers*, 5(1), p. 52. doi: 10.1038/s41572-019-0102-3.

Stephenson-Jones, M. *et al.* (2016) 'A basal ganglia circuit for evaluating action outcomes', *Nature*, 539(7628), pp. 289–293. doi: 10.1038/nature19845.

Stocco, A. (2018) 'A Biologically Plausible Action Selection System for Cognitive Architectures: Implications of Basal Ganglia Anatomy for Learning and Decision-Making Models', *Cognitive Science*, 42(2), pp. 457–490. doi: 10.1111/cogs.12506.

Tai, C.-H. *et al.* (2003) 'Electrophysiological and metabolic evidence that high‐frequency stimulation of the subthalamic nucleus bridles neuronal activity in the subthalamic nucleus and the substantia nigra reticulata', *The FASEB Journal*, 17(13), pp. 1820–1830. doi: 10.1096/fj.03-0163com.

Tan, K. R. *et al.* (2012) 'GABA Neurons of the VTA Drive Conditioned Place Aversion', *Neuron*, 73(6), pp. 1173–1183. doi: 10.1016/j.neuron.2012.02.015.

Tan, S. K. H. *et al.* (2011) 'High frequency stimulation of the subthalamic nucleus increases c-fos immunoreactivity in the dorsal raphe nucleus and afferent brain regions', *Journal of Psychiatric Research*, 45(10), pp. 1307–1315. doi: 10.1016/j.jpsychires.2011.04.011.

Temel, Y. *et al.* (2005) 'The functional role of the subthalamic nucleus in cognitive and limbic circuits', *Progress in Neurobiology*, 76(6), pp. 393–413. doi: 10.1016/j.pneurobio.2005.09.005.

Temel, Y. *et al.* (2006) 'Behavioural changes after bilateral subthalamic stimulation in advanced Parkinson disease: A systematic review', *Parkinsonism & Related Disorders*, 12(5), pp. 265–272. doi: 10.1016/j.parkreldis.2006.01.004.

Tian, J. *et al.* (2018) 'Optogenetic Stimulation of GABAergic Neurons in the Globus Pallidus Produces Hyperkinesia', *Frontiers in Behavioral Neuroscience*, 12. doi: 10.3389/fnbeh.2018.00185.

Tooley, J. *et al.* (2018) 'Glutamatergic Ventral Pallidal Neurons Modulate Activity of the Habenula–Tegmental Circuitry and Constrain Reward Seeking', *Biological Psychiatry*, 83(12), pp. 1012–1023. doi: 10.1016/j.biopsych.2018.01.003.

Trudeau, L.-E. *et al.* (2014) 'The multilingual nature of dopamine neurons', in *Progress in Brain Research*. Elsevier, pp. 141–164. doi: 10.1016/B978-0-444-63425-2.00006-4.

Tsien, J. Z. (2016) 'Cre-Lox Neurogenetics: 20 Years of Versatile Applications in Brain Research and Counting…', *Frontiers in Genetics*, 7. doi: 10.3389/fgene.2016.00019.

Tysnes, O.-B. and Storstein, A. (2017) 'Epidemiology of Parkinson's disease', *Journal of Neural Transmission*, 124(8), pp. 901–905. doi: 10.1007/s00702-017-1686-y.

Unal, C. T., Pare, D. and Zaborszky, L. (2015) 'Impact of Basal Forebrain Cholinergic Inputs on Basolateral Amygdala Neurons', *The Journal of Neuroscience*, 35(2), pp. 853–863. doi: 10.1523/JNEUROSCI.2706-14.2015.

Vertes, R. P. (1992) 'PHA-L analysis of projections from the supramammillary nucleus in the rat', *The Journal of Comparative Neurology*, 326(4), pp. 595–622. doi: 10.1002/cne.903260408.

Viereckel, T. *et al.* (2016a) 'Midbrain Gene Screening Identifies a New Mesoaccumbal Glutamatergic Pathway and a Marker for Dopamine Cells Neuroprotected in Parkinson's Disease', *Scientific Reports*, 6(1), p. 35203. doi: 10.1038/srep35203.

Viereckel, T. *et al.* (2016b) 'Midbrain Gene Screening Identifies a New Mesoaccumbal Glutamatergic Pathway and a Marker for Dopamine Cells Neuroprotected in Parkinson's Disease', *Scientific Reports*, 6(1), p. 35203. doi: 10.1038/srep35203.

Viereckel, T., Konradsson-Geuken, Å. and Wallén-Mackenzie, Å. (2018) 'Validated multi-step approach for *in vivo* recording and analysis of optogenetically evoked glutamate in the mouse globus pallidus', *Journal of Neurochemistry*, 145(2), pp. 125–138. doi: 10.1111/jnc.14288.

Vitek, J. L. (2002) 'Deep Brain Stimulation for Parkinson's Disease', *Stereotactic and Functional Neurosurgery*, 78(3–4), pp. 119–131. doi: 10.1159/000068959.

Vizcarra, J. A. *et al.* (2019) 'Subthalamic deep brain stimulation and levodopa in Parkinson's disease: a meta-analysis of combined effects', *Journal of Neurology*, 266(2), pp. 289–297. doi: 10.1007/s00415-018-8936-2.

Wade, C. L. *et al.* (2017) 'High-Frequency Stimulation of the Subthalamic Nucleus Blocks Compulsive-Like Re-Escalation of Heroin Taking in Rats',

Neuropsychopharmacology, 42(9), pp. 1850–1859. doi: 10.1038/npp.2016.270.

Wallace, M. L. *et al.* (2017) 'Genetically Distinct Parallel Pathways in the Entopeduncular Nucleus for Limbic and Sensorimotor Output of the Basal Ganglia', *Neuron*, 94(1), pp. 138-152.e5. doi: 10.1016/j.neuron.2017.03.017.

Wallace, M. L. *et al.* (2020) 'Anatomical and single-cell transcriptional profiling of the murine habenular complex', *eLife*, 9, p. e51271. doi: 10.7554/eLife.51271.

Wallén-Mackenzie, Å. *et al.* (2020) 'Spatio-molecular domains identified in the mouse subthalamic nucleus and neighboring glutamatergic and GABAergic brain structures', *Communications Biology*, 3(1), p. 338. doi: 10.1038/s42003-020-1028-8.

Webster, J. F. *et al.* (2020) 'Disentangling neuronal inhibition and inhibitory pathways in the lateral habenula', *Scientific Reports*, 10(1), p. 8490. doi: 10.1038/s41598-020-65349-7.

Whittier, J. R. and Mettler, F. A. (1949) 'Studies on the subthalamus of the rhesus monkey. II. Hyperkinesia and other physiologic effects of subthalamic lesions, with special reference to the subthalamic nucleus of Luys', *The Journal of Comparative Neurology*, 90(3), pp. 319–372. doi: 10.1002/cne.900900304.

Williams, K. A. and Swedo, S. E. (2015) 'Post-infectious autoimmune disorders: Sydenham's chorea, PANDAS and beyond', *Brain Research*, 1617, pp. 144–154. doi: 10.1016/j.brainres.2014.09.071.

Winter, C., Mundt, A., *et al.* (2008) 'High frequency stimulation and temporary inactivation of the subthalamic nucleus reduce quinpirole-induced compulsive checking behavior in rats', *Experimental Neurology*, 210(1), pp. 217–228. doi: 10.1016/j.expneurol.2007.10.020.

Winter, C., Lemke, C., *et al.* (2008) 'High frequency stimulation of the subthalamic nucleus modulates neurotransmission in limbic brain regions of the rat', *Experimental Brain Research*, 185(3), pp. 497–507. doi: 10.1007/s00221-007-1171-1.

Witt, K., Daniels, C. and Volkmann, J. (2012) 'Factors associated with neuropsychiatric side effects after STN-DBS in Parkinson's disease', *Parkinsonism & Related Disorders*, 18, pp. S168–S170. doi: 10.1016/S1353-8020(11)70052-9.

Wulff, A. B. *et al.* (2019) 'Ventral pallidal modulation of aversion processing', *Brain Research*, 1713, pp. 62–69. doi: 10.1016/j.brainres.2018.10.010.

Yetnikoff, L. *et al.* (2015) 'Sources of input to the rostromedial tegmental nucleus, ventral tegmental area, and lateral habenula compared: A study in rat: RMTg, VTA, and LHb afferents compared', *Journal of Comparative Neurology*, 523(16), pp. 2426–2456. doi: 10.1002/cne.23797.

Yoon, H. H. *et al.* (2014) 'Optogenetic Inactivation of the Subthalamic Nucleus Improves Forelimb Akinesia in a Rat Model of Parkinson Disease':, *Neurosurgery*, 74(5), pp. 533–541. doi: 10.1227/NEU.0000000000000297.

Yoon, H. H. *et al.* (2016) 'Optogenetic Inhibition of the Subthalamic Nucleus Reduces Levodopa-Induced Dyskinesias in a Rat Model of Parkinson's Disease', *Stereotactic and Functional Neurosurgery*, 94(1), pp. 41–53. doi: 10.1159/000442891.

Zahm, D. S. (1989) 'The ventral striatopallidal parts of the basal ganglia in the rat—II. Compartmentation of ventral pallidal efferents', *Neuroscience*, 30(1), pp. 33–50. doi: 10.1016/0306-4522(89)90351-5.

Zhang, L. *et al.* (2018) 'A GABAergic cell type in the lateral habenula links hypothalamic homeostatic and midbrain motivation circuits with sex steroid signaling', *Translational Psychiatry*, 8(1), p. 50. doi: 10.1038/s41398-018-0099-5.

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 1958

Editor: The Dean of the Faculty of Science and Technology

A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. (Prior to January, 2005, the series was published under the title "Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology".)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-417746

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2020