UNIVERSITÄT ZU LÜBECK

UNIVERSITÄT ZU LÜBECK
GRADUATE SCHOOL FOR COMPUTING
IN MEDICINE AND LIFE SCIENCES

From the Department of Neurosurgery of the University of Luebeck

Director: Prof. Dr. med. Volker Tronnier

Neurophysiological and neurohumoral changes by High Frequency Stimulation (HFS) of Nucleus Accumbens (NAc)

Dissertation for

Fulfillment of Requirements for the

Doctoral Degree of the University of Luebeck

from the Department of Natural Sciences

Submitted by

Ramya Varatharajan Thirugnanasambantham

Born in Veeramanipatti-TN, India

Luebeck, 2015

Ramya Varatharajan Thirugnanasambantham

Department of Neurosurgery

University of Luebeck

Ratzeburger allee 160

23562, Luebeck

Germany

First referee: Prof. Dr. Volker Tronnier

Second referee: Prof. Dr. Thomas Martinetz

Date of oral examination: 23.09.2015

Approved for printing, Luebeck: 30.09.2015

Acknowledgements

The hard work of so many people is involved in successful completion of this thesis, that a genuine list is virtually impossible.

I convey my thanks and gratitude to Prof. Dr. med. Volker Tronnier, Department of Neurosurgery, for providing this opportunity to work under his guidance with this interesting topic.

I express my deepest gratitude to Prof. Dr. med. Andreas Moser and Prof. Dr. rer. nat. Ulrich G. Hofmann for providing Laboratory Infrastructure, financial and moral support. Beyond scientific supervision, I was lucky to have the people from liquorlab, guiding me through all matters during my stay in Luebeck. I am thankful to Katharina Schnackenberg whose excellent experience in the laboratory and generous practical help was essential for the successful work at the lab. Thanks to Karin Wiegers, Marie Luis Reher and Gisa Brunk for being like friends and taking good care of me.

I am very much thankful to Krishnakumar, Kevin Joseph, Sonya Neto, Mehrnaz Hazrati, Yijing Xie and Susanne Loeffler for their support, encouragement and timely suggestions.

I would like to acknowledge the support by the Graduate School for Computing in Medicine and Life Sciences funded by Germany's Excellence Initiative [DFG GSC 235/1].

Last but not the least; I am extremely grateful to my parents and brother for their affection and constant encouragement without which I would not have come to this position in my life.

Abstract

High frequency deep brain stimulation (DBS) of the nucleus accumbens region (NAc) is an effective treatment for several psychiatric disorders that are not responsive to traditional treatment strategies. But the mechanism by which DBS achieves therapeutic effects is still unclear. From several in vitro and in vivo experiments clear evidence exists that electrical high frequency stimulation (HFS) is activating GABAergic neurons and therefore produces neuronal inhibition. Characterization of the effect of high frequency stimulation was performed using simultaneous and collocated microdialysis and electrical high frequency stimulation with pulses of 124 Hz and 0.5 mA current in the nucleus accumbens of conscious and freely moving rats. Memantine, a NMDA receptor antagonist and Quinpirole, a D_2 and D_3 receptor agonist were injected subcutaneously to freely moving rats and the neurotransmitter outflow was sampled. The neurotransmitters: GABA, glutamate, dopamine (DA), serotonin (5-HT) and their metabolites were quantified by means of HPLC with electrochemical detection. This thesis provides evidence that the effect of high frequency stimulation is characterized by an increase in GABA outflow in the nucleus accumbens. Memantine significantly increased basal GABA and DA outflow. Memantine seemed to mask the HFS effect on GABA whereas DA was decreased by HFS in memantine injected animals. Similar to memantine, quinpirole also increased the basal GABA and DA outflow. The HFS induced increase in GABA was seen in quinpirole injected animals. Also, HFS increased DA outflow in quinpirole injected rats. Both memantine and quinpirole had significant effect on the behavior of the animals leading to an increase in the overall activity. HFS decreased the behavioral activity induced by memantine and quinpirole. The above results suggest that HFS specifically affects GABAergic neurons in the nucleus

accumbens of the rat. In addition, NMDA and dopamine receptors might be able to modulate the HFS effect.

Zusammenfassung

Die Hochfrequente Tiefenhirnstimulation (THS) der Nucleus accumbens-Region (NAc) stellt eine innovative Behandlungsform für unterschiedliche psychiatrische Störungen dar. Der Mechanismus der THS, durch den die therapeutische Wirkung zustande kommt, ist bislang nicht eindeutig geklärt. Allerdings finden sich tierexperimentelle Hinweise darauf, dass die elektrische Hochfrequenzstimulation (HFS) zu einer Aktivierung GABAerger (Inter-)Neuronen führt, was eine Hemmung neuronaler Netzwerkaktivität zur Folge hat. Ziel dieser Arbeit ist die Charakterisierung der Wirkung der HFS unter Verwendung gleichzeitiger Mikrodialyse im Nucleus accumbens der Ratte in vivo. Darüber hinaus wurden Memantine, ein NMDA-Rezeptorantagonist und Quinpirol, ein D_2 und D_3-Rezeptor Agonist subkutan in die sich frei bewegende Ratte injiziert, um den potentiellen HFS-Effekt zu modulieren. Die Neurotransmitter wurden mittels HPLC mit elektrochemischer Detektion quantifiziert. Es zeigte sich, dass eine HFS des NAc zu einer Erhöhung der extrazellulären GABA-Konzentration im Nucleus accumbens führt. Memantine erhöhte die basale GABA und Dopamin (DA) Konzentration deutlich. Memantine maskiert dabei die HFS Wirkung auf GABA. Ähnlich wie Memantine, erhöhte auch Quinpirol die basale GABA und DA Konzentrationen. Im Gegensatz zu Memantine, führte Quinpirol zu einer weiteren Erhöhung der GABA Konzentrationen unter HFS. Sowohl Memantine als auch Quinpirol zeigten signifikante Effekte auf das Verhalten der Tiere mit einer Zunahme der motorischen Gesamtaktivität. HFS verringerte diese motorische Aktivität, die durch Memantine und Quinpirol verursacht wurde. Die obigen Ergebnisse legen nahe, dass der Effekt der HFS auf eine spezifische Wirkung auf das GABA-erge System zurückzuführen ist. Darüber hinaus sind NMDA und Dopamin Rezeptoren in der Lage, diesen Effekt zu modulieren.

Contents

1. Introduction

1.1 Deep Brain Stimulation

Neurological disorders are treated by several means ranging from behavioral therapy to surgery. The lesion-making approaches are nowadays replaced by electrical stimulation of targeted subcortical structures with implanted electrodes, a methodology usually referred to as deep brain stimulation (DBS). DBS has been widely accepted for the treatment of non-psychiatric neurological diseases, namely, Parkinson disease (PD), pain, dystonia, and tremor. The application of DBS for psychiatric disease is an effective and relatively safe therapeutic tool. Several recent studies and case reports show hope, in particular, for the treatment of severe, medically refractory OCD (Anderson and Ahmed, 2003; Bais et al., 2014; Kisely et al., 2014).

1.1.1 Principle of Deep Brain Electrical Stimulation

During DBS, an electrode made of metal (platinum-iridium in commercially available leads for clinical use) is introduced into the tissue that has to be stimulated. The different properties of electrode and the tissue form an interface for the transmission of charge to the tissue from the stimulation electrode. The tissue consists of ion- conducting solution which acts as an electrolyte for the conduction of charge. The interaction between the metal and electrolyte results in the formation of capacitance on the surface of the electrode. An electric field is formed between the electrolyte and tissue due to the change in electric potential caused by current flow. This produces a current flow through electrochemical reactions thus resulting in a transport of charge. Thus in the metal electrode the electrons acts as the charge carriers whereas in the electrolyte, the charge is carried by the ions. The two primary mechanisms by which charge transfer takes place

at the electrode/electrolyte interface are: non-Faradaic reaction and Faradaic reaction. In non-faradaic reaction there are no electrons transferred between electrode and electrolyte but there is a redistribution of charged chemicals in the electrolyte. In a Faradaic reaction electrons are transferred between the electrode and electrolyte, resulting in reduction or oxidation of chemicals in the electrolyte.

In non-Faradaic reaction, the redistribution of charge in the electrode/electrolyte interface can be modeled as a simple double layer capacitor (Helmholtz, 1853). Several physical phenomena may lead to the formation of the double layer capacitor. When a metal electrode is in electrolyte, charge redistribution due to the combination of the metal ions in the electrolyte and the electrode occurs. This redistribution involves transfer of electrons between the two phases, resulting in plane of charge on the surface of metal electrode and a plane of opposite charge (counter ions) in the electrolyte, thus forming a double layer capacitor. Specific chemical species such as halide anions may adsorb to the solid electrode which acts to separate charge and forms a double layer. Another reason for the double layer formation is the polar molecules which may have a specific orientation at the interface, and this net orientation of polar molecules separates charge (Chapman, 1913; Gouy, 1910; Grahame, 1947; Merrill et al., 2005; Stern, 1924). The charge transport in this double layer is determined by the charging and discharging of the capacitor which is optimal for the tissue as this does not result in any damage to the tissue or electrode.

There are two types of Faradaic reactions. They are reversible and irreversible Faradaic reaction. A Faradaic reaction is reversible if the kinetics (electron transfer at the interface) is very fast relative to the rate of mass transport. In irreversible Faradaic reaction, the kinetics is slow relative to the rate of mass transport. In reversible reactions, due to the fast kinetics, the

electrochemical products are bound to the electrode surface and does not get into the tissue or move away from the electrode thus resulting in less damage to the tissue and electrode. But in case of irreversible reactions, due to the slow kinetics, reactants diffuse into the tissue or move away from the electrode forming new chemical compounds that are harmful to the tissue or the electrode. Thus the key objective of electrical stimulation design is to avoid irreversible Faradaic reactions (Merrill et al. 2005).

Several studies have been made in the recent years in order to find the exact area of tissue affected by stimulation. Due to the lower resistance, white matter substance is more easily stimulated than grey matter in the brain. But the exact scope of the tissue region affected by stimulation is still unclear and will need further extensive studies (Butson et al., 2006).

1.1.2 Electrode types and electrode materials

The stimulation electrode acts as the interface between the biological structures and stimulation systems. They transform the charge from the metal phase of the electrode to the liquid phase of the tissue. The amount of charge passed to the tissue depends upon the impedance of the electrode. The impedance of the electrode is affected by several factors such as the electrode surface, connection between the stimulator and the electrode, conductivity and density of the encapsulation around the electrode and the conductivity of the tissue. In order to improve the functionality of the electrode in the tissue, it is important to ensure that the electrode is biocompatible. Only if the electrode is biocompatible, the stability, efficiency and the safety can be ensured (Horch and Dhillon, 2004) . There are two types of electrodes namely polarizable and non-polarizable electrodes. An ideal polarizable electrode is modeled as a pure capacitor and all the current is transferred through capacitive action rather than electron transfer between the

electrode and the tissue. The ideal non-polarizable electrode has zero resistance, so only the resistance by the tissue is present. So in non-polarizable electrodes the charge is injected into the tissue by Faradaic reactions (Merrill et al., 2005).

Depending upon the research areas there are a wide range of electrode materials in use. For electrical stimulation, the electrodes are often made of noble metals like platinum, gold or iridium as they are naturally resistant to corrosion and oxidation unlike other base metals. The most commonly used electrode for intra-cortical stimulation is the platinum-iridium electrode since it is mechanically stable due to a combination of the two noble metals and they have a large storage capacity for the applied load. Electrodes made of other metals such as stainless steel, cobalt or nickel are characterized by a thin passive layer that protects them against corrosion. This passivation layer is degraded once a positive electrode potential is reached, thus making the electrode vulnerable to corrosion. So due to corrosion the metal of the electrode is dissolved irreversibly, resulting in damage to both the electrode and tissue. Noble metal electrodes also lose their natural corrosion protection after sometime. However, the noble metal electrodes have a long lifetime and safe stimulation due to their natural corrosion resistance (Merrill et al., 2005).

1.1.3 Characteristics of high frequency deep brain stimulation

High frequency stimulation is used increasingly for the treatment of several neurological and psychiatric disorders like Parkinson's disease, epilepsy, depression or OCD (Obsessive Compulsive Disorder). The target regions for HFS are usually subcortical areas like basal ganglia or thalamic structures. Since the target regions for stimulation are deeper regions of brain, HFS is referred as deep brain stimulation (DBS) in clinical terms (Perlmutter and Mink, 2006; Wichmann and DeLong, 2006; Williams, 2015).

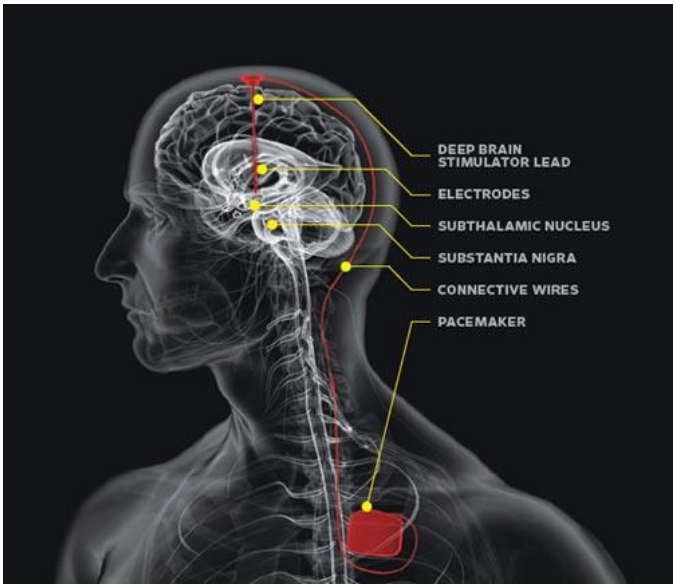


Figure 1: Representation of Deep Brain Stimulation for the treatment of idiopathic Parkinson's disease. Stimulation electrode connected to a pulse generator (pacemaker) is implanted in the human brain.

(Courtesy:http://biomed.brown.edu/Courses/BI108/BI108_2008_Groups/group07/Parkinsons.html).

The principle of HFS of deep brain regions (Figure 1) is based on a continuous and long-term application of electric pulses via one or two stimulation electrodes implanted in the brain. A pulse generator (pace maker) is responsible for the parameters like frequency, pulse width and intensity of the stimulation. These parameters differ based on the individual and the purpose of stimulation. However, in the treatment of neurological disorders the frequency of electrical pulse used for DBS is greater than 100Hz, which is above the characteristic firing frequency of neurons. Therefore, for such stimulation with high frequencies the term high frequency stimulation (HFS) was established. The pulses used for stimulation are either monopolar or bipolar. In monopolar stimulation (between the lead and the pulse generator), the distance between the anode and cathode is more resulting in an electric field spreading over a large area

affecting large number of neurons. In bipolar stimulation (between two or more electrode contacts of a lead), the electric field is relatively small, thus, affecting only a specific amount of neurons due to the small distance between the anode and cathode. The current standard parameters for stimulation that is established after several years of medical usage is as follows : monopolar or bipolar, 130-180 Hz pulses with width of 60-180μs and voltage between 1-3.5V (Perlmutter and Mink, 2006).

1.1.4 Mechanism of action of high frequency stimulation

About 50 years ago, the first experiments for treating movement disorders using high frequency deep brain stimulation were performed. In various works, such as that of Taira and Hori (2003), the overactive regions of basal ganglia were regulated by lesions. These lesions, achieved either by radio frequency or thermo-coagulation had some effect in the treatment of movement disorders (Svennilson et al., 1960). These kinds of lesions resulted in an irreversible damage to the brain which also could lead to serious side effects. At that time HFS was used only temporarily during the procedure to mimic the lesional effect. In the late 80s Benabid et al. (1991) worked to find whether an effective therapy that is reversible can be achieved for movement disorders. They conducted studies in Parkinson's patients with severe, treatment-resistant tremor (involuntary trembling). They were the first to observe that the electrical stimulation of the nucleus ventralis intermedius thalamus (Vim) with 100Hz resulted in the improvement of tremor (Benabid et al., 1991, 1987). By Benabid’s studies it became clear that lesions and HFS have similar clinical effects. This in turn led to the assumption that HFS leads to a functional inhibition of the stimulated brain region. However, the inhibition caused by HFS is reversible, since they can be terminated by switching off the stimulation generator, while

inhibition caused by lesions is irreversible. In HFS, the stimulation parameters can be adjusted based on the condition of the patient whereas lesions of several brain regions are permanent. The reversibility and adaptation are the greatest advantages of HFS compared to the procedures involving lesions.

The molecular mechanisms underlying the effect of HFS are still not fully understood. It has been proposed that high-frequency stimulation of the sub-thalamic nucleus for the treatment of Parkinson's disease silences cell bodies through an effect on calcium channels, but stimulates efferent axons (Garcia et al., 2005; Perlmutter and Mink, 2006). This would produce an effective blockade on the transmission of neural information through that structure, and impose a new output on its downstream targets.

In the particular case of DBS for OCD, several fundamental questions remain largely unexplored. For example, some groups have noted a possible delay in the beneficial effects of stimulation of weeks to months (Abelson et al., 2005; Aouizerate et al., 2004a; Nuttin et al., 2003; Rauch et al., 2006), unlike the immediate and dramatic effects of DBS for PD. Likewise, acute capsular stimulation may result in increased frontal metabolism (Tass et al., 2003), in contrast to chronic stimulation (Abelson et al., 2005; Aouizerate et al., 2004a; Nuttin et al., 2003). Most crucially, the occasional need to use relatively high voltages to achieve therapeutic effects in OCD compared with PD suggests that the "real" target might be some distance away, maybe in the ventral caudate (Rauch et al., 2006). Others have reported preliminary evidence suggesting the effective target may be the nearby nucleus accumbens (Khan and Shuaib, 2001). DBS as it exists today is a relatively non-specific tool to manipulate brain function, because besides neurons in the grey matter, also afferent and efferent fibers are stimulated.

1.2 Obsessive Compulsive Disorder (OCD)

Obsessive Compulsive Disorder (OCD) is a severe psychiatric disorder, affecting 2-3% of the population (Aouizerate et al., 2004b; Kopell et al., 2004; Nuttin et al., 2003). Intrusive thoughts (obsessions) cause extensive fear that patients try to relieve by certain repetitive, ritualistic behavior (compulsions) (Aouizerate et al., 2004b). The most common compulsions are checking, ordering, praying, counting, touching, collecting, hoarding, washing and cleaning (Math and Janardhan Reddy, 2007). Compulsions vary per person and are often carried out to neutralize the anxiety caused by the obsessions, or until it 'feels right' (Björgvinsson et al., 2007).

Development of OCD is influenced by learning principles, as well as it is related to biological, genetic and familial factors (Björgvinsson et al., 2007). The three main circuits involved in OCD are shown in figure 2. According to several studies, the metabolic activity of the orbito-frontal cortex (OFC) and striatum appear to be higher in OCD patients. Besides, during symptom provocation, an even greater increase of activity was found. After successful treatment, a decrease was seen (El Mansari and Blier, 2006). Therefore, brain areas appearing to be involved in OCD are the ventral prefrontal cortex, dorsal/ventral striatum (comprising the nucleus accumbens and olfactory tubercle), thalamus and ventral globus pallidus (Rauch et al., 2006) (Figure 2).

Cortico-striato-thalamocortical interactions are implicated in the pathogenesis of OCD due to its symptoms and response to treatment. Clinically and genetically related to OCD is Gilles de la Tourette-Syndrome, a disorder characterized by multiple physical tics and coprolalia. Also in that disorder, a central role of the basal ganglia is suggested (Kopell et al., 2004).

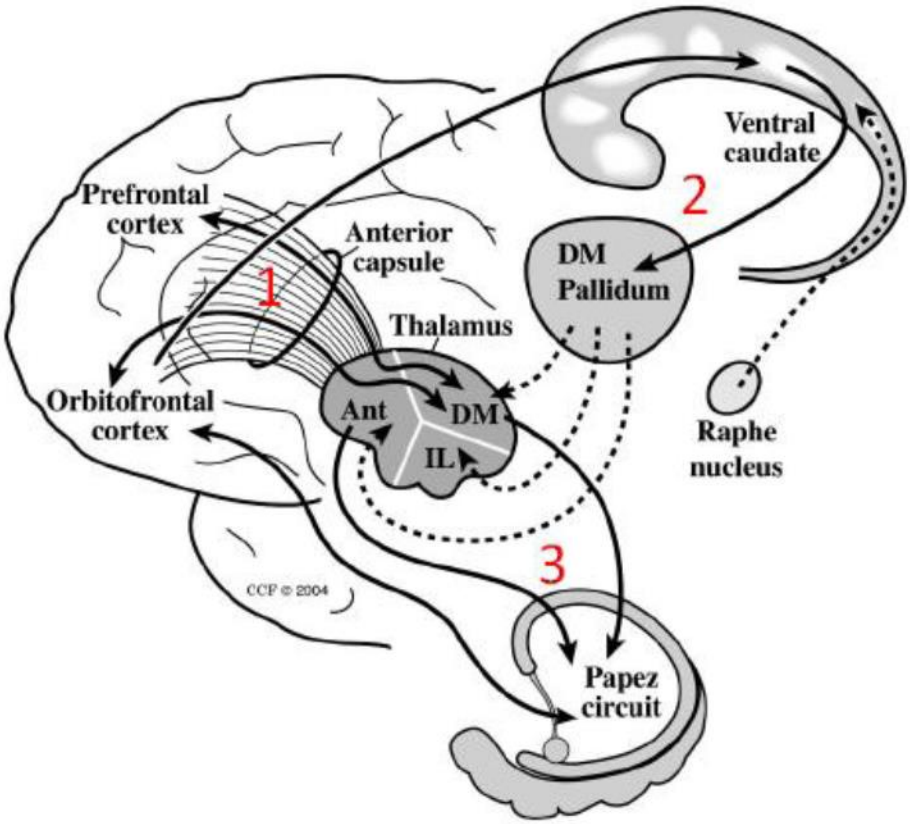


Figure 2: Schematic anatomy of the OCD neural circuitry (Kopell et al., 2004). *Three circuits (loops) seem to be mainly involved in the disorder. Circuit 1 is a positive feedback loop involving the orbital and prefrontal cortex and the dorsomedial thalamic nucleus, by way of the anterior limb of the internal capsule. Circuit 2 comprises the orbitofrontal cortex (OFC) cortico-striato-thalamocortical loop; involving the OFC, ventral caudate, dorsomedial pallidum and intralaminar (IL), anterior (ant) and dorsomedial (DM) thalamic nuclei. Circuit 3 consists of the limbic system and circuit of Papez (with projections from the anterior cingulate cortex (ACC) to the nucleus accumbens (NAc) region of striatum)* (Kopell et al., 2004)

The exact pathophysiologic process that underlies OCD has not been established. Research and treatment trials suggest that abnormalities in serotonin (5-HT) neurotransmission in the brain are involved in this disorder. This is strongly supported by the efficacy of serotonin reuptake inhibitors (SRIs) in the treatment of OCD (Dold et al., 2013; Greist et al., 1995; Kobak et al., 1998).

Evidence also suggests abnormalities in dopaminergic transmission in at least some cases of OCD. In the past decade, attention has focused on glutamatergic abnormalities and possible glutamatergic treatments for OCD (Pittenger et al., 2006). Although modulated by serotonin and other neurotransmitters, the synapses in the cortico-striato-thalamo-cortical circuits thought to be centrally involved in the pathology of OCD, principally employ the neurotransmitters glutamate and gamma-aminobutyric acid (GABA). Preclinical studies and several case reports and small clinical trials have provided some preliminary support for the therapeutic use of specific glutamatergic agents (Coric et al., 2005; Greenberg et al., 2009).

There are different treatment options for OCD ranging from behavioral therapy to surgery. Behavioral therapy includes training the subject to endure the anxiety and not conduct the compelling behavior. This behavioral training slowly breaks the cycle of obsession, anxiety and action. Behavioral therapy works well in subjects that are otherwise disciplined. Another treatment option of OCD is through prescription of medicines such as antidepressants and anti-psychotics. Although these medicines are quite effective in certain patients, the downside is that a patient can become dependent on these. For patients who do not respond well to the behavioral therapy or medicines, electroconvulsive therapy (ECT), deep brain stimulation or brain surgery are possible treatment options. Due to its intrusive nature, surgery is the last resort for treatment

of OCD (Fontenelle et al., 2015; Koran et al., 2007). Some of the most effective surgical interventions are anterior capsulotomy or anterior cingulotomy (Greenberg et al., 2003). Later in the 90's, Nuttin and Cosyns published about the series of patients with treatment-resistant OCD treated with deep brain stimulation (DBS) (Nuttin et al., 1999). Besides the work of Nuttin's group, another controlled study of four patients and a case series of ten patients including outcomes of up to three years in patients with treatment-resistant OCD receiving DBS have also been published (Abelson et al., 2005; Greenberg et al., 2006; Pepper et al., 2015). Bilateral stimulation was applied in the anterior limb of the internal capsule in the published cases. In some cases the stimulation area was extended to the adjacent ventral striatal regions which include the nucleus accumbens (NAc). Cortico-striato-thalamo-cortical (CSTC) circuit's dysfunction may implicate the pathogenesis underlying OCD, which is caused by a failure of inhibition of the ventral striatum (Alexander et al., 1986). The NAc is part of the ventral striatum, along with other parts of the tuberculum olfactorium, ventromedial parts of the nucleus caudatus, and the putamen. Thus, the NAc can be a promising target location for DBS, because of its predominant role in modulating these circuits (Nicola, 2007).

1.3 Nucleus Accumbens (NAc)

Since many years, the ventral striatum and the NAc have attracted the interest of various researchers (De Olmos and Heimer, 1999; Heimer et al., 1997). The theory about the involvement of dopamine in schizophrenia led to focusing on the nucleus accumbens and its role in psychiatric diseases (Matthysse, 1973; Stevens, 1997). The NAc can be considered as a target for deep brain stimulation for treating OCD. It is located at a point where the caudate and anterior portion of the putamen meets each other. The NAc together with the olfactory tubercle is

called the ventral striatum. The ventral striatum is a part of the basal ganglia (Sturm et al., 2003a).

Figure 3 illustrates the position of nucleus accumbens in human brain. The nucleus accumbens is divided into two principal parts: a central core and a peripheral shell. These parts have different morphology and different function. The core is associated with the extrapyramidal motor and the shell is associated with the limbic system. The core–shell-dichotomy is well established in rodents, but in man, both parts are poorly characterized (Heimer, 2000).

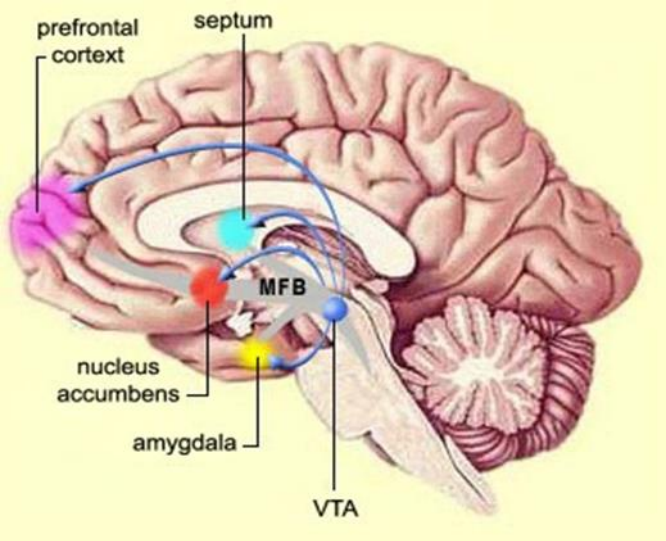


Figure 3: Slice of Human Brain showing the nucleus accumbens. [Median Forebrain Bundle (MFB), Ventral tegmental area (VTA)] (Courtesy: http://thebrain.mcgill.ca/)

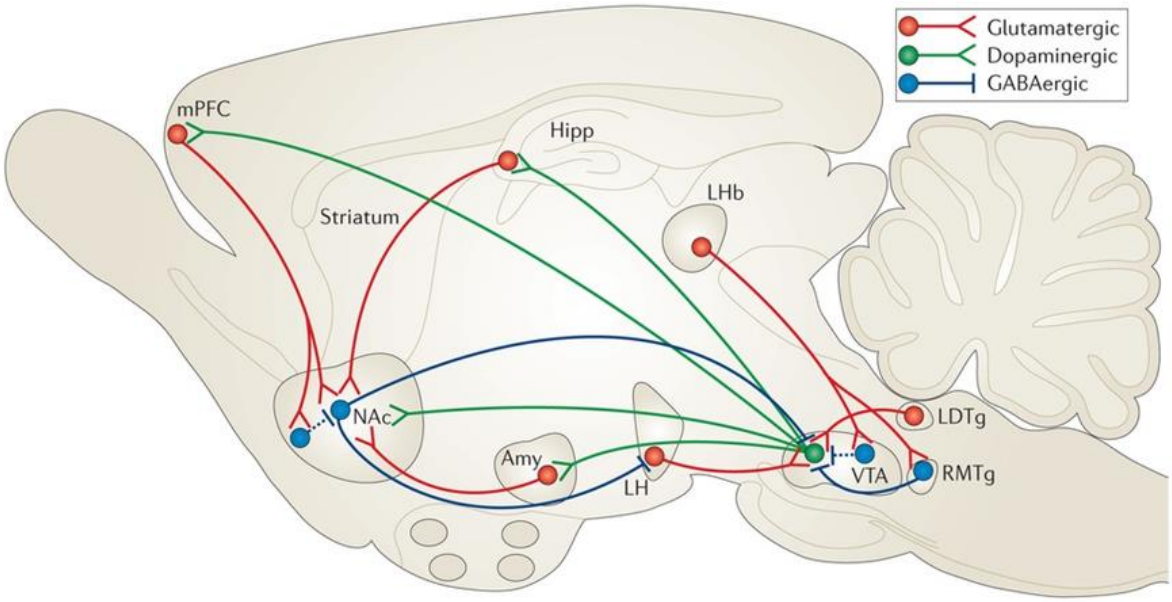


Figure 4: Slice of Rat brain showing the nucleus accumbens and the connections.[Ventral tegmental area (VTA), medial prefrontal cortex (mPFC), hippocampus (Hipp), amygdala (Amy), lateral dorsal tegmentum (LDTg), lateral habenula (LHb),lateral hypothalamus (LH)](Russo and Nestler, 2013).

The shell region has histological and biochemical properties similar to the central amygdaloid nucleus (De Olmos and Heimer, 1999). The shell contains relatively small cells with higher concentrations of D_1- and D_3-receptors than other regions of the NAc and the ventral striatum (Heimer, 2000).Within the NAc, information is transmitted from shell to core. Together with the ventral striatum, the nucleus accumbens, especially the shell region, receives a strong dopaminergic input from the ventral tegmental area (VTA) and the dorsal region of the substantia nigra (Haber, 2003; Haber et al., 2000). The NAc has many medium spiny neurons which produce the inhibitory neurotransmitter GABA. Figure 4 shows the nucleus accumbens and the various projections to it in a rat brain. Due to the central position of the NAc between the amygdaloid complex, basal ganglia, mediodorsal thalamic nucleus and prefrontal cortex which

are all involved in the pathophysiology of anxiety-disorders (Shumyatsky et al., 2002) and OCD (Saxena and Rauch, 2000) it can be considered as a target for treating OCD with deep brain stimulation (Sturm et al., 2003a) .

1.4 Neurotransmitters

Neurotransmitters are organic compounds, such as peptides, amino acids, derivatives of amino acids, or other biological agents. They help in the transmission of signals within the nervous system. The neurotransmitters are synthesized in one neuron and stored in synaptic vesicles. Depolarization of the presynaptic cell by an action potential leads to the opening of the voltage dependent calcium channels (Ca^{2+}). The Ca^{2+} influx cause the vesicles to fuse with the membrane resulting in the release of neurotransmitter into the synaptic cleft. The released neurotransmitter binds to the receptors on the postsynaptic membrane causing an excitatory or inhibitory effect. The neurotransmitter can also bind to other receptors present on the presynaptic or dendritic region. The localization of the neurotransmitter receptor has a functional importance. The neurotransmitters considered in this work are amino acids and catecholamines. The amino acids taken into account were gamma -amino-butyric acid (GABA) and glutamate. The other neurotransmitters analysed were dopamine, serotonin and their metabolites 3, 4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindole-3-acetic acid (HIAA) respectively.

1.4.1 GABA and Glutamate

GABA is one of the major inhibitory neurotransmitter of the brain, occurring in 30-40% of all synapses. It is most highly concentrated in the substantia nigra and globus pallidus nuclei of the

basal ganglia, followed by the hypothalamus and the hippocampus. The medium spiny neurons are the main GABAergic neurons.

The GABA system is the target of a wide range of drugs active on the CNS, including anxiolytics, sedative-hypnotics, general anesthetics, and anticonvulsants (Macdonald and Olsen, 1994; Richard W Olsen and Timothy M DeLorey., 1999). It is synthesized by a specific enzyme, L-glutamic acid decarboxylase (GAD), in one step from L-glutamate. Much of the glutamate and GABA used as neurotransmitter is derived from glial storage pools of glutamine (http://www.benbest.com/science/anatmind/anatmd10.html). GABA interacts with two receptor families, the ionotropic $GABA_A$ and the metabotropic $GABA_B$ receptors. $GABA_A$ opens direct chloride channels whereas $GABA_B$ increases the opening probability of K^+-channels and decreases it for Ca^{2+}-channels via G-proteins, resulting in hyperpolarisation of postsynaptic membrane. Another type of ionotropic receptors which is insensitive to allosteric modulators of $GABA_A$ receptor channels such as benzodiazepines and barbiturates were called $GABA_C$ receptors (Bormann and Feigenspan, 1995; Sivilotti and Nistri, 1991). But since the $GABA_C$ receptors have structure, sequence and functions closely related to $GABA_A$ receptors besides containing the ρ subunits, the IUPHAR (International Union of Basic and Clinical Pharmacology) has recommended that the $GABA_C$ receptors be called as ρ subfamily of the $GABA_A$ receptors ($GABA_A$-ρ)(Olsen and Sieghart, 2008). GABA contributes to motor control, vision, and many other cortical functions. It also regulates anxiety.

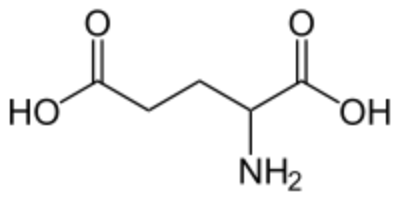


Figure 5: Structure of Glumatic acid (Glutamate)

Glutamate (Figure 5) is the most common neurotransmitter in the brain. It is always excitatory, usually due to simple receptors that increase the flow of positive ions by opening ion-channels.

N-Methyl-D-Aspartate is a selective agonist that binds specifically to the NMDA glutamate receptor (http://www.benbest.com/science/anatmind/anatmd10.html). NMDA receptors are most densely concentrated in the cerebral cortex, amygdala, and basal ganglia (Morón et al., 2002). Glutamate is associated with learning and memory. It is also thought to be associated with Alzheimer's disease, whose first symptoms include memory malfunctions (Yavich et al., 2007).

1.4.2 Dopamine and its metabolites

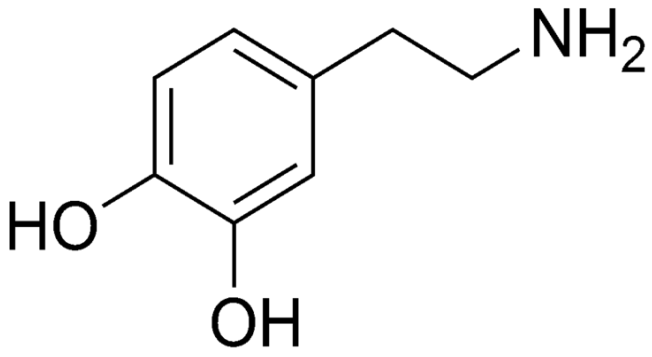


Figure 6: Structure of Dopamine

Dopamine (Figure 6) is a catecholamine and monoamine compound. It works as a neurotransmitter and activates five types of dopamine receptors - D_1, D_2, D_3 , D_4 and D_5 and its variants.

Dopamine is produced in various areas of brain including the substantia nigra and ventral tegmental region. Neural pathways transmit dopamine from one region to another. Four major dopaminergic pathways were identified (Figure 8).

First, the Mesolimbic Pathway through which the dopamine is transmitted from the midbrain, ventral tegmental area (VTA) to the NAc in the limbic system.

Second, the Mesocortical Pathway, in this pathway dopamine is transmitted from the VTA to the frontal cortex.

Third, the Nigrostriatal Pathway - This pathway transmits the dopamine from substantia nigra (structure in the midbrain) to the striatum (subcortical part of cerebrum). This pathway is related to motor control. Dysfunction or degeneration of this pathway leads to Parkinson's disease.

Fourth, the Tuberoinfundibular Pathway - This pathway, which influences the secretion of certain hormones, including prolactin, transmits dopamine from hypothalamus to the pituitary gland.

Dopamine is biosynthesized by the hydroxylation of the amino acid L-tyrosine to L-DOPA by the enzyme tyrosine 3-monooxygenase, also known as tyrosine hydroxylase, and then by the decarboxylation of L-DOPA by aromatic L-amino acid decarboxylase (which is often referred to as dopa decarboxylase). Two major degradation pathways exist for dopamine. In most areas of

the brain, including the striatum and basal ganglia, dopamine is inactivated by reuptake by the dopamine transporter –DAT1, followed by the enzymatic breakdown into 3, 4-dihydroxyphenylacetic acid by monoamine oxidase (MAOA and MAOB) (Figure 7) [http://www.buzzle.com/articles/dopamine-neurotransmitter.html].

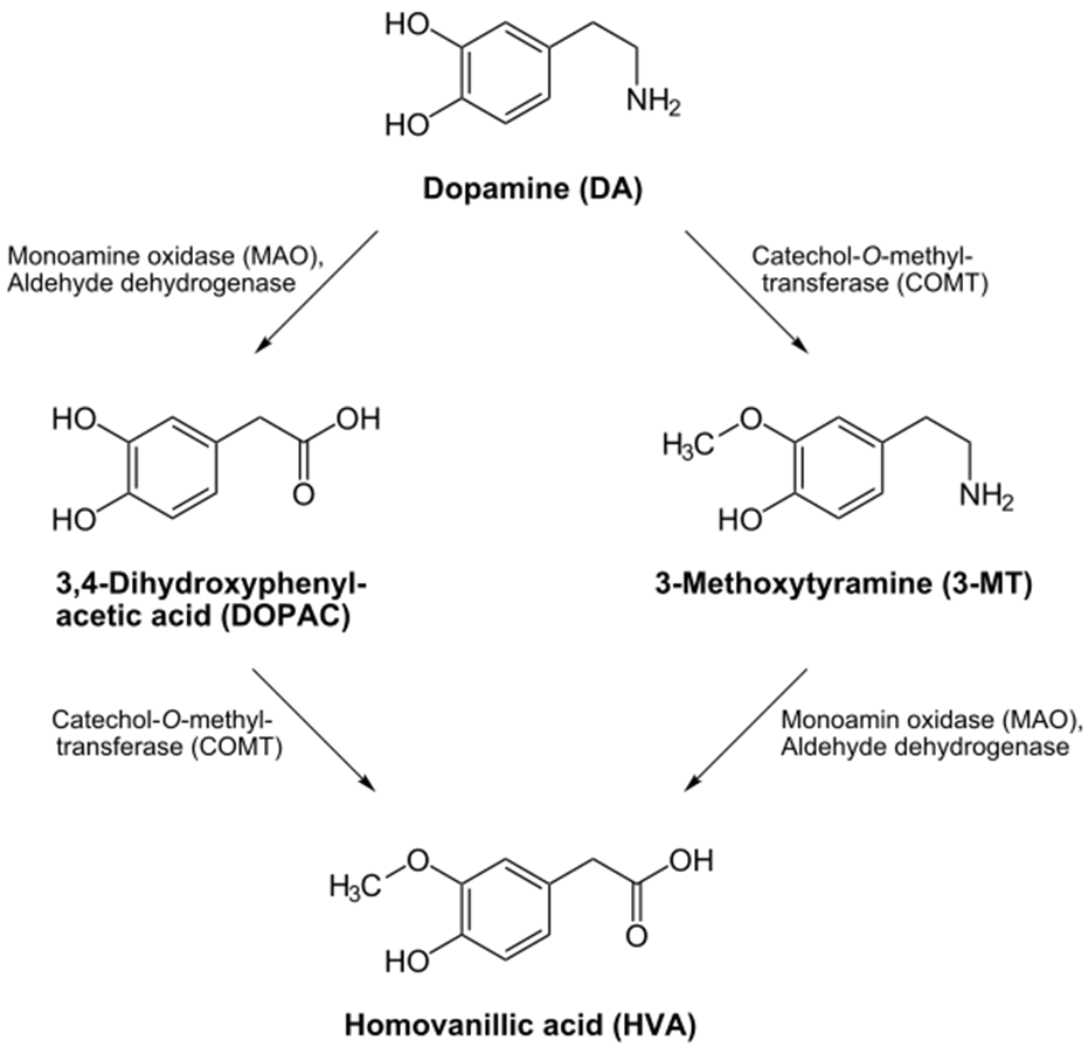


Figure 7: Biodegradation of dopamine.

Dopamine has significant roles associated with behavior and cognition, motivation, reward and voluntary movement. Dopaminergic neurons located in the midbrain are the main source of

dopamine. Dopamine is also responsible for the flow of information from different areas of the brain to the frontal lobe. Dopamine-related disorders can cause a dip in memory, problem solving and attention. The reduction in the concentration of dopamine in the pre-frontal cortex is most likely responsible for attention deficit disorder [http://www.buzzle.com/articles/dopamine-neurotransmitter.html].

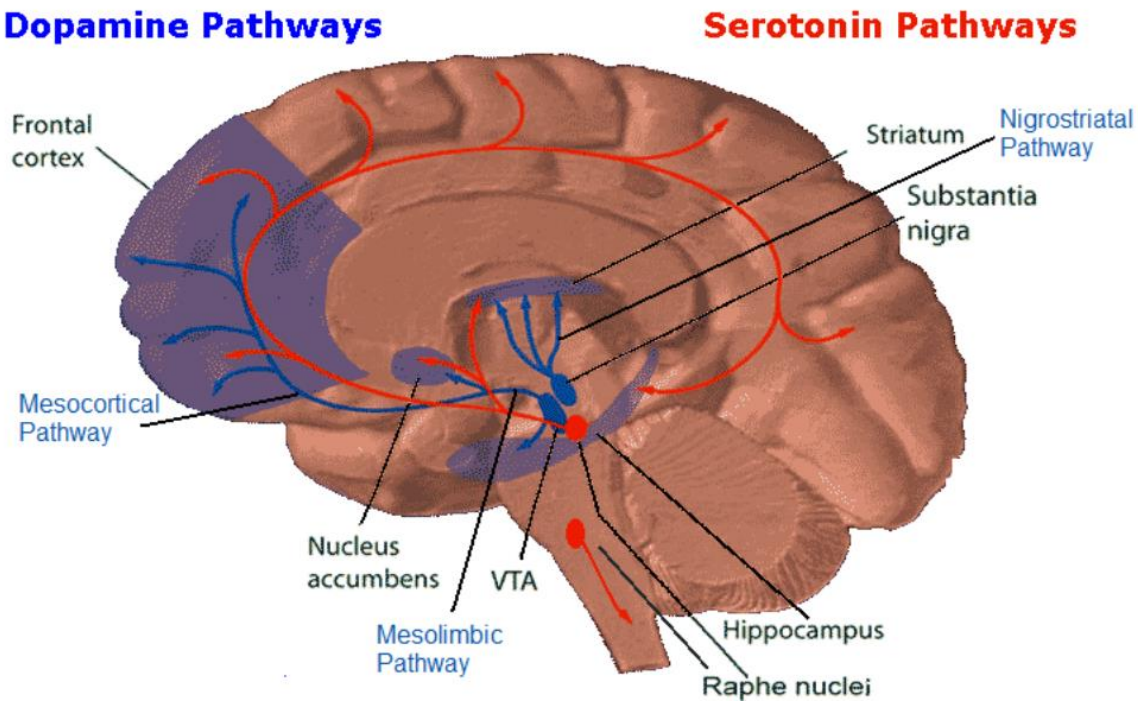


Figure 8: Dopamine and Serotonin pathways in human brain. Ventral tegmental area (VTA)

1.4.3 Serotonin and its Metabolites

Serotonin (5-hydroxytryptamine, 5-HT) (Figure 9) is a neurotransmitter in the brain that has an enormous influence over many brain functions. It is synthesized from the amino acid L-tryptophan (Figure 10). Serotonin is found in three main areas of the body: the intestinal wall, large constricted blood vessels and the central nervous system. The most widely studied effects

have been those on the central nervous system. The functions of serotonin are numerous and appear to involve control of appetite, sleep, memory and learning, temperature regulation, mood, behavior (including sexual and hallucinogenic behavior), cardiovascular function, muscle contraction, endocrine regulation, and depression (Ronald F.Borne,1994).

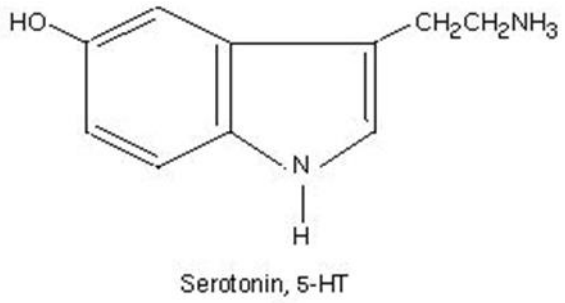


Figure 9: Structure of Serotonin

Serotonin is mainly metabolized to 5-HIAA, chiefly by the liver. Metabolism starts with oxidation of serotonin to the corresponding aldehyde by monoamine oxidase (MAO). This is followed by a second oxidation to 5-HIAA, the indole acetic acid derivative by aldehyde dehydrogenase.

The activity of serotonin arises in the brainstem from clusters of neurons known as the raphe nucleus. The serotonin pathway is shown in figure 8. From the brain, serotonin neurons extend to virtually all parts of the central nervous system making the branching of the serotonin network the most expansive neurochemical system in the brain. There are at least seven populations of receptors for serotonin: 5-HT_1- 5-HT_7 (Hoyer et al., 1994).

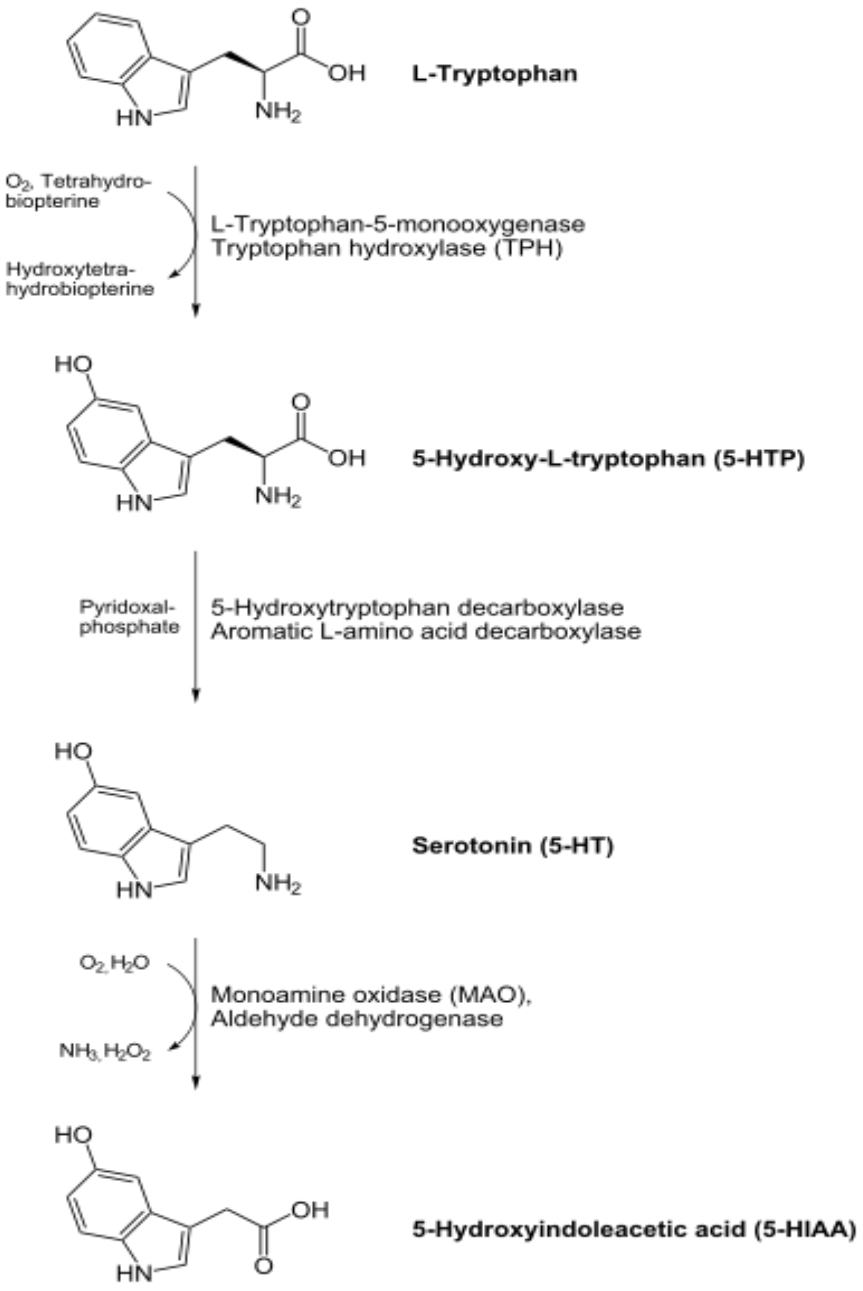


Figure 10: Synthesis of serotonin from tryptophan.

1.5 Intracerebral Microdialysis

Intracerebral microdialysis involves the insertion of a microdialysis probe into a selected area of the brain. Substances around the semipermeable part of the probe will diffuse into or out of the perfusate, into the direction of the lowest concentration. Subsequently the dialysate is collected for analysis.

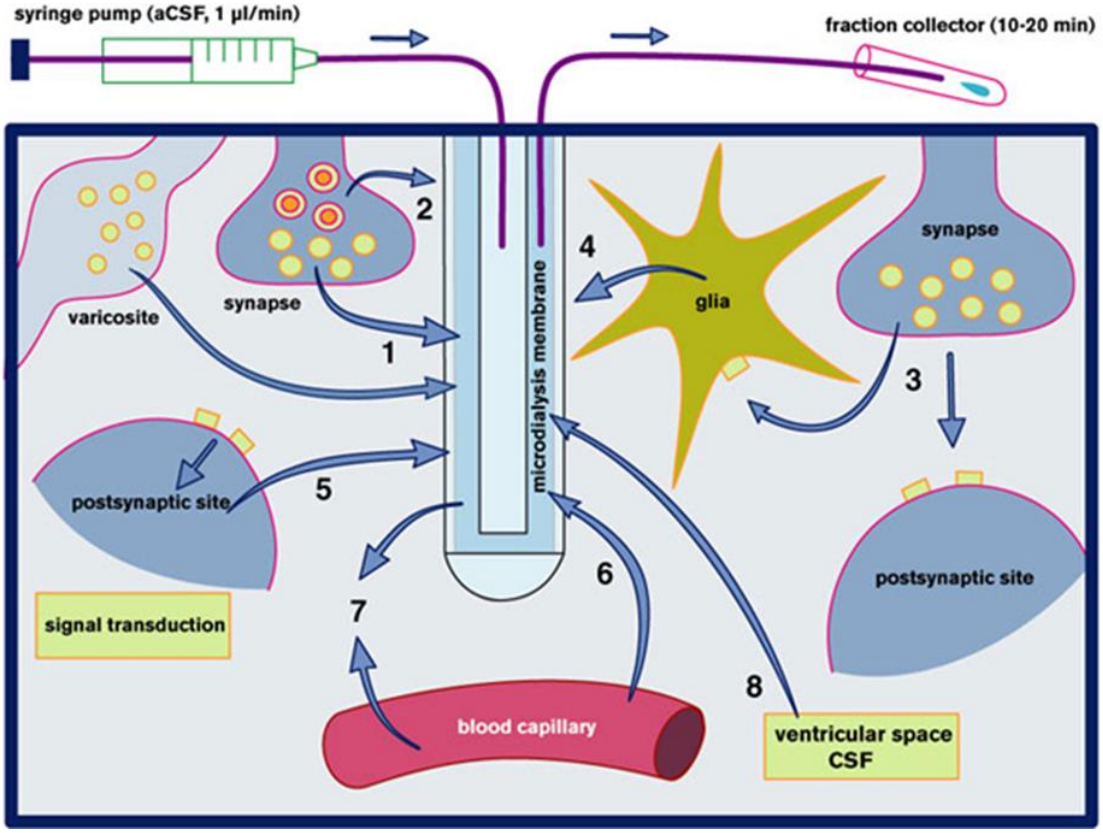


Figure 11: Principle of Microdialysis. The studied molecules are : 1,2,3 - neurotransmitters, neuromodulators and neuropeptides; 3,4 - the neuron-glia interactions, glutamate and GABA, large molecules such as interleukins and trophic factors; 5 - second messengers cAMP, cGMP or arachidonic acid metabolites; 6 - molecules transported from blood capillaries - glucose, nutrients, drugs; 7 - neuro-vascular communication - NO; 8 - molecules transported from or into the CSF.(Courtesy: Kehr J, Yoshitake T (2006) Monitoring brain chemical signals by

microdialysis. In: Encyclopedia of Sensors (Eds CA Grimes, EC Dickey and MV Pishko) American Scientific Publishers, USA, pp 287-312.)

The principle of microdialysis and the substances that can be obtained in the dialysate is shown in figure 11. Neurotransmitter concentrations in brain dialysate reflect concentrations in brain extra cellular fluid (ECF). This technique has a number of advantages like concentration versus time profiles can be obtained from freely moving individual animals and concentrations can be determined in a distinct region [http://www.chromatography-online.org].

Microdialysis techniques have been developed to monitor changes in the chemistry of the extracellular space in living tissues. Intracerebral microdialysis(IC-MD) is a novel and popular technique for studying a wide range of substances, crossing a semi-permeable intracerebral microdialysis probe(IC-MDP) membrane, and getting dialysates continuously from the ECF (Extracellular Fluid) of the conscious animal's brain to determinate the concentrations of drugs and their metabolites, and to monitor the different levels of neurotransmitters relatively (de Lange, 1997). The dialysate can be gained on real time through IC-MDP, which is inserted into certain tissue of brain. The dialysate obtained through microdialysis can be analyzed using high pressure liquid chromatography (HPLC).

1.6 Local Field Potentials (LFPs)

Extracellular recordings may refer to single or multiple events of neural discharge depending upon the distance between the recording electrode and the neural membrane. If the recording electrode is far away from a single spiking neuron then a sum of potentials from several neural cells is recorded. This multi-unit recording includes fast spiking activity from cells and slow

integrative activity from the dendritic tree. The different components in the multi-unit recordings can be distinguished by their frequencies. In these two components, a low-pass cut off at 300 Hz contributes to the local field potentials which are the integrative activity of synapse and dendritic tree whereas multi-unit spikes activity can be obtained by applying a high-pass cut off filter at 300 Hz (Belitski et al., 2008; Kelly et al., 2010). Local field potentials which represent the low-frequency component of multi-unit activity were reported to reflect a weighted average of synchronized dendrosynaptic components from neural populations which are within 0.5-3 mm range from the electrode tip (Juergens et al., 1999; Mitzdorf, 1987, 1985). The local field potential activity is divided into delta (0.4-4 Hz), theta (4-12 Hz), beta (13-30 Hz) and gamma (30-70 Hz) band activity similar to the electroencephalogram (EEG) recordings. Each band activity can be strongly correlated to specific behavioral state (Logothetis, 2003; Silva et al., 1991).

1.7 Aim of the study

The aim of this work was to characterize the effect of high frequency stimulation (HFS) on neurotransmitters such as GABA, glutamate, dopamine, serotonin and their metabolites in the nucleus accumbens of the rat brain in vivo. The influence of subcutaneous injection of memantine on the above neurotransmitters was also studied. Quinpirole, a selective D2 and D3 receptor agonist, which induces compulsive checking behavior in rats, was also used to check the effect of HFS on these rats. For this work, microdialysis and high frequency stimulation were performed simultaneously in the nucleus accumbens of conscious and freely moving rats. In order to find the influence of the high frequency stimulation on the local field potentials (LFPs) of the rat brain, electrophysiological recordings were performed immediately before and after

HFS as well as one hour after stimulation. Stereotactic surgery was performed to implant a guide cannula into the rat brain. The implant was fixed on the skull of the rat using dental cement. This guiding implant was used for holding the microdialysis probe and the stimulation electrode during the experiment in conscious rats. During the experiment the microdialysis probe was constantly perfused with artificial cerebrospinal fluid (aCSF). Neurotransmitters were collected in 24µl fractions from the extracellular fluid (ECF) every 20 min. The concentration of the neurotransmitters in the collected samples was determined using HPLC with electrochemical detectors (ECD). The electrophysiological recordings were undertaken before, after and one hour after HFS and analyzed using MATLAB.

2 Materials and Methods

2.1 Materials

2.1.1 General Lab Equipment

Name	Company
Analytical Balance Satorious Reasearch R200D	Satorius AG, Goettingen, Germany
Balance Mettler PM2000	Mettler Waagen GmbH, Giessen,Germany
Graduated beakers : 25 ml,50 ml,100 ml	DURAN Group GmbH, Wertheim/Main, Germany
Griffin beakers : 3000 ml,5000 ml	VITLAB GmbH, Grossostheim, Germany
Magnetic Stirrer MR 3001	Heidolph, Kelheim, Germany
Measuring cylinder : 100ml,1000ml,2000ml	DURAN Group GmbH, Wertheim/Main, Germany
Plastic disposable Spatula	Carl Roth GmbH, Karlsruhe, Germany
pH Meter 761 Calimatic	Knick Electronische Messgeraete, Berlin, Germany
Ultrasonic Bath Bandelin Sonorex TK52H	Schalltec GmbH, Moerfelden-Walldorf, Germany
Vortexer VF2	Janke & Kumkel IKA®Labortechnik, Staufen, Germany

2.1.2 Surgery Equipment

Name	Company
Bone Cutter	FST®Fine Science Tools, Heidelberg, Germany
Bone Screws (0.85 x 4.0 mm)	FST®Fine Science Tools, Heidelberg, Germany
Connecting Rod for Clips	CMA/Microdialysis, Stockholm, Sweden
Delicate Forceps	FST®Fine Science Tools, Heidelberg, Germany
Digital camera	eo® Edmund Optics® , Karlsruhe, Germany
Electrical Shaver	
Feather Disposable Scalpel	Feather Safety Razor Co., Osaka, Japan
FEP Tubing (1.2µl/100mm)	CMA/Microdialysis, Stockholm, Sweden
High Speed Micro Drill	FST®Fine Science Tools, Heidelberg, Germany
Hypodermic Needles BD Microlance 3 (0.5x25mm, 0.6x25mm, 0.9x40mm)	Becton Dickinson S.A., Fraga, Spain
Injection Syringe TP5-100-71 (A0726)	Knauer, Berlin, Germany
Intra Cerebral Guiding Cannula CMA 11	CMA/Microdialysis, Stockholm, Sweden
Intra Cerebral Guiding Cannula MAB 4.15.IC	AgnTho's ,Sweden
Micro Drill Steel Burrs : 2.1mm, 1.4mm, 0.9mm	FST®Fine Science Tools, Heidelberg, Germany
Olsen-Hegar Needle Holder	FST®Fine Science Tools, Heidelberg, Germany

Probe Clips	CMA/Microdialysis, Stockholm, Sweden
Q-Tips	Carl Roth GmbH, Karlsruhe, Germany
Rat Adapter (Tooth plate & nose clamp)	TSE Systems, Bad Homburg, Germany
Rat Ear Bars	TSE Systems, Bad Homburg, Germany
Scissors	FST® Fine Science Tools, Heidelberg, Germany
Semken Forceps	FST® Fine Science Tools, Heidelberg, Germany
Stereotactic Frame	TSE Systems, Bad Homburg, Germany
Sutures Resolon® blue	Resorba® , Nuernberg, Germany
Syringe BD Plastipak (1 ml)	Becton Dickinson S.A., Madrid, Spain
Tissue Forceps BD557	AESCULAP, Center Valley, USA
Tissues ECO 67	IGEFA Handelsgesellschaft mbH & Co.KG, Berlin, Germany
Tissue Pehazell	Paul Hartmann AG, Heidenheim, Germany

2.1.3 Chronic Experiment (Experiment in freely moving rats) Equipment

Name	Company
Bowl Test cage (diameter 500 mm, depth 250 mm)	TSE Systems, Bad Homburg, Germany
Concentric Bipolar electrode CBCBG30	FHC Inc. USA
Microdialysis Probe MAB 4.15.1.Cu	AgnTho‘s, Sweden

Oscilloscope-TDS2004B	Tektronix , USA
Perfusion pump(Type 540101)	TSE Systems, Bad Homburg, Germany
Electrical Stimulators-ISO STIM ^{TM} A320	World Precicion Instrument GmbH, Berlin, Germany
RZ2 Multi-Channel Neurophysiology Workstation	Tucker –Davis Technologies, USA

2.1.4 HPLC Systems

2.1.4.1 System A for Catecholamine

Parts	Company
Column Heater	Knauer, Berlin, Germany
Injection & Switching Valve A1357	Knauer, Berlin, Germany
CLC100 Electrochemical Detector	Chromsystems, Gräfelfing, Germany
Smartline HPLC Pump 1050	Knauer, Berlin, Germany
Vertex Plus Column Eurosphere 100-5 C18 (pre column : 30 x 4 mm ; main column: 250 x 4 mm)	Knauer, Berlin, Germany

2.1.4.2 System B for Amino Acids

Parts	Company
Column Heater	Knauer, Berlin, Germany
Injection & Switching Valve A1357	Knauer, Berlin, Germany

Waters 460 Electrochemical Detector	Millipore, Eschborn, Germany
WellChrom HPLC Pump K501	Knauer, Berlin, Germany
Vertex Plus Column Eurosphere 100-5 C18 (pre column : 30 x 4 mm ; main column: 250 x 4 mm)	Knauer, Berlin, Germany

2.1.5 Chemicals

2.1.5.1 Bio chemicals and Solutions for HPLC

Name	Company
3,4-Dihydroxyphenyl Acetic Acid (DOPAC, $C_8H_8O_4$)	Sigma Aldrich, St.Louis ,USA
4-Hydroxy-3-Methoxy-Phenylacetic Acid (HVA, Homovanillic Acid, $C_9H_{10}O_4$)	Sigma Aldrich, St.Louis ,USA
5-Hydroxyindole-3-Acetic Acid (HIAA, $C_{10}H_9NO_3$)	Sigma Aldrich, St.Louis ,USA
Acetic Acid (100%, CH_3COOH), 'Baker HPLC Analyzed'	J.T.Baker Deventer, Netherlands
Di-Sodium Tetraborate ($Na_2B_4O_7$)	Merck, Darmstadt, Germany
Dopamine ($C_8H_{11}NO_2$*HCl)	Sigma Aldrich, St.Louis ,USA
Ethylendiamine Tetraborate Acid (EDTA, $C_{10}H_{16}N_2O_8$)	Serva Electrophoresis GmbH, Heidelberg, Germany
Ethanol (70%, C_2H_6O)	Merck, Darmstadt, Germany
γ-Aminobutyric Acid (GABA, $C_4H_9NO_2$)	Sigma Aldrich, St.Louis ,USA
Hydrochloric Acid (HCl)	Merck, Darmstadt, Germany

L-Ascorbic Acid	Sigma Aldrich, St.Louis ,USA
L-Glutamic Acid Potassium Salt Monohydrate ($C_5H_8KNO_4*H_2O$)	Sigma Aldrich, St.Louis ,USA
L-Glutamine ($C_5H_{10}N_2O_3$)	Sigma Aldrich, St.Louis ,USA
LiChrosolv Ethanol absolute	Merck, Darmstadt, Germany
Magnesium Sulfate ($MgSO_4*H_2O$)	Merck, Darmstadt, Germany
Methanol, HPLC Analyzed	J.T.Baker Deventer, Netherlands
o-Phtaldialdehyde (OPA,$C_8H_6O_2$)	Sigma Aldrich, St.Louis ,USA
Perchloric Acid 0.33M	DiaSys GmbH, Holzheim, Germany
Potassium Chloride (KCl)	Merck, Darmstadt, Germany
Potassium Dihydrogen Phosphate (KH_2PO_4)	Merck, Darmstadt, Germany
Serotonin (5-HT,5-Hydroxytryptamin,$C_{10}H_{12}N_2O$)	Sigma Aldrich, St.Louis ,USA
Sodium Acetate (CH_3COONa)	Merck, Darmstadt, Germany
Sodium Chloride (NaCl)	J.T.Baker Deventer, Netherlands
Sodium Dihydrogen Phospate (NaH_2PO_4)	Sigma Aldrich, St.Louis ,USA
Sodium Hydrogen Carbonate ($NaHCO_3$)	Merck, Darmstadt, Germany
Sodium Hydroxide Solution (NaOH, Titrisol)	Merck, Darmstadt, Germany
Sodium Octane Sulfonic Acid ($C_8H_{17}NaO_3S$)	Merck, Darmstadt, Germany
Sodium Sulfite (Na_2SO_3)	Sigma Aldrich, St.Louis ,USA
Water, HPLC Analyzed	J.T.Baker Deventer, Netherlands

2.1.5.2 Bio chemicals and Solutions for Surgery

Name	Company
Bepanthen Eye and Nose ointment (Active ingredient : Dexpanthenol)	Bayer Vital, Leverkusen, Germany
Betaisadonna (active ingredient : Polyvidon-Iod)	Mundipharma GmbH, Limburg (Lahn), Germany
Glucose Solution(5%[w/v])	SERAG Wiessner KG, Naila, Germany
Isoflurane (100%)	Baxter Deutschland GmbH, Unterscleissheim, Germany
Isotone NaCl Solution (0.9%[w/v])	Berlin-Chemie AG, Berlin, Germany
Ketavet® (100mg/ml Ketaminhydrochloride)	Pfizer Deutschland GmbH, Berlin, Germany
Memantine hydrochloride	TOCRIS bioscience, USA
Quinpirole hydrochloride	Sigma Aldrich, St.Louis ,USA
Bicuculline hydrochloride	Sigma Aldrich, St.Louis, USA
Palapress Cold-curing Resin (Fluid and Powder)	Heraeus Kreuzer, Hanau, Germany
Rompun® (2%[w/v],active ingredient : Xylazinhydrochloride)	Bayer Vital, Leverkusen, Germany
Xylocain (4%[w/v],active ingredient : Lidocainhydrochloride)	AstraZeneca GmbH, Wedel, Germany

2.1.6 Buffers and Solutions

Dervatisation Solution	16.4 mM OPA 10 mM Na_2SO_3 Ethanol 2%(v/v) 90 mM Disodium Tetraborate, pH 10.4
Mobile phase for HPLC Eluent IV for Catecholamines	0.15M CH_3COONa,pH 4.0 0.014g/l EDTA 0.1 mM Octanesulfonic Acid 10.7% HPLC Analyzed Methanol 10 min He degassed
Mobile phase for HPLC Eluvent V for aminoacids	0.1M NaH_2PO_4,pH 4.5 0.5 mM EDTA 25% HPLC Analyzed Methanol 10 min He degassed
Krebs Ringer Bicarbonate Buffer (nKRBP)	125 mM NaCl 5 mM KCl 1mM $MgSO_4*7H_2O$ 1.25 mM KH_2PO_4 2 mM $CaCl_2*2H_2O$ 0.0001 mM Ascorbic Acid 25 mM $NaHCO_3$ 10 mM Glucose pH 7.4

	20 min CO_2 degassed
Fixation Solution for Brain	Buffered formaldehyde 4.5 %

2.2 Methods

Animal experiments were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The experimental protocol was approved by the animal welfare department - Ministerium für Natur and Umwelt des Landes Schleswig-Holstein, Germany (Schleswig-Holstein, State Office of Nature and Environment, Permit Number: V312-72241.122-13).

2.2.1 Stereotactic Coordinates

The target region for microdialysis and HFS in this project was the NAc. The stereotactic coordinates for the NAc was determined from the Paxinos Rat atlas (Paxinos and Watson, 2007)**.** The reference points for the stereotaxy were the bregma and the internal aural point. Bregma is the point of intersection of the sagittal suture with the curve of best fit along the coronal suture. The interaural point is the midpoint of the curve of best fit along the lamboid suture (Figure 12). The coordinates of the accumbens were determined based on the bregma and the interaural point.

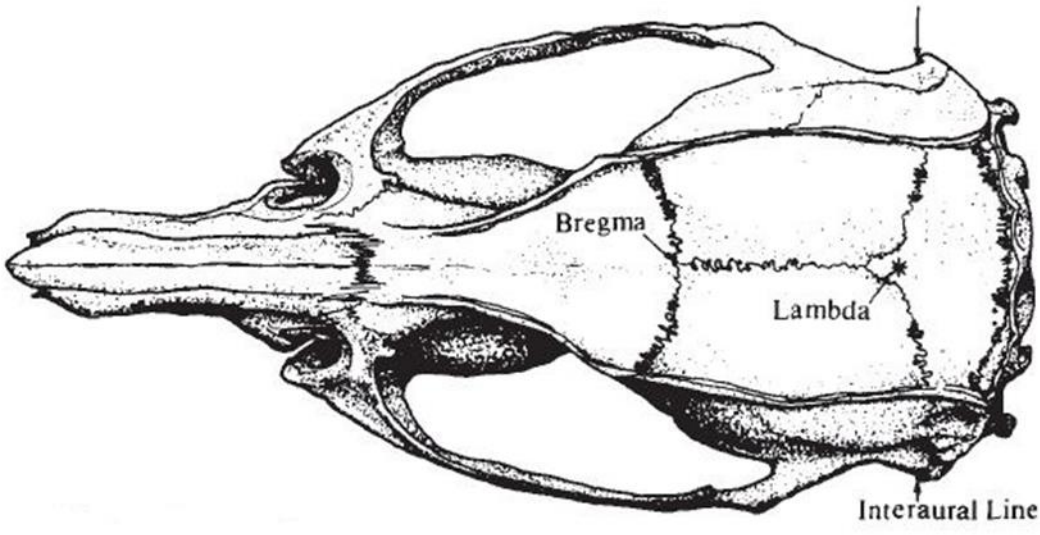


Figure 12: Dorsal view of the skull of a 290g male Wistar rat showing bregma, lambda and interaural line (Paxinos et al., 1985)

The coordinates for the nucleus accumbens based on the bregma are: anterior-posterior (AP) + 0.17cm, medial-lateral (ML)-0.39cm and dorsal-ventral (DV)-0.75cm with an angle of 20º.

2.2.2 Guiding Tubes

The day before the surgery, the guiding tubes necessary for the implantation were prepared. Therefore, one of the guiding tubes was modified such that it can hold the electrode. The modification was done by replacing the tube in the guiding tube by a 1 cm long tube with one end widened in order to fit into the hole of the guiding tube. Then the modified guiding tube and an original one from the company (*CMA/Microdialysis, Stockholm, Sweden*) were glued together using epoxy (Figure 13). Such that there is certain angle between the two tubes so that when the electrode and microdialysis probes are inserted, the distance between their tips is less than 1mm (Figure 14).

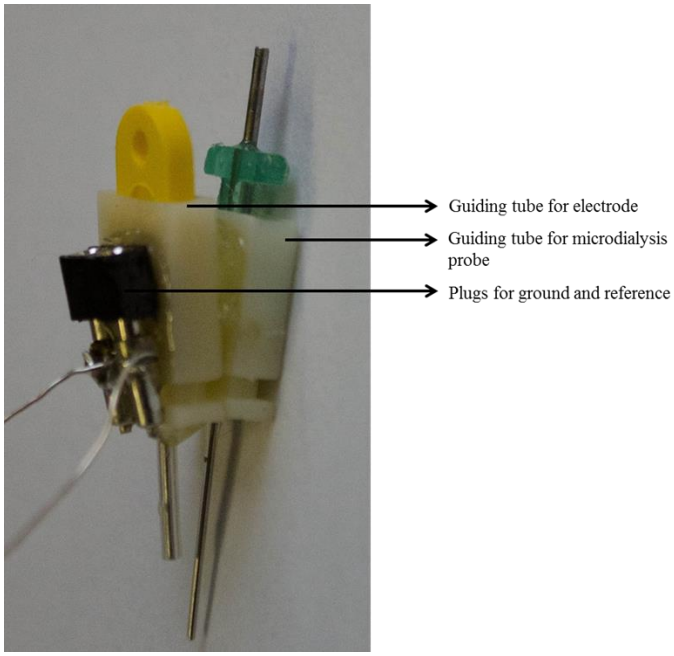


Figure 13: Guiding Tubes for electrode and probe glued together with plugs for ground and reference

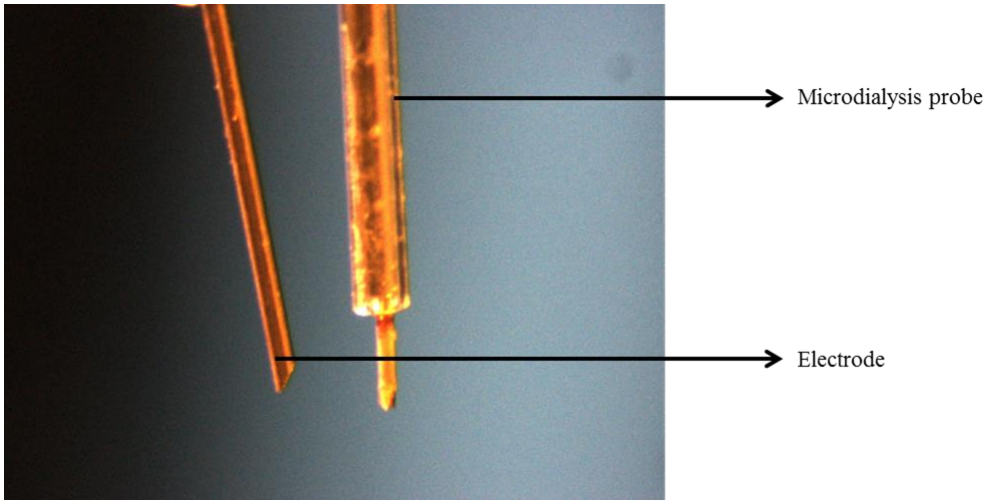
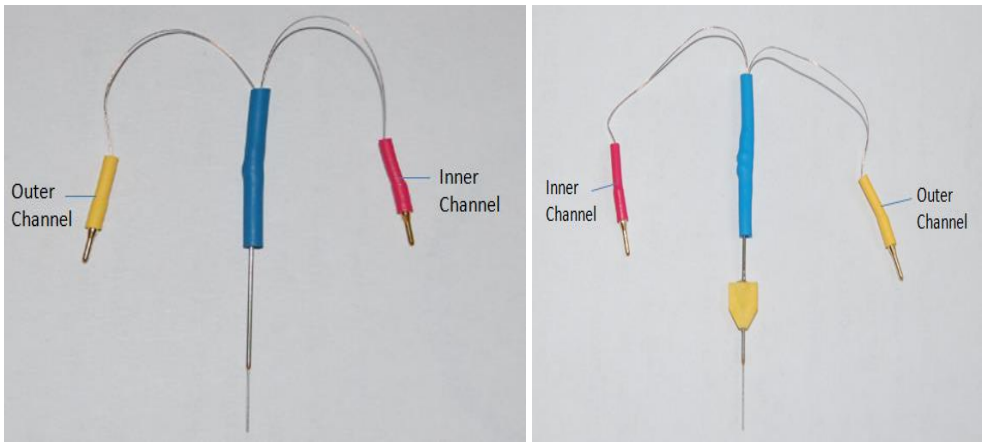


Figure 14: Tip of the electrode and probe

This modification was done in order to make sure that both the electrode and microdialysis probes are in the target region. Two plugs soldered with the ground and the reference screws were glued together with the guiding tubes. The ground and reference screws were necessary for

the electrophysiological recordings. The implant was prepared in such a manner that there was no glue at the tip of the guiding tubes. The electrode was prepared by gluing a support so that it can fit into the guiding tubes (Figure 15B).



A **B**

Figure 15: A) Original FHC electrode. B) Modified electrode with the support.

2.2.3 Anaesthesia

Male Wistar rats with a body weight of 350-500 g were used for the experiment. In order to inject the narcotic substances, the rat was first put in a box with the inhalation anaesthetic substance isoflurane. Once the rat was sedated, the intraperitoneal injection of the narcotic substances ketamine hydrochloride (100 mg/kg) and xylazinhydrochloride (50 mg/kg) was performed. The rats were injected with 500µl of 0.9% sodium chloride solution and 500 µl of glucose (5%) whenever necessary in order to prevent dehydration.

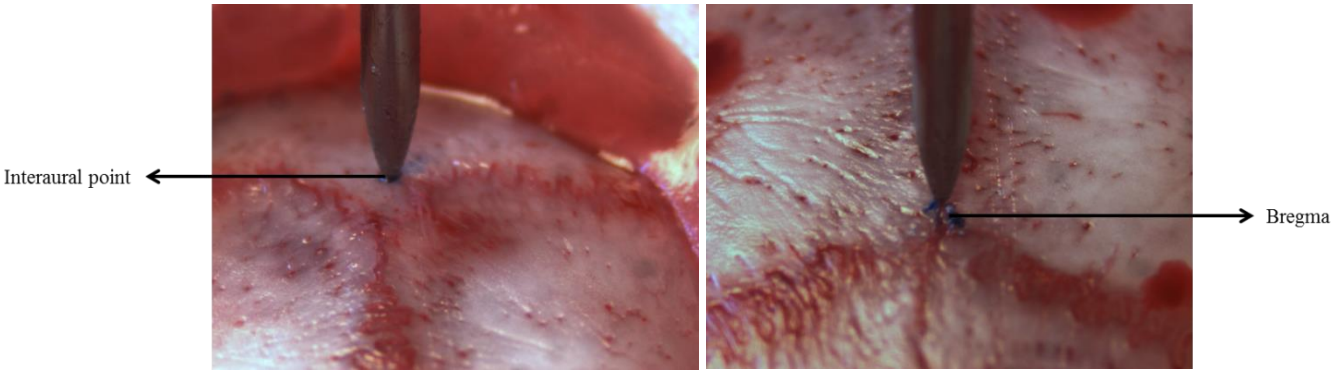
2.2.4 Stereotaxy

First, the head of the anaesthetised rat was shaved so that the skin was exposed. In order to maintain the body temperature constant at 35° C the rat was placed on a heating (40° C) water pad. Then the rat was mounted in the stereotaxic frame using the ear bars (Figure 16). Then the skin was cut around 3 cm long using a scalpel. The skin was then opened and fixed aside using

suture material. The periosteum was removed with Q-tips. The skull was cleaned by rinsing with sodium chloride solution and rubbing with Q-tips.

Figure 16: Rat mounted on the stereotaxic frame.

When the surface of the skull is clear enough such that the bregma and lambda, the two crossing points of the coronal suture and the sagittal suture can be seen, the reference points bregma and lambda were marked using a marker (Figure 17). The difference between the DV (Dorsal Ventral) measurement of the bregma and lambda should not exceed 0.02. If this difference was greater, then the nose bar in the stereotaxic frame was adjusted to make the difference lesser. This was done to make sure that the skull of the rat was flat and horizontal.



A **B**

Figure 17: A) Interaural point B) Bregma

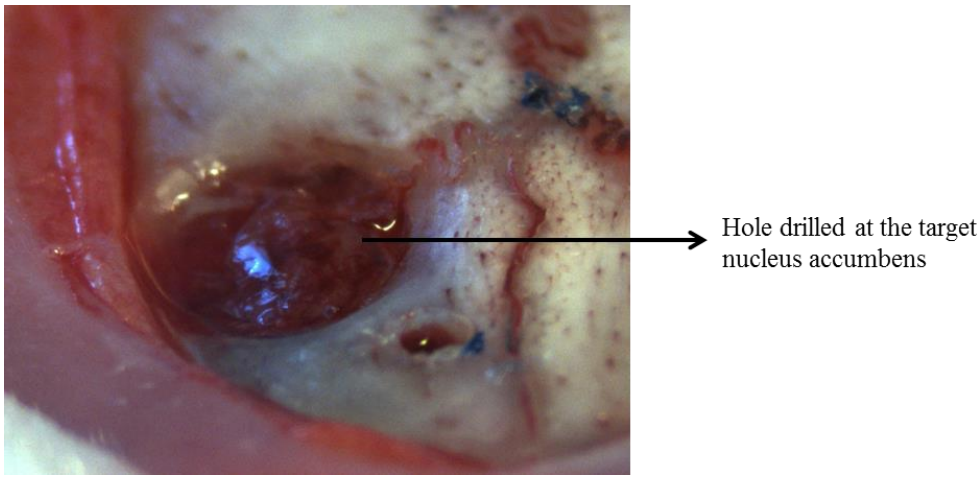


Figure 18: Hole drilled at the target nucleus accumbens.

The position of the target accumbens was marked in relation to the bregma with the coordinates AP + 0.17cm, ML-0.39cm and DV-0.75cm. The position for the two fixation screws each with +0.3cm and –0.4 cm from AP of the NAc and +0.3cm from ML of the NAc for both the screws were marked. The position for the ground screw was marked in relation to the interaural point with the coordinates AP + 0.25 cm and ML + 0.25 cm. During the drilling the skull was cooled from time to time by spilling sodium chloride solution. Holes were drilled at the marked positions using 0.9mm Micro Drill Steel Burr and the High Speed Micro Drill. The hole at the

target (NAc) was made wider using 2.1mm Micro Drill Steel Burr. After drilling the hole the duramater was removed carefully using a bent needle and surgical scissors (Figure 18). One of the two fixation screws that were required to hold the implant was put in one of the holes drilled for it and tightened using the screw drivers. Then the guiding tubes were mounted to the probe clip that was attached to the stereotaxic bars and the bar was tilted to a 20º angle in order to reach the accumbens with a certain trajectory. The tilted bar was then lowered carefully into the hole that was drilled at the target region (Figure 19). The ground and the reference screws attached to the plugs were placed in their respective holes and screwed tightly.

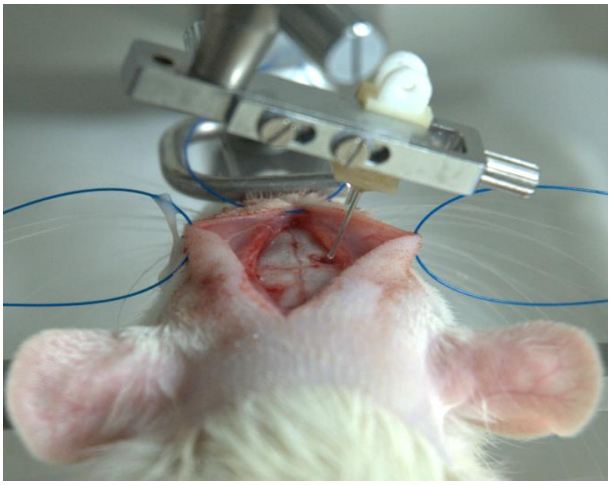


Figure 19: Lowering the guiding cannula into the brain tissue

The implant was fixed to the skull using dental cement. The dental cement was applied in such a manner that there were no sharp edges as this may hurt the skin when the wound is closed. The sharp edges were cut using a bone cutter. After making sure that the implant was fixed in place the probe clips were removed. The skull was then closed by making 2 or 3 sutures in the front and back. The bleeding was cleaned using Q-tips and the wound was disinfected using Polyvidon-Iod [Poly(1-vinyl-2-pyrrolidon)-Iod complex]. Then, the animal along with the

heating water pad was put back in the cage and kept under observation until it was awake from the anaesthesia.

2.2.5 Animal Groups

High frequency stimulation (HFS) was applied in 11 animals (awake). In addition to HFS, the $GABA_A$ receptor antagonist bicuculline (0.1mM in aCSF) was used as a perfusate in 4 experiments in which the experiment time was extended to another 1 hr and bicuculline was added in the final 1 hr. The control group (n=9, awake) consisted of implanted rats without HFS. The other groups received memantine with HFS, memantine without HFS, quinpirole with HFS, quinpirole without HFS, NaCl (saline) injection with HFS and NaCl (saline) injection without HFS with 7-8 animals in each group. All the injections were made subcutaneously (s.c).

2.2.5.1 Drugs Used

Bicuculline hydrochloride, a $GABA_A$ receptor antagonist (antagonist- drug that blocks agonist-mediated responses rather than provoking a biological response itself upon binding to a receptor) – 0.1 mM in aCSF was used as the perfusate. Memantine hydrochloride, a NMDA receptor antagonist - 5 mg/kg was dissolved in saline (0.9% NaCl) and injected subcutaneously (s.c.) immediately after probe insertion on the day of the experiment. In previous works, our group has shown the involvement of NMDA receptors in the HFS effect in the rat caudate nucleus (Varatharajan et al., 2014). Therefore, in order to further investigate the involvement of NMDA receptors in the HFS effect we used an antagonist in this work. Quinpirole hydrochloride, a high-affinity agonist (agonist- drug that binds to a receptor and activates the receptor to produce a biological response) of dopamine D_2 and D_3 receptors was also used in our studies. This was

used to check the involvement of the D_2 and D_3 receptors in the HFS mechanism. Also quinpirole induces compulsive behavior in rats which is similar to the compulsive symptom in OCD.

2.2.6 Perfusion Liquid

The perfusion liquid or artificial cerebrospinal fluid (aCSF) which has similar concentration as the cerebrospinal fluid (CSF) was used in studies involving in vivo microdialysis in the NAc.

Table 1: *Salt Concentration in perfusion liquid*

Salt	Concentration
NaCl	125 mM
Ascorbic Acid	0.10mM
$NaHCO_3$	25 mM
KCl	5 mM
$CaCl_2$* $2H_2O$	2 mM
KH_2PO_4	1.25 mM
$MgSO_4$ * $7H_2O$	1 mM

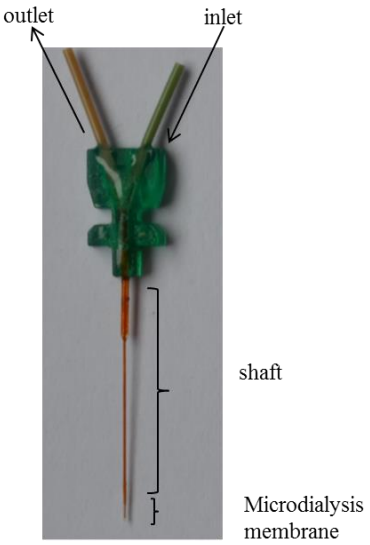
The salt concentrations in perfusion liquid are shown in the Table 1. The solutions NaCl, KCl, $MgSO_4$*$7H_2O$, KH_2PO_4, $Cacl_2$*$2H_20$ were always prepared before and stored at -20° C. But $NaHCO_3$ and Ascorbic acid were always prepared fresh. All these solutions were prepared using HPLC water (see 2.1.5.1 in Materials).

The perfusion solution with the salt concentration as shown in Table 1 was prepared freshly before application. The pH was adjusted by adding 0.1M hydrochloric acid in steps of 10µl. The pH meter from the company *Knick Electronische Messgeraete, Berlin, Germany* was used. After that the liquid was ultrasonified for 5 min and then stored at 4° C. The prepared perfusion liquid should be used for experiment within 24 hr.

2.2.7 Microdialysis and High Frequency Stimulation

The chronic experiments were performed 3 days after the implantation of the guiding tubes in order to make sure that the animals were fully recovered from the surgery. The experiments were performed from morning 9:00 until evening 16:30. During the experiment the animals were in full access to food. The reaction tubes required for the sample collection were prepared the day before the experiment. The samples were collected in 5µl of perichloric acid. The microdialysis probe (Figure 20(A)) was also made ready by flushing it with water in order to make sure that the tubes were clean before the experiment.

A



B

Figure 20: A) Microdialysis Probe B) Pump used for microdialysis

On the day of the experiment, a 1ml syringe was filled with the aCSF and loaded into the pump (Figure 20-B). Flow rate and volume were set to 1.2µl/min and 700µl, respectively. Also, a timer and video recorder were started. The parameters required for stimulation during the experiment were set at frequency 124Hz, current 0.5mA, pulse width 60µs and pulse interval 8ms. The TDT system used for recording was switched on and kept ready.

The animals were shortly anaesthetised using isoflurane. Only then the microdialysis probe and the electrode were inserted. The animals were put in the bowl test cage (*TSE Systems, Bad Homburg, Germany*) which was supplied with straw and some food. After a stabilization period of 2 h, 10 consecutive dialysis samples were collected in 5 µl 3.3% perchloric acid with a sampling period of 20 min each. Exactly eighty minutes after stabilization period, HFS was applied for 40 min (Figure 21).

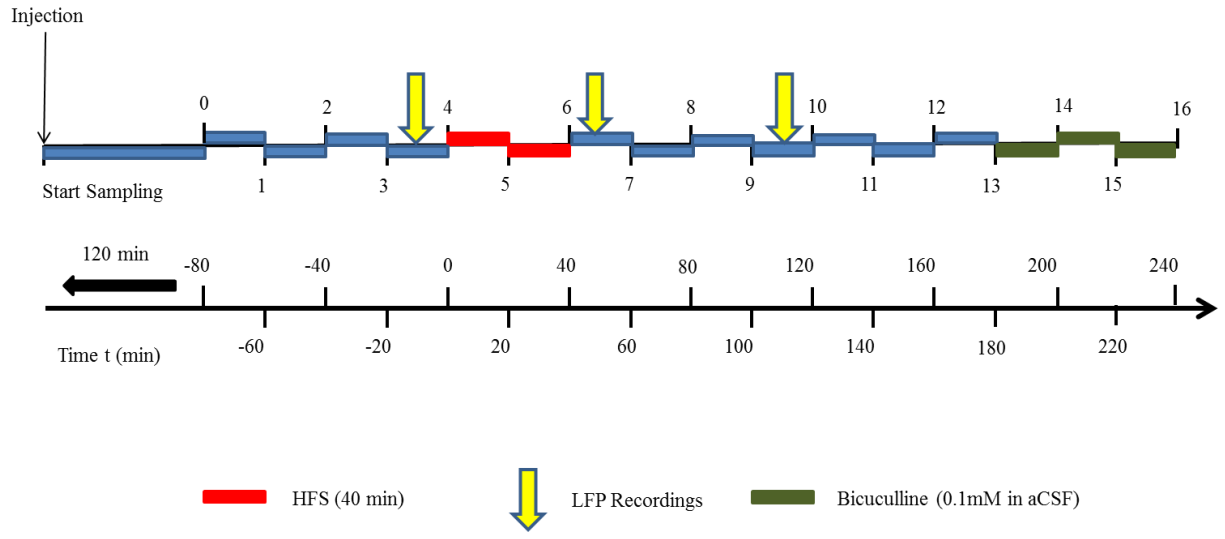


Figure 21: Experimental protocol.

The electrophysiological recordings were done before, after and one hour after stimulation respectively. Until the measurement of neurotransmitter concentration by means of HPLC the samples were stored at -20° C.

After the experiments, rats were deeply anesthetised and decapitated and the brains were removed. The brains were washed using wash buffer (Fixation solution without Formalin-see 2.1.6. in Materials) and then kept in fixation solution until histology.

2.2.8 High Pressure Liquid Chromatography (HPLC)

High pressure liquid chromatography was used to determine the concentration of neurotransmitters present in the samples collected by microdialysis. Chromatography is a separations method that relies on differences in partitioning behaviour between a flowing mobile phase and a stationary phase to separate the components in a mixture. Liquid chromatography (LC) is an analytical chromatographic technique that is useful for separating ions or molecules that are dissolved in a solvent.

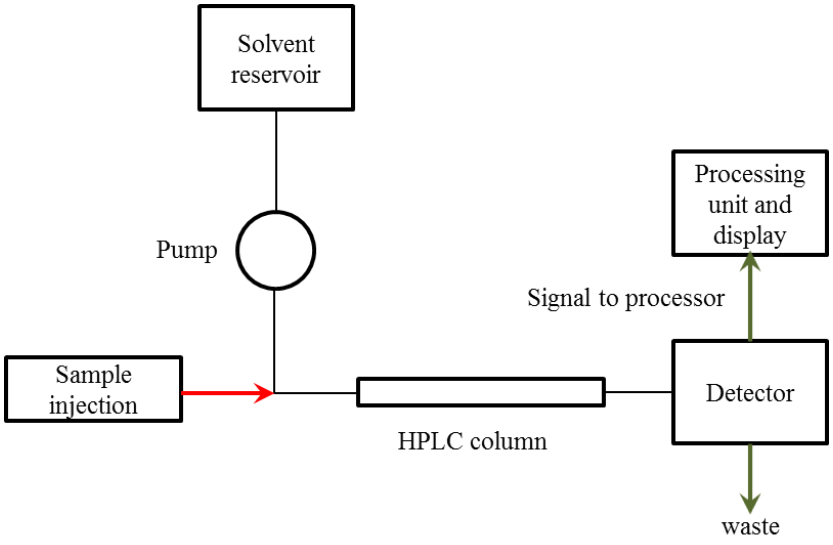


Figure 22: Flow scheme of HPLC

HPLC is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector (Figure 22). Compounds are separated by injecting the sample mixture onto the column. The different components in the mixture pass through the column at different rates due to differences in their partitioning behaviour between the mobile (liquid) phase and the stationary phase. Electrochemical detectors, which are based on the electrochemical oxidation or reduction of the analyte, can be applied to the analysis of selected compounds [http://www.chromatography-online.org]. The electrochemical detector requires three electrodes, the working electrode (where the oxidation or reduction takes place), the auxiliary electrode (which together with working electrode provides a circuit over which either current is measured or applied) and the reference electrode (which compensates for any changes in the background conductivity of the mobile phase) (Hanai, 1999). In the oxidation process, a current depending on the redox potential of the molecule is discharged and measured. The measured current is converted into potential corresponding on the used sensitivity. This potential is plotted against the retention time resulting in the chromatogram results. The measurement of the dialysate samples using HPLC is explained below.

2.2.8.1 Preparation and derivatisation of samples for HPLC

The electrochemical detection (ECD) of the microdialysis samples gives only the potentials. In order to determine the concentration and retention time of the substances of interest standards solution of each amino acids and catecholamine with known concentrations (0.1µM, 0.25µM and 0.5µM) were prepared. These standard solutions were first measured in the HPLC before the actual sample measurement in order to define the potential and retention time of the amino acids

and catecholamines. A stock solution of 0.01M was prepared for all the amino acids and catecholamines. From these solutions dilutions of 1:100 and 1:10000 were made to obtain concentrations of 0.1mM and 1.0µM, respectively. From these dilutions the standards were made as shown in Table 2.

Table 2: *Preparation of standards for HPLC*

Standard c [µM]	**Stock solution**		**HPLC Water [µl]**
	c [µM]	**V [µl]**	
0.1	1.0	100	900
0.25	1.0	250	750
0.5	100	5	995

c –concentration [µM], V – volume [µl]

Table 3: Preparation of standard pools

	Amino Acids			**Catecholamines**		
Pools c [µM]	**Stock Solution**		**HPLC Water**	**Stock Solution**		**HPLC Water**
	c [µM]	**V [µl]**	**V [µl]**	**c[µM]**	**V [µl]**	**V [µl]**
0.1	1.0	100	700	1.0	100	300
0.25	1.0	250	250	100	2.5	983
0.5	100	5	975	100	5	965

c –concentration [µM], V – volume [µl]

Similarly, for the measurement of every experiment, standard pools containing all the substances were made with concentrations of 0.1µM, 0.25µM and 0.5µM respectively as described in the below table (Table 3). The standard pools were used as a control to get the concentrations of each samples measured in the HPLC system.

Two separate HPLC systems (System A and System B) were used for the sample measurement (see 2.1.4 in Materials section). System A was used for the measurement of catecholamines: dopamine, serotonin, DOPAC, HVA, and HIAA; the System B was used for the amino acids: glutamate and GABA. Both systems contained an injection valve, a column heater, a pre and a main column, a pump and an electrochemical detector.

Around 50 µl diluted sample was injected into the appropriate HPLC system with a 100 µl injection syringe via the injection valve. Since the system uses only 20µl of the injected sample for analysis the remaining liquid was disposed as waste. The columns were constantly warmed to a temperature of 30°C by the column heaters. The sample was pumped via the columns with approximately 17 MPa pressure at system A and approximately 22 MPa pressure at system B. The Electrochemical detector (ECD) determined the quantities of the substances. ECD measures easily reducible or oxidizable substances. The ECD measures in terms of potentials. The measured potential is plotted against the retention time resulting in a chromatogram.

The Chromsystems CLC 100 detector (System A) and Waters 460 Electrochemical Detector (System B) , both contain a glassy carbon electrode against an Ag/AgCl reference electrode with an applied oxidizing potential of 0.9 mV. Catecholeamines are easily oxidizable whereas amino acids do not oxidize so easily. Therefore, amino acids were pretreated for the measurement. Pre-column derivatisation with o-phthaldialdehyde (OPA) is a simple means to get detectible amino

acids. In addition, OPA makes the amino acids more hydrophobic and therefore better separable. For the derivatisation of the samples, the OPA-sulfite method was chosen with sodium sulfite (Na_2SO_3) as reducing agent.

The derivatisation solution was composed of OPA, Na_2SO_3, ethanol (absolute) and disodium tetraborate with the concentrations listed in section 2.1.6 in Materials. From Na_2SO_3 and disodium tetraborate, 1M and 0.1M stock solutions were prepared with HPLC water. The tetraborate buffer should have a pH of 10.4, which was adjusted using 5 M NaOH. The stock solutions can be stored about one month at room temperature - (tetraborate buffer) and at 4° C (Na_2SO_3), respectively. However, the derivatisation solution were made freshly every day and stored at 4° C in darkness because OPA is sensitive to UV illumination and oxidation. For the solution, 0.0022 g OPA was dissolved in 50 µl 1M Sodium sulfite. After addition of 50 µl absolute ethanol a precipitate became visible. Then, 900 µl 0.1M tetraborate buffer was added and the solution was mixed using the vortexer to dissolve the precipitate. For the pre-column derivatisation, 1 µl derivatisation solution was added to 50µl diluted sample and mixed. After 15 min incubation at room temperature, the sample or standard was analyzed in the HPLC.

In case of catecholamines, samples were diluted with HPLC water to get a final volume of 30 µl. Amount of HPLC water added was depending on the original sample volume available, which differed slightly because of interferences during the microdialysis.

2.2.9 Behavior Analysis

Behavioral changes of freely moving rats were assessed before, during and after high frequency stimulation. Behavioral scoring was done using the videos recorded during the experiment.

Different behavioral patterns were documented as previously described by various authors (Andersen et al., 1987; Kelley and Lang, 1989; Westerink et al., 1987). The behavioral activity was classified in three categories of arbitrary units scored from 0 to 2 (0=absent,1=moderate,2=marked).The following criteria were used : levels of locomotion(A), climbing behavior(B), head bobbing(C), grooming(D) , sniffing over a large area of the cage (E). An activity score of 0 meant that the rat was behaviorally active for less than 1 min of the 20 min period; a score of 1 indicated activity of more than 1 min but less than 10 min and a score of 2 indicated activity for more than 10 min. Each animal was scored individually and the means of each group was calculated.

2.2.10 Histology

The removed brains stored in 4% formalin for fixation were used for further histological analysis. Histological examinations of these brains were done to ensure the correct positioning of the microdialysis probe and stimulation electrode. The brains were embedded in paraffin and coronal sections of 16 µm thickness were made. These sections were stained with hematoxylin and eosin and examined under a light microscope. No significant damage to the neuronal tissue or edema around the tip of the electrode was detected in any rat.

2.2.11 LFP recordings and analysis

The chronic recordings were performed 3 days after the surgery together with the microdialysis experiments (section 2.2.7 and Figure 21) in order to make sure that the animals were fully recovered from the surgery. On the day of the experiment, concentric bipolar electrode used for both stimulation and recording was inserted through the implanted guide cannula into the

nucleus accumbens. For LFP recordings an RZ5 BioAmp Processor (Tucker Davis Technologies) was used. Two battery powered 16-channel Medusa pre amplifiers (RA16PA, Tucker Davis Technologies) were used. The pre amplifiers provide 24.414 kHz maximum sampling rate. HFS was applied at 124 Hz with standard parameters current 0.5 mA, and pulse width 60 µs. The sham group had just the electrodes without any stimulation. HFS was applied for 40 min and LFPs were recorded for 1 min at a number of different time points: pre-HFS baseline, immediately after HFS OFF and 1 hr after the stimulation was switched off. There were no recordings during the stimulation period. These recordings were analyzed using MATLAB and the power spectrum was drawn using 'gnuplot'.

2.2.12 Statistics

The peak of the neurotransmitters in mV were obtained from the chromatogram and entered in Excel sheet. From these values the concentration of the substances present in each sample was calculated using the following formula. The dilution of the sample and the addition of 5µl of perichloric acid were also considered during the calculation of the concentration.

$$Cs = \frac{\mathrm{PHs} * Cstd * DF}{PHstd}$$

Cs – Concentration of the sample

Cstd-Concentration of the standard

PHs-Peak height of sample

PHstd-Peak height of standard

DF-Dilution Factor

$$DF = DF_{HPLC} * DF_{PCA}$$

DF_{HPLC} = 30/15 or 50/10

DF_{PCA} = 29/24

The average of baseline samples (Basal samples: -80 to 0 min – 4 samples) preceding the HFS was defined as 100% of basal neurotransmitter outflow. The samples from 0 to 60 min (HFS samples– 3 samples) and 60 to 120 min (Post HFS samples – 3 samples) were considered for the HFS and post HFS data (Figure 21). Stimulation induced outflow values are expressed as percentage of the basal outflow (100%).Significant differences in neurotransmitters induced by HFS and differences between groups were analyzed using one-way analysis of variance with follow up t-test for pairwise comparisons. Similarly for the behavior analysis, the difference between means and control groups were tested with one-way analysis of variance and follow up t-test were performed.

3 Results

3.1 Electrode and probe localization

The implantation of the double guide cannula proved to be a technical challenge as the nucleus accumbens is a small region in the rat brain. In order to confirm the correct positioning of the electrode and the probe histology was performed on the removed brain slices as mentioned in section 2.2.10. Histological verification was also necessary to detect any potential tissue damage induced by the guide cannula implantation and probe insertion. The brain slices showed that there was no significant tissue damage or hemorrhage induced by the implantation or probe insertion (Figure 23b).

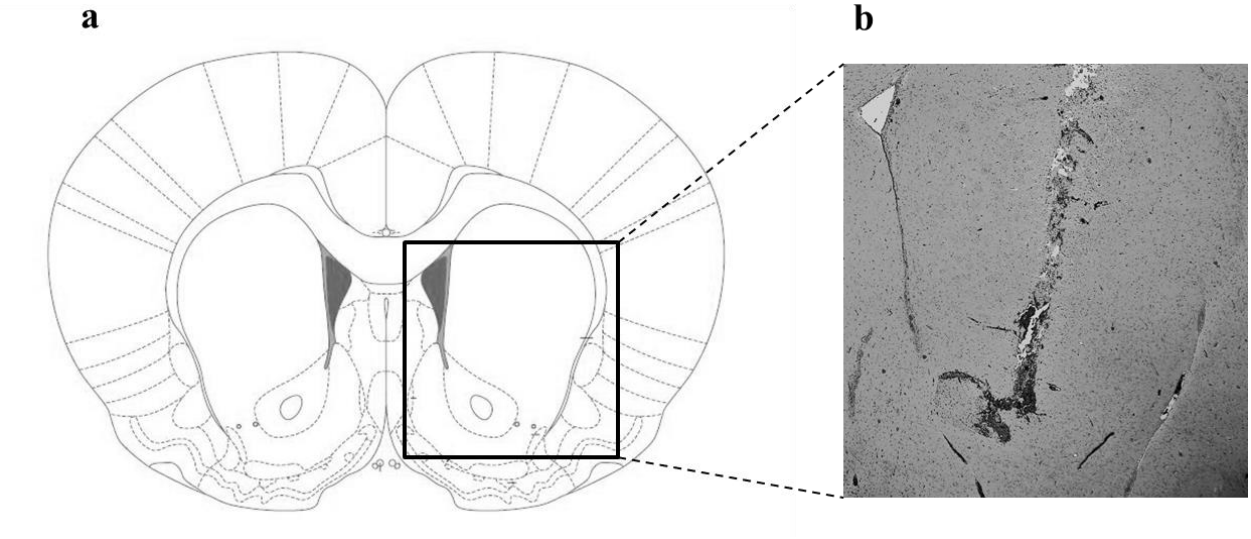


Figure 23. Coronal section of rat brain showing the probe placement in the nucleus accumbens. Figure adapted from Paxinos and Watson rat atlas (2007).

Only animals in which the electrode and microdialysis probe tips were found within the nucleus accumbens were considered for the final data analysis. Similarly animals that had any kind of tissue damage or hemorrhage were not included for the data analysis.

3.2 Effects of HFS on neurotransmitters in the NAc

In these experiments, the effects of high frequency stimulation on basal neurotransmitter outflow were studied. In the HFS group (n=11), high frequency stimulation of 124Hz was performed for 40 minutes (Figure 21). The corresponding control group (n=9) did not have any HFS in order to observe the changes in the basal neurotransmitter levels without any stimulation.

Prior to the start of HFS, there was no significant difference between the basal neurotransmitter levels in both the HFS and control group (Table 4).

Table 4: Mean basal levels (mean ± SEM) in both control (without HFS) and HFS group expressed in nM in dialysate from the accumbens nucleus of freely moving rats.

Dialysate	Without HFS	HFS
GABA	39.56±7.01	45.35±3.9
Glutamate	8514±392.9	8203±122.8
Dopamine	36.63 ±17.23	40.85 ±7.96
DOPAC	80.32 ± 24.17	84.48 ±9.64
Serotonin	5.27± 1.57	6.22± 0.48
HIAA	22.30± 3.74	27.48± 2.37
HVA	55.31± 10.82	58.42± 3.51

High inter- individual variability was observed in the neurotransmitter levels in both the control and the HFS group. In order to compare between groups, the neurotransmitter values were normalized in relationship to the individual basal values prior to stimulation defined as 100%. In HFS group, GABA outflow increased significantly during HFS whereas in the control group without HFS the GABA levels remained unchanged (Figure 24). The GABA levels remained high (123%) even after stimulation but when 0.1mM bicuculline ($GABA_A$ receptor antagonist)

was added in the perfusate later during the final minutes (180 – 240 min) of the experiment, the levels decreased to 71%**.**

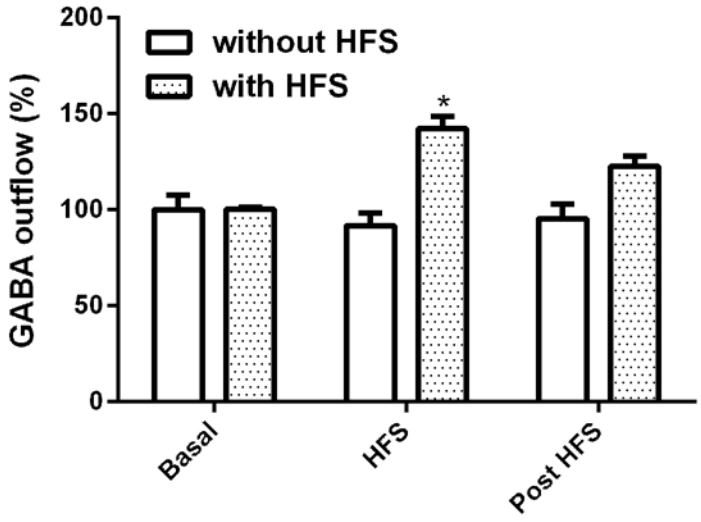


*Figure 24.GABA outflow was measured in dialysate from the accumbens nucleus of freely moving rats by HPLC with electrochemical detection. Data are mean ± SEM expressed in percent of basal values (=100%).*p<0.001 compared to control group without HFS.*

The glutamate outflow was not affected in both the HFS group and the control group (Figure 25). Similarly, dopamine (DA), Serotonin (5-HT) and their metabolites were unaffected by the HFS (Table 5).

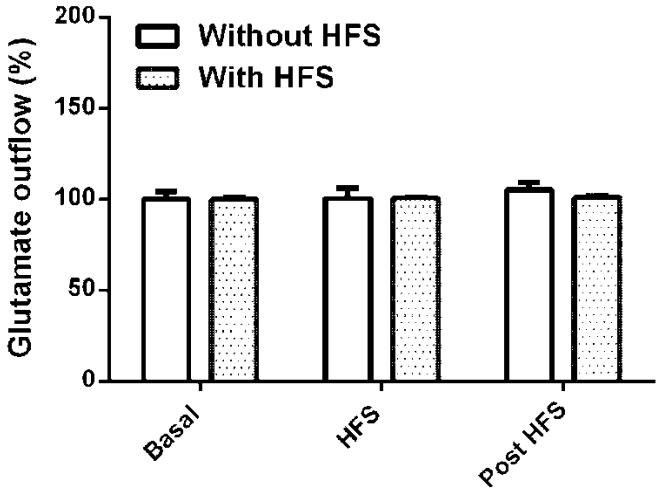


Figure 25.Glutamate outflow was measured in dialysate from the accumbens nucleus of freely moving rats by HPLC with electrochemical detection. Data are mean ± SEM expressed in percent of basal values (=100%).

*Table 5. Data are mean ± SEM expressed in percent of basal values (=100%) in dialysate from the nucleus accumbens of freely moving rats. Neurotransmitters and their metabolite outflow in animals with HFS (n=11) and control group without HFS (n=9). *p<0.04 compared to control group without HFS.*

Dialysate	Without HFS		With HFS	
	HFS (0 - 60 min)	Post HFS (60 -120 min)	HFS (0 - 60 min)	Post HFS (60 -120 min)
GABA (%)	91.6± 6.6	95.3±7.6	142.0±11.1*	123.6 ± 11.8*
Glutamate (%)	100.7± 5.4	105.3±4.3	100.7± 0.9	101.1± 1.2
DA (%)	94.9± 3.9	101.0±5.5	100.6± 1.1	99.9± 1.1
DOPAC (%)	104.5± 3.2	101.5±6.1	100.3± 1.0	100.9± 1.1
HVA (%)	106.1± 2.1	101.1±3.4	101.5± 0.6	99.9± 1.0
5-HT (%)	95.1± 5.4	106.0±6.1	101.8± 1.0	101.5± 1.0
HIAA (%)	105.2± 5.3	103.3±5.6	97.8± 0.8	97.7± 1.0

3.3 Effects of NMDA antagonist Memantine on basal neurotransmitter levels in the NAc

In the presence of NMDA antagonist memantine, the overall outflow of GABA significantly increased to 157% *(p-value < 0.0001)* compared to the control group with NaCl (saline) injection (here the values were normalized in relationship to the concentration values in control group as 100%). There was no significant change in the glutamate (Glu) outflow in between the groups *(p-value = 0.0567)* (Figure 26).

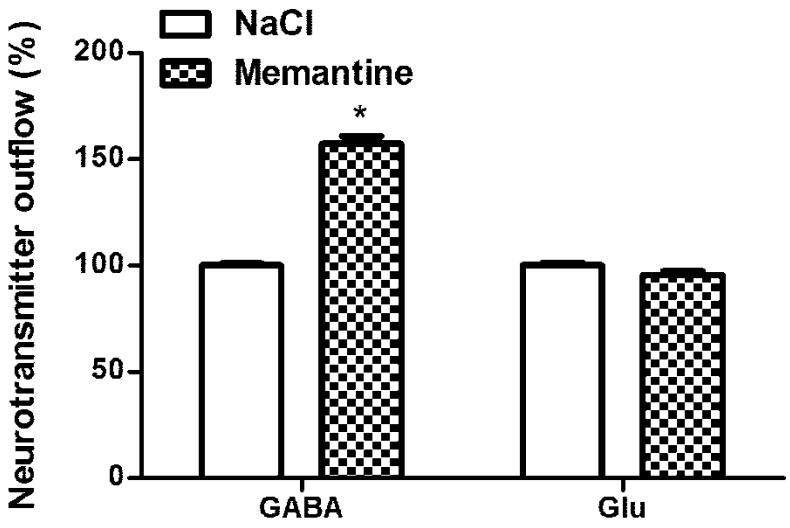


*Figure 26.GABA and Glu outflow in control (NaCl) group (n=7) and memantine group (n=7). Data are mean ± SEM expressed in percent of control (NaCl) group values (=100%).*p<0.0001 compared to control group (NaCl injection).*

When NMDA antagonist memantine was injected subcutaneously, it led to a significant increase in DA outflow to 146% *(p-value < 0.0001)* compared to the DA levels in the NaCl injected control group. 5-HT was not affected by the memantine injection (Figure 27).

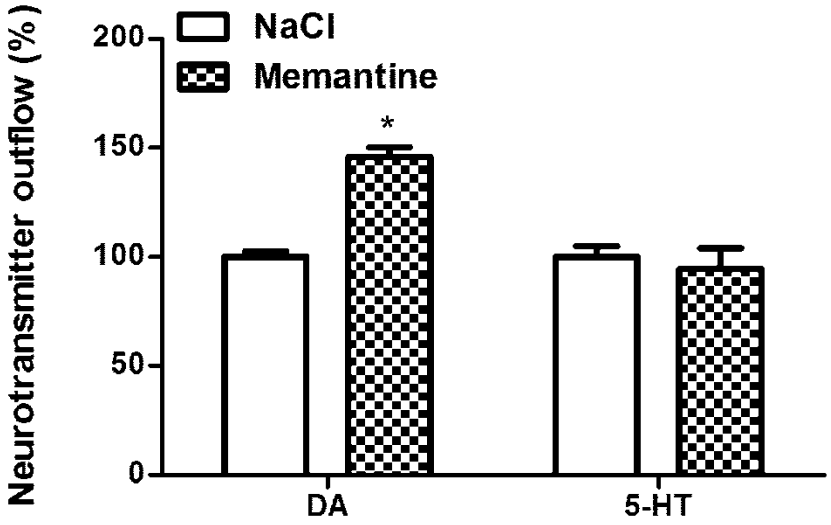


*Figure 27.DA and 5-HT outflow in control (NaCl) group (n=7) and memantine group. Data are mean ± SEM expressed in percent of control (NaCl) group values (=100%).*p<0.0001 compared to control group (NaCl injection).*

The metabolites DOPAC, HVA and HIAA were also not affected by memantine. There was no significant *(p-value> 0.05)* difference between the memantine group and the control group. The normalized values of DOPAC, HVA and HIAA were 108%, 96% and 92% compared to control group values of 100%.

3.4 Effects of NMDA antagonist Memantine on the HFS effect in the NAc

In order to study the role of NMDA receptors on the HFS induced neurotransmitter outflow, NMDA antagonist memantine was injected subcutaneously at the beginning of the microdialysis experiment. As shown in figure 25, memantine increased the basal levels of GABA. But the HFS induced increase in GABA was not found in animals injected with memantine, implying that the HFS effect was masked by the NMDA antagonist (Figure 28).

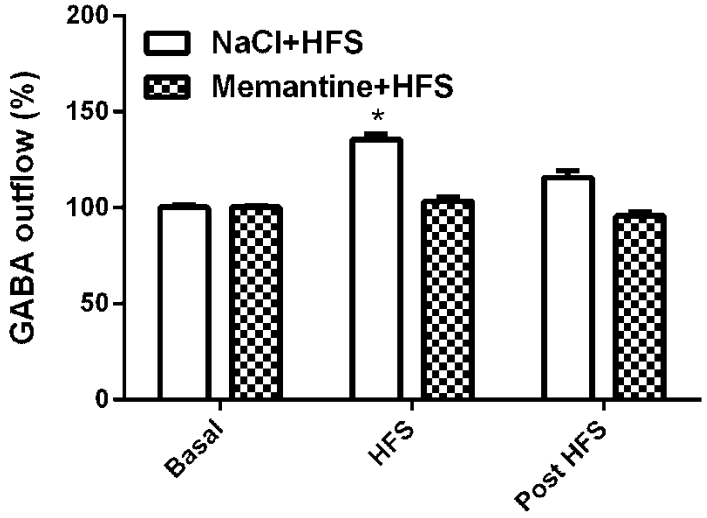


*Figure 28.GABA outflow in NaCl+HFS (n=7) and Memantine+HFS (n=7) groups. Data are mean ± SEM expressed in percent of basal values (=100%).*p<0.0001 compared to basal GABA levels.*

Glutamate levels were not affected by HFS in both the NaCl injected control group and the memantine group *(p-value >0.05)* (Figure 29).

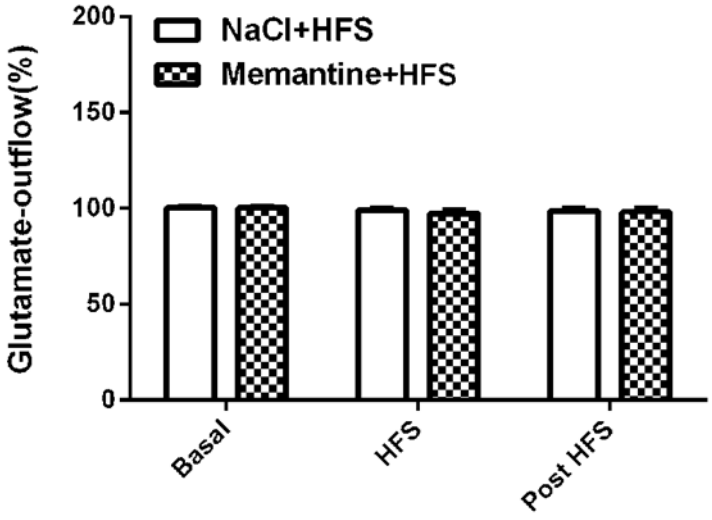


Figure 29.Glutamate outflow in NaCl+HFS (n=7) and Memantine+HFS (n=7) groups. Data are mean ± SEM expressed in percent of basal values (=100%).

*Table 6. Data are mean ± SEM expressed in percent of basal values (=100%) in dialysate from the nucleus accumbens of freely moving rats. DOPAC, HVA and HIAA outflow in animals with NaCl+HFS (n=7) and Memantine+HFS (n=7). *p<0.001 compared to basal values (=100%).*

Dialysate	NaCl + HFS		Memantine + HFS	
	HFS (0 - 60 min)	Post HFS (60 -120 min)	HFS (0 - 60 min)	Post HFS (60 -120 min)
DOPAC (%)	98.04± 1.09	99.53±1.91	86.88± 2.16*	82.95± 1.36*
HVA (%)	98.55± 1.27	98.83±1.27	98.37± 1.26	99.46± 1.21
HIAA (%)	99.46±1.61	100.4±1.24	97.53± 0.97	100.0± 1.26

Dopamine levels significantly decreased to 82% *(p-value < 0.001)* during stimulation in animals injected with memantine whereas stimulation did not have any effect on dopamine levels in control group animals injected with NaCl (Figure 30). Similarly, levels of DOPAC also decreased significantly to 86% *(p-value < 0.001)* during stimulation in memantine injected animals (Table 6) whereas HVA was not affected by stimulation. Serotonin and its metabolite HIAA were not affected by stimulation in both the groups (Figure 31) (Table 6).

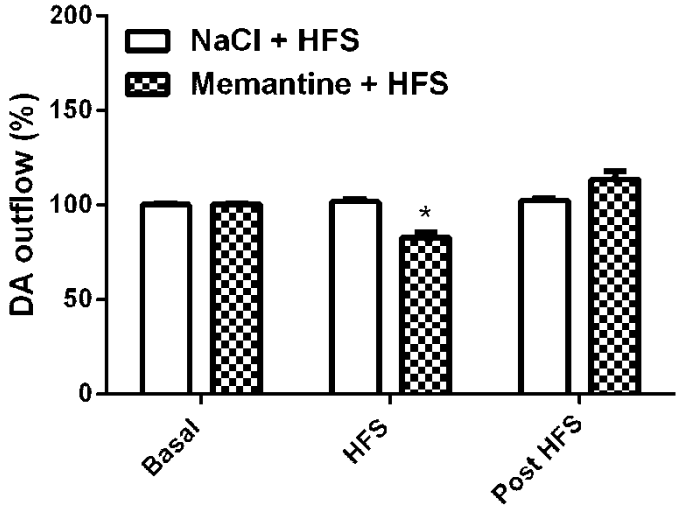


*Figure 30.DA outflow in NaCl+HFS (n=7) and Memantine+HFS (n=7) groups. Data are mean ± SEM expressed in percent of basal values (=100%).*p<0.0001 compared to basal DA levels.*

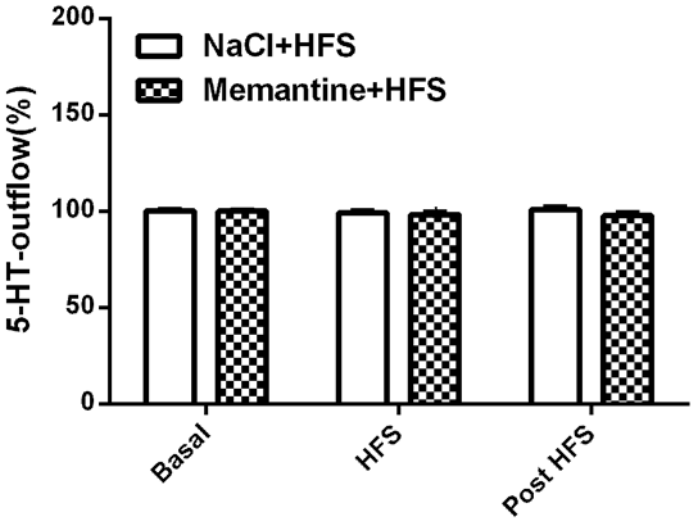


Figure 31. 5-HT outflow in NaCl+HFS (n=7) and Memantine+HFS (n=7) groups. Data are mean ± SEM expressed in percent of basal values (=100%).

3.5 Effects of Quinpirole on basal neurotransmitter levels in the NAc

In the presence of DA receptor agonist quinpirole, the overall outflow of GABA significantly increased to 121% *(p-value < 0.0001)* compared to the control group with NaCl (saline) injection (here the values were normalized in relationship to the concentration values in control group as 100%). There was no significant change in the glutamate (Glu) outflow in between the groups *(p-value = 0.885)* (Figure 32).

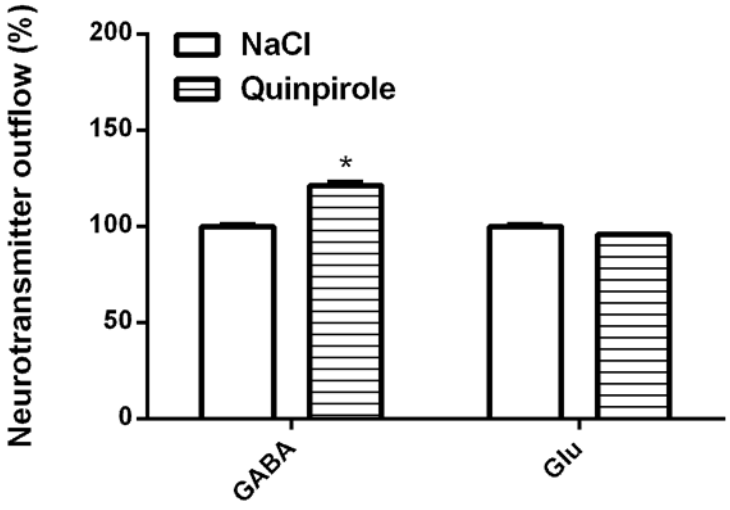


*Figure 32.GABA and Glu outflow in control (NaCl) group (n=7) and quinpirole group (n=7). Data are mean ± SEM expressed in percent of control (NaCl) group values (=100%).*p<0.0001 compared to control group (NaCl injection).*

When quinpirole was injected subcutaneously, it led to a significant increase in DA outflow to 134% *(p-value < 0.0001)* compared to the DA levels in the NaCl injected control group. In contrast to DA, its metabolites DOPAC and HVA were decreased significantly *(p-value < 0.0001)* in animals injected with quinpirole. The normalized values for DOPAC and HVA were

61% and 43% respectively. Serotonin (5-HT) and its metabolite HIAA were not affected by the quinpirole injection *(p-value = 0.73)* (Figure 33).

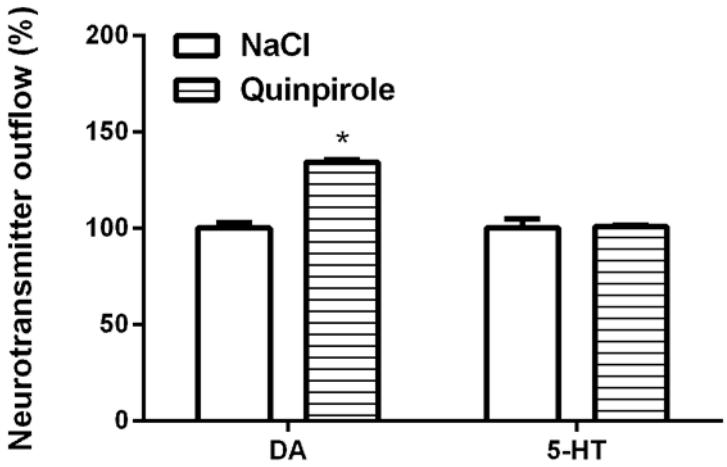


*Figure 33.DA and 5-HT outflow in control (NaCl) group (n=7) and quinpirole group(n=7). Data are mean ± SEM expressed in percent of control (NaCl) group values (=100%).*p<0.0001 compared to control group (NaCl injection).*

3.6 Effects of Quinpirole on the HFS effect in the NAc

In order to study the role of DA receptors on the HFS induced neurotransmitter outflow, dopamine receptor agonist quinpirole which induces compulsive checking behavior in rats was injected subcutaneously in a group of animals. HFS induced increase in GABA was also found in animals injected with quinpirole (Figure 34).

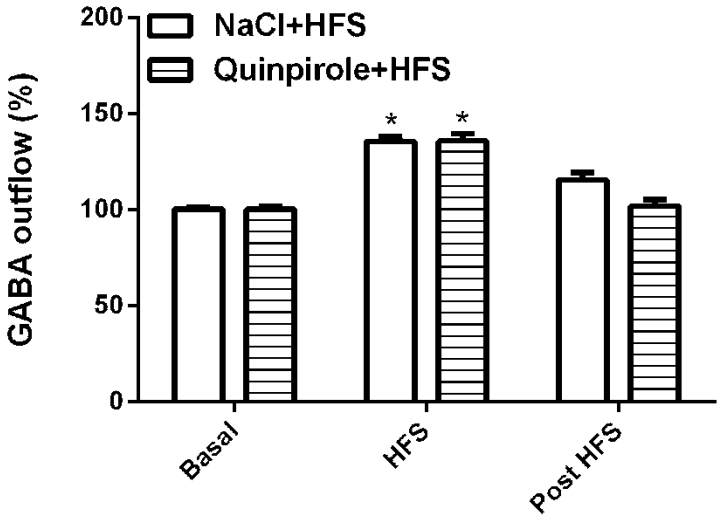


*Figure 34.GABA outflow in NaCl+HFS (n=7) and Quinpirole+HFS (n=7) groups. Data are mean ± SEM expressed in percent of basal values (=100%).*p<0.0001 compared to basal GABA levels.*

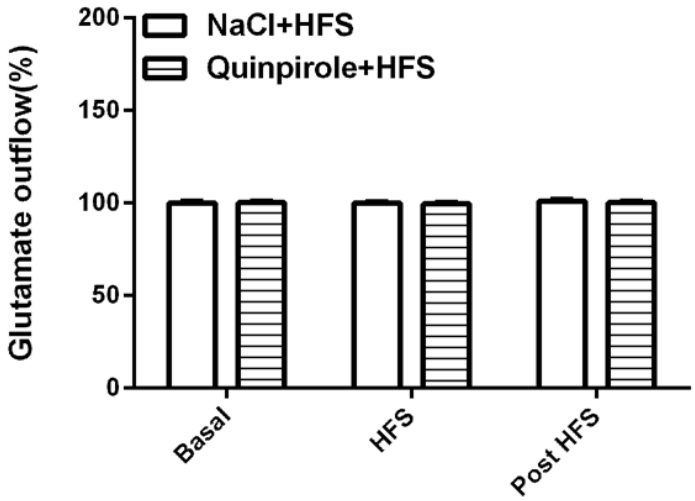


Figure 35.Glutamate outflow in NaCl+HFS (n=7) and Quinpirole+HFS (n=7) groups. Data are mean ± SEM expressed in percent of basal values (=100%).

Glutamate levels were not affected by high frequency stimulation in both the NaCl injected control group and the quinpirole group *(p-value >0.05)* (Figure 35).

Dopamine levels significantly increased to 123% *(p-value < 0.001)* during stimulation in animals injected with quinpirole whereas stimulation did not have any effect on DA levels in control group animals injected with NaCl (Figure 36). Levels of DOPAC and HVA were not affected by stimulation in both the groups (Table 7). Serotonin and its metabolite HIAA was not affected by stimulation in both the groups (Figure 37) (Table 7).

Table 7. Data are mean ± SEM expressed in percent of basal values (=100%) in dialysate from the nucleus accumbens of freely moving rats. DOPAC, HVA and HIAA outflow in animals with NaCl+HFS (n=7) and Quinpirole+HFS (n=7).

Dialysate	NaCl + HFS		Quinpirole + HFS	
	HFS (0 - 60 min)	Post HFS (60 -120 min)	HFS (0 - 60 min)	Post HFS (60 -120 min)
DOPAC (%)	99.04± 1.19	98.53±1.01	100.8± 0.86	99.43± 0.75
HVA (%)	99.55± 0.97	99.83±0.27	99.73± 0.62	99.18± 0.68
HIAA (%)	99.32±1.24	101.4±1.21	99.42± 0.44	100.1± 0.99

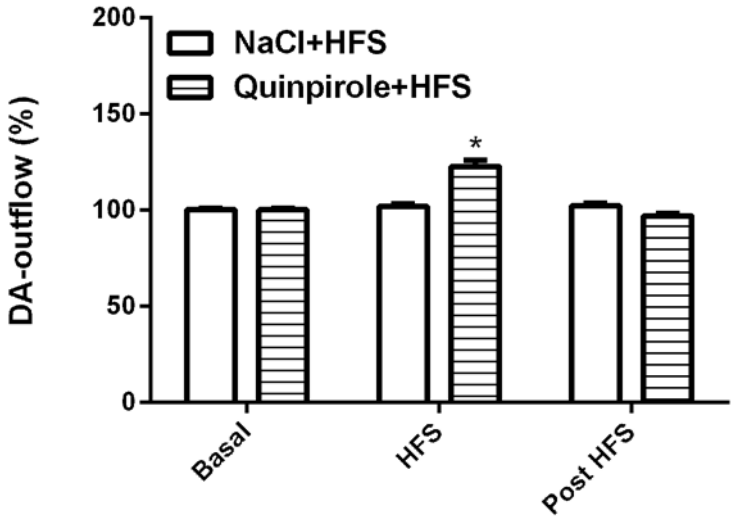


*Figure 36.DA outflow in NaCl+HFS (n=7) and Quinpirole+HFS (n=7) groups. Data are mean ± SEM expressed in percent of basal values (=100%).*p<0.001 compared to basal DA levels.*

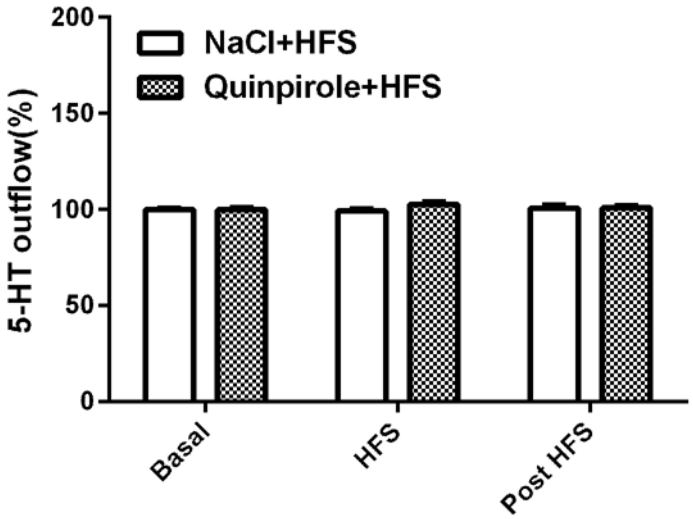


Figure 37.5-HT outflow in NaCl+HFS (n=7) and Quinpirole+HFS (n=7) groups. Data are mean ± SEM expressed in percent of basal values (=100%).

3.7 Effect of HFS on Behavior of the animals

The behaviors of the rats were assessed using the videos recorded before, during and after high frequency stimulation in all the groups. There was no significant difference between the mean behavioral score in the animals with HFS (n=10) and the control group without HFS (n=7) (Table 8).

Table 8. Behavioral scores (mean ± SEM) in both HFS group and control group without HFS.

Group	Behavioral Score
HFS	1.250 ± 0.3354
Control	1.692 ± 0.2371

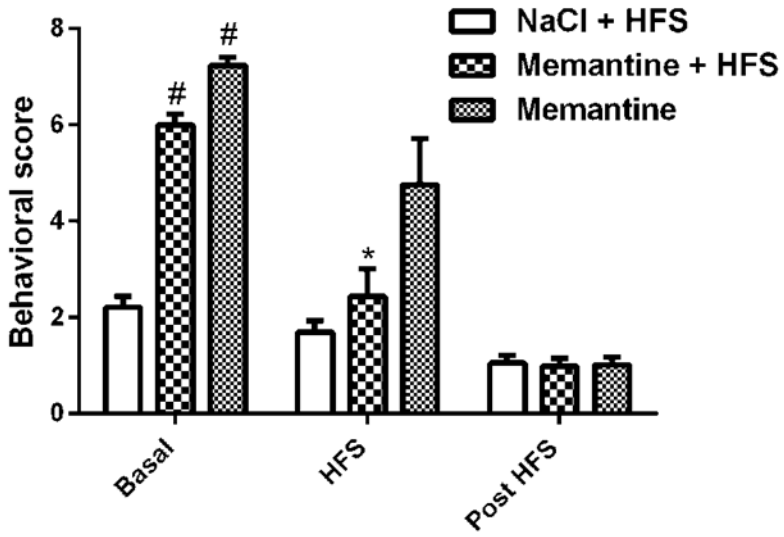


*Figure 38. Behavioral scores (mean ± SEM) in control (NaCl+ HFS) (n=7), Memantine+ HFS (n=7) and Memantine (n=7) groups. # p <0.0001 compared to control group (NaCl+ HFS) and *p<0.0001 compared to basal values.*

In the animals injected with NMDA antagonist memantine the overall activity increased significantly compared to the animals with NaCl injection *(p-value < 0.0001)* (Figure 38). The overall increase in activity was significantly decreased during HFS in the memantine injected group *(p-value < 0.0001)* (Figure 38).

In the animals injected with dopamine receptor agonist quinpirole the overall activity increased significantly compared to the animals with NaCl injection *(p-value < 0.0001)* (Figure 39). The activity was not significantly affected during HFS in the quinpirole injected group but the mean behavioral score was significantly decreased during the time period after stimulation compared to the basal values before stimulation (*p-value < 0.0001)* whereas in the quinpirole group without HFS the mean behavioral score was not affected during the same time period (Figure 39).

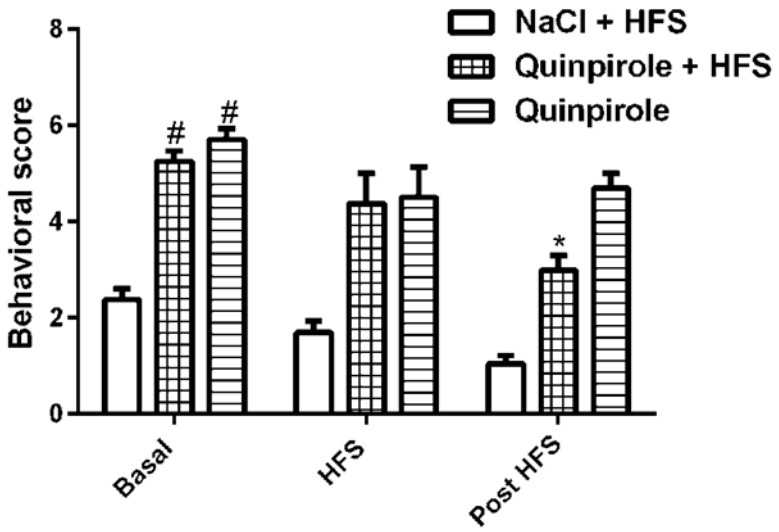


*Figure 39. Behavioral scores (mean ± SEM) in control (NaCl+ HFS) (n=7), Quinpirole+ HFS (n=7) and Quinpirole (n=7) groups. # p <0.0001 compared to control group (NaCl+ HFS) and *p<0.0001 compared to basal values.*

3.8 Effects of HFS on LFPs

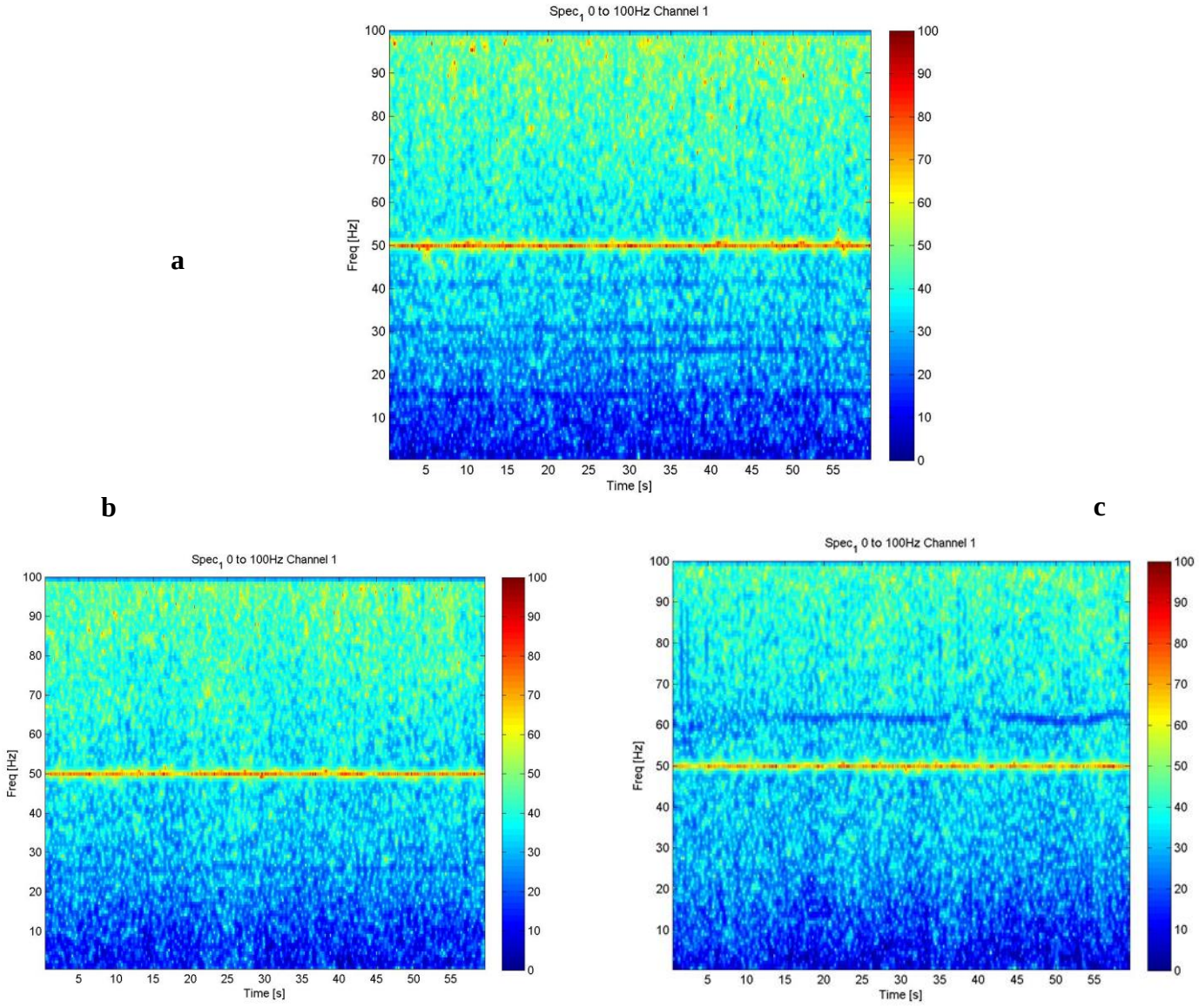


Figure 40. Spectrograms of electrophysiological recordings of local field potentials (LFPs) in the nucleus accumbens region. A) Recordings from -20-0 min – baseline before HFS. B) Recordings from 40-60 min – after stimulation. C) Recordings from 100-120 min – 1 hour after stimulation. The X-axis is Frequency in hertz (Hz) and Y-axis is Time in seconds. Color bar represents the energy levels in the spectrogram- red for high energy, yellow for medium energy, light blue for low energy, and dark blue for very low energy.

The low frequency range of 0-100Hz was filtered using MATLAB in the recorded signals in order to see any changes induced by the HFS. The 50Hz power line noise was filtered from all the signals. Here individual spectrograms of 1 min recordings of baseline, after HFS and 1 hour after HFS from one of the rats are shown. No possible changes induced by HFS can be seen in the spectrograms (Spectrogram of all the time points together in Appendix).

4 Discussion

Deep brain stimulation (DBS) has been an effective treatment method for several movement disorders like Parkinson disease and psychiatric disorders. However, there are several discussions about the mechanism underlying the beneficial effects of DBS. Many investigators have been studying the mechanism of DBS in several ways. Different studies about the effect of DBS on the network and how it disrupts the pathological activity has been made. Electrophysiological studies in the DBS target region and their ultimate effect on the postsynaptic neurons were done by many research groups (Dostrovsky et al., 2000; Hashimoto et al., 2003). Some of the other approaches were the measurement of neurotransmitters in the stimulated regions and their target structures by means of in vivo microdialysis techniques (Benabid et al., 2002; Windels et al., 2005).

Target selection and probe localization

The present study investigates the effect of high frequency stimulation (HFS) on nucleus accumbens (NAc). The NAc together with the olfactory tubercle and the ventral caudate putamen (CPU) is a functional unit, called ventral striatum, which acts as a limbic-motor interface (Cardinal et al., 2002; Joseph et al., 2003; Mogenson et al., 1980). GABAergic medium spiny neurons are the principal neurons of NAc. Major inhibitory GABAergic projections from the NAc are towards the ventral tegmental area (VTA) and the lateral hypothalamus (LH) (Russo and Nestler, 2013). It receives glutamatergic input from different cortical areas, mainly the medial prefontal cortex (mPFC) but also the amygdala, the ventral hippocampus and the LH (Pennartz et al., 1994). The major dopaminergic input to the NAc is received from the ventral tegmental area (Russo and Nestler, 2013). The NAc contains a larger proportion of cells with

high concentrations of D_1- and D_3-receptors and a distribution of many neuropeptides like VIP, CCK, enkephalins, substance P, neurotensin (Heimer, 2000). Thus, the nucleus accumbens attains, a central position between limbic as well as mesolimbic dopaminergic structures, basal ganglia, mediodorsal thalamus and prefrontal cortex (Sturm et al., 2003b). Thus, because of its central location and important role in the pathophysiology of OCD, NAc was chosen as the target in our study.

The results described in our study imply that simultaneous and collocated microdialysis and high frequency stimulation is possible in the nucleus accumbens of freely moving rats. The experimental setup is similar to the work described by Hiller et al. (2007) but, it is one of the first of its type to be done in the NAc region of the rat brain besides a recent paper by van Dijk et al. (2011) and Yan et al. (2013).

HFS increases GABA outflow in NAc

We observed that when HFS was applied for 40min, extracellular GABA levels increased in the NAc. Xie et al. (2014) showed that HFS induces a decrease in spontaneous neuronal activity in rat nucleus accumbens slices. Furthermore, the inhibitory effects were related to increased inhibitory neurotransmission via $GABA_B$ receptors, which is supported by our findings of HFS induced GABA release. Feuerstein et al. (2011) proposed that HFS selectively induces GABA release which was supported by several in vivo and in vitro experiments (Hiller et al., 2007; Li et al., 2006, 2004; Mantovani et al., 2006). Probably HFS interacts with the neuronal terminals of GABAergic medium spiny neurons which make up to 90-95% of the nucleus accumbens (Belleau and Warren, 2000), this is also in agreement with the increase in GABA release in our results. However, Yan et al. (2013) reported that HFS significantly increased GABA levels only

in rats pre-treated with morphine. The discrepancy between their results and our observation could have arisen from the longer experiment time (14 days) and the specific targeting of the nucleus accumbens core which was not the case in our work. In our experiments this GABA effect of HFS was specific and reversible and could be modulated by the $GABA_A$ receptor antagonist bicuculline. The presence of $GABA_A$ autoreceptors in the nucleus accumbens was already reported by Matsumoto (Matsumoto, 1989). The hypothesis of the involvement of GABA receptors in HFS effect can be supported by the results of Mantovani et al. (2009) and Xie et al. (2014) ; in experiments performed in human neocortical slices and rat brain slices respectively. HFS did not affect the glutamate levels in our present study. This result is comparable to the results published by Hiller et al. (2007). Similarly there was no significant change in the dopamine, serotonin and their metabolites levels due to HFS.

Memantine increases GABA and Dopamine levels

In a previous study in the rat caudate nucleus, we showed that the NMDA receptor agonist RS-tetrazol-5yl-glycine (RS-TG) increased GABA outflow and blocked the GABAergic activation induced by HFS (Varatharajan et al., 2014). In order to further investigate the role of NMDA receptors in the HFS effect, we used the NMDA receptor antagonist memantine in our present in vivo study. Administration of memantine led to an increase of overall basal GABA levels in the NAc. These apparent contradicting results, of both the NMDA agonist and antagonist increasing the basal GABA levels can be attributed to the difference in the regions in which the microdialysis was performed. In addition to the GABA increase, we observed that the overall basal DA levels also increased implying the involvement of NMDA receptors in the DA release. The increase in DA due to NMDA antagonist is in agreement with several other studies (Imperato et al., 1990; Mathé et al., 1999; Yoshida et al., 1998). Mathé et al. (1999) reported a

NMDA antagonist induced increase in DA which was related to the increase in dopamine neuronal activity. In addition to being a NMDA receptor antagonist, memantine is also a 5-HT_3 receptor antagonist (Rammes et al., 2008). But the 5-HT_3 receptor antagonism of memantine is not discussed in detail here as we did not see any significant change in the 5-HT levels induced by memantine.

HFS decreases DA levels in the presence of memantine

In the presence of memantine, HFS decreased DA, whereas in control groups with only HFS and saline administration, HFS did not affect the DA levels. Our results showing decrease of DA during HFS is in line with the results of Moser et al. (2003) , in which a HFS induced decrease of DA outflow was observed in rat striatal slices. However, the inhibitory effect of HFS could not be seen in the control group, which could be due to the lower basal levels of DA. Also, the HFS effect of increased GABA outflow was not observed in the memantine group with HFS, which may be explained by the increase in overall basal GABA levels induced by memantine, which in turn masked the increase induced by the HFS. These results show the involvement of NMDA receptors in the HFS effect.

Memantine and HFS effect on behavior

In the present study, HFS did not have any specific effect on the behavior of animals as there was no significant difference between the behavioral scores in the control group and HFS group. But when NMDA receptor antagonist memantine was injected subcutaneously, there was an increase in the overall activity of the animals leading to the significant difference between the behavioral score of sham injected and memantine injected animals. The behavioral scores can be related to neurotransmitter levels, as the increased activity in memantine treated animals is most

likely caused by the increased DA levels, since direct DA injections in the nucleus accumbens have been demonstrated to increase locomotor activity (Tzschentke, 2001). This has also been shown by other studies, which have suggested the involvement of the dopaminergic system in the NMDA antagonist induced behavioral effects (Bubser et al., 1992; Ikeda et al., 2012). The HFS induced decrease in the behavior may be partially explained by the lower DA levels during HFS.

Quinpirole increases GABA and Dopamine levels

Quinpirole is a high-affinity agonist of D_2 and D_3 receptors (Levant et al., 1993; Rowlett et al., 1995). Also, it has been suggested that the behavioral changes induced by quinpirole in rats, is most reminiscent of many features seen in obsessive-compulsive disorder (OCD) (Szechtman et al., 1998). DA neurotransmission can be modulated by activity at the D_2 receptors by affecting the synthesis, release or uptake of dopamine in important parts of the brain like the VTA and the NAc. Therefore, in order to study the role of D_2 receptors in HFS effect quinpirole was used in the present study. It was also used because of its property to serve as a probable model for OCD. The current results showed that injecting quinpirole subcutaneously led to an increase in basal levels of GABA and DA. Several evidences have shown the localization, distribution and function of D_1 and D_2 receptors in the NAc (Allin et al., 1989; Bardo and Hammer, 1991; Shetreat et al., 1996; White and Wang, 1986). The GABAergic medium spiny neurons in the accumbens express both D_1 and D_2 receptors (Russo and Nestler, 2013). Thus, the increase in basal GABA levels induced by quinpirole D_2 receptor agonist can be explained by the vast expression of the DA receptors in the NAc. Similar to the accumbens, D_1 and D_2 receptors are also highly expressed in the VTA (Adell and Artigas, 2004; Bouthenet et al., 1987; Wamsley et al.). The major dopaminergic input to the NAc is from the ventral tegmental area (VTA)

(Shirayama and Chaki, 2006). Therefore, the increase in basal DA levels can be attributed to the agonist's activity on the DA receptors in VTA. The quinpirole induced increase in DA is in line with the results published by Rahman et al. (2001) and Sullivan et al. (1998). In contrast to DA, its metabolites were significantly decreased in the animals injected with quinpirole compared to the control group with saline injection. Thus, the increase in DA levels can also be attributed to the reduction in the metabolism.

HFS increases both GABA and DA in the presence of quinpirole

HFS in quinpirole injected rats led to an increase in GABA outflow similar to the effect seen in the control group with NaCl injection. This result implies that the HFS effect on the GABAergic neurons is not affected by the D_2 receptor agonist quinpirole. Though quinpirole injection resulted in significant increase in the basal GABA levels, the HFS effect on GABA levels was not affected by this increase. This result is in contrast to the above result concerning the NMDA receptor antagonist in which the HFS effect was masked by the increased basal GABA levels. This difference can be explained by the amount of increase in the basal GABA outflow in both groups. In case of NMDA receptor antagonist - memantine injected rats, the basal GABA levels were around 157% compared the 100% in NaCl injected control group whereas in the quinpirole injected group, the basal GABA levels were 121% compared to the control group. Thus, this difference in basal GABA levels can explain the possible difference between the HFS effects in both groups.

DA levels were increased by HFS in the quinpirole injected animals. Several studies have reported the increase of dopamine induced by HFS in other regions of brain like mPFC, orbital prefrontal cortex and VTA (Sesia et al., 2010; Van Dijk et al., 2012; Winter et al., 2008a). In

these studies the stimulation targets were subthalamic nucleus (STN) and NAc. Winter et al. (2008a) have reported that the DA increase in the NAc induced by STN-HFS can be attributed to the reduced inhibitory GABAergic activity on the DAergic neurons in the VTA from which the accumbens receive majority of its DAergic input. But in the current study, we see an increase of GABA also which should have resulted in increased inhibitory activity on the VTA DAergic neurons. There are many conflicting results regarding HFS and DA release. Some studies have reported no effect on DA release due to HFS (van Dijk et al., 2011) while other studies have reported a decrease in DA (Moser et al., 2003). Thus, inorder to clearly explain the result of DA increase induced by HFS in the quinpirole injected animals further detailed studies with DA receptor antagonist is necessary.

Quinpirole and HFS effect on behavior

D_2 receptor agonist, quinpirole is known to induce compulsive checking behavior in rats (Szechtman et al., 1998; Winter et al., 2008b). Though we did not have any specific behavioral study designed for observing the compulsive checking behavior, we recorded videos of the overall activity of the rats injected subcutaneously with quinpirole. The analysis of these videos showed that the behivoral score increased significantly in rats injected with quinpirole compared to the NaCl injected control group. The increase in the overall activity can be attributed to the increase in dopamine levels which involves the role of D_2 receptor. Dreher and Jackson (1989) have shown that the depletion of catecholamines led to the blocking of the locomotor activity induced by quinpirole confirming the role of D_2 receptor in the quinpirole induced activity. Quinpirole induced compulsive checking behavior is one of the well established models of OCD. This model is widely used because of its good face validity and strong predictive validity (Albelda and Joel, 2012). Due to the above reason, we chose to study the effect of HFS in the

quinpirole injected animals. Although there was decrease in the overall activity during HFS in the quinpirole injected rats, this decrease was not significant. But after the stimulation the behavioral score decreased significantly compared to the basal score, this decrease was not seen in the group with only quinpirole injection and no HFS. This reduction in activity can be related to the results reported by Mundt et al. (2009) and Winter et al. (2008b). In their work, they showed that HFS of the NAc resulted in decrease of compulsive checking behavior of rats induced by quinpirole.

Local Field Potential (LFP) analysis

Previous studies have shown that HFS of subthalamic nucleus and globus pallidus reduced the overall activity of the targeted regions (Benazzouz et al., 2004; Filali et al., 2004; Meissner et al., 2005) . The reduction in activity has been attributed to the excitation of inhibitory afferents (Bacci et al., 2004; Benazzouz et al., 2004; Meissner et al., 2007), direct inhibition of targeted cell bodies or modulation of the efferent projections of the stimulated region (Benabid et al., 2005; McIntyre et al., 2004; Stefani et al., 2005). McCracken and Grace (2007) have shown that HFS supresses neuronal activity and modulates afferents in the rat orbitofrontal cortex. Similarly, Xie et al. (2014) showed that HFS supresses accumbens interneurons in acute rat brain slices. In the present study, LFPs were recorded before , after and one hour after stimulation. These data were analysed but there was no detectable differences induced by HFS on the LFPs. The reason that for no changes may be due to the problems in the overall experimental setup. Since the changes in neruonal activity lasts only a few micro seconds, the manual switch between the stimualtion and recording could be the reason for these changes being not detectable. Thus, further studies are required with a better experimental setup to study the actual changes induced

by HFS on the LFPs. These studies would give a better insight into the mechanism of action of HFS as it can be compared to the changes in the neurotransmitters induced by HFS.

In summary, we conclude that HFS in the nucleus accumbens primarily affects the GABAergic medium spiny neurons and NMDA and DA receptors might play a modulating role in the HFS effect. The results also imply that the HFS has a specific effect on GABA rather than an unspecific change. Richter *et al.* suggested that OCD is associated with deficient inhibition and excessive facilitation in the cortex (Richter et al., 2012). Since HFS increased the release of GABA in our work, it can be assumed that HFS may be used an effective treatment option for OCD.

5 References

Abelson, J.L., Curtis, G.C., Sagher, O., Albucher, R.C., Harrigan, M., Taylor, S.F., Martis, B., Giordani, B., 2005. Deep brain stimulation for refractory obsessive-compulsive disorder. Biol. Psychiatry 57, 510–516.

Adell, A., Artigas, F., 2004. The somatodendritic release of dopamine in the ventral tegmental area and its regulation by afferent transmitter systems. Neurosci. Biobehav. Rev. 28, 415–31.

Albelda, N., Joel, D., 2012. Animal models of obsessive-compulsive disorder: exploring pharmacology and neural substrates. Neurosci. Biobehav. Rev. 36, 47–63.

Alexander, G.E., DeLong, M.R., Strick, P.L., 1986. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu. Rev. Neurosci. 9, 357–381.

Allin, R., Russell, V., Lamm, M., Taljaard, J., 1989. Regional distribution of dopamine D1 and D2 receptors in the nucleus accumbens of the rat. Brain Res. 501, 389–391.

Andersen, P.H., Jansen, J.A., Nielsen, E.B., 1987. [3H]GBR 12935 binding in vivo in mouse brain: labelling of a piperazine acceptor site. Eur. J. Pharmacol. 144, 1–6.

Anderson, D., Ahmed, A., 2003. Treatment of patients with intractable obsessive-compulsive disorder with anterior capsular stimulation. Case report. J. Neurosurg. 98, 1104–1108.

Aouizerate, B., Cuny, E., Martin-Guehl, C., Guehl, D., Amieva, H., Benazzouz, A., Fabrigoule, C., Allard, M., Rougier, A., Bioulac, B., Tignol, J., Burbaud, P., 2004a. Deep brain

stimulation of the ventral caudate nucleus in the treatment of obsessive-compulsive disorder and major depression. Case report. J. Neurosurg. 101, 682–686.

Aouizerate, B., Guehl, D., Cuny, E., Rougier, A., Bioulac, B., Tignol, J., Burbaud, P., 2004b. Pathophysiology of obsessive-compulsive disorder: a necessary link between phenomenology, neuropsychology, imagery and physiology. Prog. Neurobiol. 72, 195–221.

Bacci, J.-J., Absi, E.H., Manrique, C., Baunez, C., Salin, P., Kerkerian-Le Goff, L., 2004. Differential effects of prolonged high frequency stimulation and of excitotoxic lesion of the subthalamic nucleus on dopamine denervation-induced cellular defects in the rat striatum and globus pallidus. Eur. J. Neurosci. 20, 3331–41.

Bais, M., Figee, M., Denys, D., 2014. Neuromodulation in obsessive-compulsive disorder. Psychiatr. Clin. North Am. 37, 393–413.

Bardo, M.T., Hammer, R.P., 1991. Autoradiographic localization of dopamine D1 and D2 receptors in rat nucleus accumbens: Resistance to differential rearing conditions. Neuroscience 45, 281–290.

Belitski, A., Gretton, A., Magri, C., Murayama, Y., Montemurro, M. a, Logothetis, N.K., Panzeri, S., 2008. Low-frequency local field potentials and spikes in primary visual cortex convey independent visual information. J. Neurosci. 28, 5696–5709.

Belleau, M.L., Warren, R.A., 2000. Postnatal development of electrophysiological properties of nucleus accumbens neurons. J. Neurophysiol. 84, 2204–2216.

Benabid, A., Pollak, P., Gervason, C., Hoffmann, D., Gao, D.M., Hommel, M., Perret, J.E., De Rougemont, J., 1991. Long-term suppression of tremor by chronic stimulation of the ventral intermediate thalamic nucleus. Lancet 337, 403–406.

Benabid, A.L., Benazzous, A., Pollak, P., 2002. Mechanisms of deep brain stimulation. Mov. Disord. 17, 73–4.

Benabid, A.L., Pollak, P., Louveau, A., Henry, S., de Rougemont, J., 1987. Combined (thalamotomy and stimulation) stereotactic surgery of the VIM thalamic nucleus for bilateral Parkinson disease. Appl. Neurophysiol. 50, 344–346.

Benabid, A.-L., Wallace, B., Mitrofanis, J., Xia, R., Piallat, B., Chabardes, S., Berger, F., 2005. A putative generalized model of the effects and mechanism of action of high frequency electrical stimulation of the central nervous system. Acta Neurol. Belg. 105, 149–57.

Benazzouz, A., Tai, C.H., Meissner, W., Bioulac, B., Bezard, E., Gross, C., 2004. High-frequency stimulation of both zona incerta and subthalamic nucleus induces a similar normalization of basal ganglia metabolic activity in experimental parkinsonism. FASEB J. 18, 528–30.

Björgvinsson, T., Hart, J., Heffelfinger, S., 2007. Obsessive-compulsive disorder: update on assessment and treatment. J. Psychiatr. Pract. 13, 362–372.

Bormann, J., Feigenspan, A., 1995. GABAC receptors. Trends Neurosci. 18, 515–9.

Bouthenet, M.L., Martres, M.P., Sales, N., Schwartz, J.C., 1987. A detailed mapping of dopamine D-2 receptors in rat central nervous system by autoradiography with [125I]iodosulpride. Neuroscience 20, 117–55.

Bubser, M., Keseberg, U., Notz, P.K., Schmidt, W.J., 1992. Differential behavioural and neurochemical effects of competitive and non-competitive NMDA receptor antagonists in rats. Eur. J. Pharmacol. 229, 75–82.

Butson, C.R., Maks, C.B., McIntyre, C.C., 2006. Sources and effects of electrode impedance during deep brain stimulation. Clin. Neurophysiol. 117, 447–454.

Cardinal, R.N., Parkinson, J.A., Hall, J., Everitt, B.J., 2002. Emotion and motivation: The role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci. Biobehav. Rev. 26, 321–352.

Chapman, D.L., 1913. A contribution to the theory of electrocapillarity. Philos. Mag. 25, 475–481.

Coric, V., Taskiran, S., Pittenger, C., Wasylink, S., Mathalon, D.H., Valentine, G., Saksa, J., Wu, Y.T., Gueorguieva, R., Sanacora, G., Malison, R.T., Krystal, J.H., 2005. Riluzole augmentation in treatment-resistant obsessive-compulsive disorder: An open-label trial. Biol. Psychiatry 58, 424–428.

De Lange, E.C.M., 1997. Methodological considerations of intracerebral microdialysis in pharmacokinetic studies on drug transport across the blood–brain barrier. Brain Res. Rev. 25, 27–49.

De Olmos, J.S., Heimer, L., 1999. The concepts of the ventral striatopallidal system and extended amygdala. Ann. N. Y. Acad. Sci. 877, 1–32.

Dold, M., Aigner, M., Lanzenberger, R., Kasper, S., 2013. Antipsychotic augmentation of serotonin reuptake inhibitors in treatment-resistant obsessive-compulsive disorder: a meta-analysis of double-blind, randomized, placebo-controlled trials. Int. J. Neuropsychopharmacol. 16, 557–74.

Dostrovsky, J.O., Levy, R., Wu, J.P., Hutchison, W.D., Tasker, R.R., Lozano, A.M., 2000. Microstimulation-induced inhibition of neuronal firing in human globus pallidus. J. Neurophysiol. 84, 570–574.

Dreher, J.K., Jackson, D.M., 1989. Role of D1 and D2 dopamine receptors in mediating locomotor activity elicited from the nucleus accumbens of rats. Brain Res. 487, 267–277.

El Mansari, M., Blier, P., 2006. Mechanisms of action of current and potential pharmacotherapies of obsessive-compulsive disorder. Prog. Neuro-Psychopharmacology Biol. Psychiatry 30, 362–373.

Feuerstein, T.J., Kammerer, M., Lücking, C.H., Moser, A., 2011. Selective GABA release as a mechanistic basis of high-frequency stimulation used for the treatment of neuropsychiatric diseases. Naunyn. Schmiedebergs. Arch. Pharmacol. 384, 1–20.

Filali, M., Hutchison, W.D., Palter, V.N., Lozano, A.M., Dostrovsky, J.O., 2004. Stimulation-induced inhibition of neuronal firing in human subthalamic nucleus. Exp. brain Res. 156, 274–81.

Fontenelle, L.F., Coutinho, E.S.F., Lins-Martins, N.M., Fitzgerald, P.B., Fujiwara, H., Yücel, M., 2015. Electroconvulsive therapy for obsessive-compulsive disorder: a systematic review. J. Clin. Psychiatry. [Epub ahead of print]

Garcia, L., D'Alessandro, G., Bioulac, B., Hammond, C., 2005. High-frequency stimulation in Parkinson's disease: more or less? Trends Neurosci 28, 209–216.

Gouy, G., 1910. Sur la Constitution de la Charge Electrique a la Surface d'un Electrolyte. Compt. Rend. 149, 654.

Grahame, D., 1947. The electrical double layer and the theory of electrocapillarity. Chem. Rev. 441–501.

Greenberg, B.D., Malone, D. a, Friehs, G.M., Rezai, A.R., Kubu, C.S., Malloy, P.F., Salloway, S.P., Okun, M.S., Goodman, W.K., Rasmussen, S. a, 2006. Three-year outcomes in deep brain stimulation for highly resistant obsessive-compulsive disorder. Neuropsychopharmacology 31, 2384–2393.

Greenberg, B.D., Price, L.H., Rauch, S.L., Friehs, G., Noren, G., Malone, D., Carpenter, L.L., Rezai, A.R., Rasmussen, S.A., 2003. Neurosurgery for intractable obsessive-compulsive disorder and depression: Critical issues. Neurosurg. Clin. N. Am. 14, 199–212.

Greenberg, W.M., Benedict, M.M., Doerfer, J., Perrin, M., Panek, L., Cleveland, W.L., Javitt, D.C., 2009. Adjunctive glycine in the treatment of obsessive-compulsive disorder in adults. J. Psychiatr. Res. 43, 664–670.

Greist, J.H., Jefferson, J.W., Kobak, K.A., Katzelnick, D.J., Serlin, R.C., 1995. Efficacy and tolerability of serotonin transport inhibitors in obsessive-compulsive disorder. A meta-analysis. Arch. Gen. Psychiatry 52, 53–60.

Haber, S.N., 2003. The primate basal ganglia: Parallel and integrative networks. J. Chem. Neuroanat. 26, 317–330.

Haber, S.N., Fudge, J.L., McFarland, N.R., 2000. Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. J. Neurosci. 20, 2369–2382.

Hanai, T., 1999. HPLC: A Practical Guide, First. ed. Royal Society of Chemistry, Cambridge.

Hashimoto, T., Elder, C.M., Okun, M.S., Patrick, S.K., Vitek, J.L., 2003. Stimulation of the subthalamic nucleus changes the firing pattern of pallidal neurons. J. Neurosci. 23, 1916–1923.

Heimer, L., 2000. Basal forebrain in the context of schizophrenia. Brain Res. Rev. 31, 205–235.

Heimer, L., Harlan, R.E., Alheid, G.F., Garcia, M.M., De Olmos, J., 1997. Substantia innominata: A notion which impedes clinical-anatomical correlations in neuropsychiatric disorders. Neuroscience 76, 957–1006.

Helmholtz, H., 1853. Ueber einige Gesetze der Vertheilung elektrischer Ströme in körperlichen Leitern mit Anwendung auf die thierisch-elektrischen Versuche. Ann. Phys. Chem. 89, 211–233, 353–377.

Hiller, A., Loeffler, S., Haupt, C., Litza, M., Hofmann, U., Moser, A., 2007. Electrical high frequency stimulation of the caudate nucleus induces local GABA outflow in freely moving rats. J. Neurosci. Methods 159, 286–290.

Horch, K.W., Dhillon, G.S., 2004. Neuroprosthetics theory and practice, Series on bioengineering and biomedical engineering. World Scientific Pub Co Inc, Singapore.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R., Humphrey, P.P., 1994. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol. Rev. 46, 157–203.

Ikeda, H., Kamei, J., Koshikawa, N., Cools, A.R., 2012. Nucleus Accumbens and Dopamine-Mediated Turning Behavior of the Rat: Role of Accumbal Non-dopaminergic Receptors. J. Pharmacol. Sci. 120, 152–164.

Imperato, a, Scrocco, M.G., Bacchi, S., Angelucci, L., 1990. NMDA receptors and in vivo dopamine release in the nucleus accumbens and caudatus. Eur. J. Pharmacol. 187, 555–6.

Joseph, M., Datla, K., Young, A., 2003. The interpretation of the measurement of nucleus accumbens dopamine by in vivo dialysis: the kick, the craving or the cognition? Neurosci. Biobehav. Rev. 27, 527–541.

Juergens, E., Guettler, A., Eckhorn, R., 1999. Visual stimulation elicits locked and induced gamma oscillations in monkey intracortical- and EEG-potentials, but not in human EEG. Exp. Brain Res. 129, 247–259.

Kelley, A.E., Lang, C.G., 1989. Effects of GBR 12909, a selective dopamine uptake inhibitor, on motor activity and operant behavior in the rat. Eur. J. Pharmacol. 167, 385–395.

Kelly, R.C., Smith, M.A., Kass, R.E., Lee, T.S., 2010. Local field potentials indicate network state and account for neuronal response variability. J. Comput. Neurosci. 29, 567–579.

Khan, S.H., Shuaib, A., 2001. The technique of intracerebral microdialysis. Methods 23, 3–9.

Kisely, S., Hall, K., Siskind, D., Frater, J., Olson, S., Crompton, D., 2014. Deep brain stimulation for obsessive-compulsive disorder: a systematic review and meta-analysis. Psychol. Med. 44, 3533–42.

Kobak, K.A., Greist, J.H., Jefferson, J.W., Katzelnick, D.J., Henk, H.J., 1998. Behavioral versus pharmacological treatments of obsessive compulsive disorder: a meta-analysis. Psychopharmacology (Berl). 136, 205–216.

Kopell, B.H., Greenberg, B., Rezai, A.R., 2004. Deep brain stimulation for psychiatric disorders. J. Clin. Neurophysiol. 21, 51–67.

Koran, L.M., Hanna, G.L., Hollander, E., Nestadt, G., Simpson, H.B., 2007. Practice guideline for the treatment of patients with obsessive-compulsive disorder. Am J Psychiatry 164, 5–53.

Levant, B., Grigoriadis, D.E., DeSouza, E.B., 1993. [3H]quinpirole binding to putative D2 and D3 dopamine receptors in rat brain and pituitary gland: a quantitative autoradiographic study. J Pharmacol Exp Ther 264, 991–1001.

Li, T., Qadri, F., Moser, A., 2004. Neuronal electrical high frequency stimulation modulates presynaptic GABAergic physiology. Neurosci. Lett. 371, 117–21.

Li, T., Thümen, A., Moser, A., 2006. Modulation of a neuronal network by electrical high frequency stimulation in striatal slices of the rat in vitro. Neurochem. Int. 48, 83–6.

Logothetis, N.K., 2003. The underpinnings of the BOLD functional magnetic resonance imaging signal. J. Neurosci. 23, 3963–3971.

Macdonald, R.L., Olsen, R.W., 1994. GABAA receptor channels. Annu. Rev. Neurosci. 17, 569–602.

Mantovani, M., Moser, A., Haas, C. a, Zentner, J., Feuerstein, T.J., 2009. GABA(A) autoreceptors enhance GABA release from human neocortex: towards a mechanism for high-frequency stimulation (HFS) in brain? Naunyn. Schmiedebergs. Arch. Pharmacol. 380, 45–58.

Mantovani, M., Van Velthoven, V., Fuellgraf, H., Feuerstein, T.J., Moser, A., 2006. Neuronal electrical high frequency stimulation enhances GABA outflow from human neocortical slices. Neurochem. Int. 49, 347–350.

Math, S.B., Janardhan Reddy, Y.C., 2007. Issues in the pharmacological treatment of obsessive-compulsive disorder. Int. J. Clin. Pract. 61, 1188–1197.

Mathé, J.M., Nomikos, G.G., Hygge Blakeman, K., Svensson, T.H., 1999. Differential actions of dizocilpine (MK-801) on the mesolimbic and mesocortical dopamine systems: Role of neuronal activity. Neuropharmacology 38, 121–128.

Matsumoto, R.R., 1989. GABA receptors: are cellular differences reflected in function? Brain Res. Brain Res. Rev. 14, 203–25.

Matthysse, S., 1973. Antipsychotic drug actions: a clue to the neuropathology of schizophrenia? Fed. Proc. 32, 200–205.

McCracken, C.B., Grace, A. a, 2007. High-frequency deep brain stimulation of the nucleus accumbens region suppresses neuronal activity and selectively modulates afferent drive in rat orbitofrontal cortex in vivo. J. Neurosci. 27, 12601–10.

McIntyre, C.C., Savasta, M., Walter, B.L., Vitek, J.L., 2004. How does deep brain stimulation work? Present understanding and future questions. J. Clin. Neurophysiol. 21, 40–50.

Meissner, W., Guigoni, C., Cirilli, L., Garret, M., Bioulac, B.H., Gross, C.E., Bezard, E., Benazzouz, A., 2007. Impact of chronic subthalamic high-frequency stimulation on metabolic basal ganglia activity: a 2-deoxyglucose uptake and cytochrome oxidase mRNA study in a macaque model of Parkinson's disease. Eur. J. Neurosci. 25, 1492–500.

Meissner, W., Leblois, A., Hansel, D., Bioulac, B., Gross, C.E., Benazzouz, A., Boraud, T., 2005. Subthalamic high frequency stimulation resets subthalamic firing and reduces abnormal oscillations. Brain 128, 2372–82.

Merrill, D.R., Bikson, M., Jefferys, J.G.R., 2005. Electrical stimulation of excitable tissue: Design of efficacious and safe protocols. J. Neurosci. Methods 141, 171–198.

Mitzdorf, U., 1987. Properties of the evoked potential generators: current source-density analysis of visually evoked potentials in the cat cortex. Int. J. Neurosci. 33, 33–59.

Mitzdorf, U., 1985. Current source-density method and application in cat cerebral cortex: investigation of evoked potentials and EEG phenomena. Physiol. Rev. 65, 37–100.

Mogenson, G.J., Jones, D.L., Yim, C.Y., 1980. From motivation to action: functional interface between the limbic system and the motor system. Prog. Neurobiol. 14, 69–97.

Morón, J.A., Brockington, A., Wise, R.A., Rocha, B.A., Hope, B.T., 2002. Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: evidence from knock-out mouse lines. J. Neurosci. 22, 389–395.

Moser, A., Gieselberg, A., Ro, B., Keller, C., Qadri, F., 2003. Deep brain stimulation: Response to neuronal high frequency stimulation is mediated through GABAA receptor activation in rats. Neurosci. Lett. 341, 57–60.

Mundt, A., Klein, J., Joel, D., Heinz, A., Djodari-Irani, A., Harnack, D., Kupsch, A., Orawa, H., Juckel, G., Morgenstern, R., Winter, C., 2009. High-frequency stimulation of the nucleus accumbens core and shell reduces quinpirole-induced compulsive checking in rats. Eur. J. Neurosci. 29, 2401–12.

Nicola, S.M., 2007. The nucleus accumbens as part of a basal ganglia action selection circuit. Psychopharmacology (Berl). 191, 521–550.

Nuttin, B., Cosyns, P., Demeulemeester, H., Gybels, J., Meyerson, B., 1999. Electrical stimulation in anterior limbs of internal capsules in patients with obsessive-compulsive disorder. Lancet 354, 1526.

Nuttin, B.J., Gabriëls, L.A., Cosyns, P.R., Meyerson, B.A., Andréewitch, S., Sunaert, S.G., Maes, A.F., Dupont, P.J., Gybels, J.M., Gielen, F., Demeulemeester, H.G., Van Kuyck, K., Kopell, B., Kelly, P.J., Velasco, F., Benabid, A.L., Malone, D., Rezai, A.R., 2003. Long-term electrical capsular stimulation in patients with obsessive-compulsive disorder. Neurosurgery 52, 1263–1274.

Olsen, R.W., Sieghart, W., 2008. International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. Pharmacol. Rev. 60, 243–60.

Paxinos, G., Watson, C., 2007. The Rat Brain in Stereotaxic Coordinates Sixth Edition. Elsevier Acad. Press 170, 547–612.

Paxinos, G., Watson, C., Pennisi, M., Topple, A., 1985. Bregma, lambda and the interaural midpoint in stereotaxic surgery with rats of different sex, strain and weight. J. Neurosci. Methods 13, 139–143.

Pennartz, C.M., Groenewegen, H.J., Lopes da Silva, F.H., 1994. The nucleus accumbens as a complex of functionally distinct neuronal ensembles: an integration of behavioural, electrophysiological and anatomical data. Prog. Neurobiol. 42, 719–61.

Pepper, J., Hariz, M., Zrinzo, L., 2015. Deep brain stimulation versus anterior capsulotomy for obsessive-compulsive disorder: a review of the literature. J. Neurosurg. 122, 1028–37.

Perlmutter, J.S., Mink, J.W., 2006. Deep brain stimulation. Annu. Rev. Neurosci. 29, 229–257.

Pittenger, C., Krystal, J.H., Coric, V., 2006. Glutamate-modulating drugs as novel pharmacotherapeutic agents in the treatment of obsessive-compulsive disorder. NeuroRx 3, 69–81.

Rahman, S., McBride, W.J., 2001. D1-D2 dopamine receptor interaction within the nucleus accumbens mediates long-loop negative feedback to the ventral tegmental area (VTA). J. Neurochem. 77, 1248–55.

Rammes, G., Danysz, W., Parsons, C.G., 2008. Pharmacodynamics of memantine: an update. Curr. Neuropharmacol. 6, 55–78.

Rauch, S.L., Dougherty, D.D., Malone, D., Rezai, A., Friehs, G., Fischman, A.J., Alpert, N.M., Haber, S.N., Stypulkowski, P.H., Rise, M.T., Rasmussen, S.A., Greenberg, B.D., 2006. A functional neuroimaging investigation of deep brain stimulation in patients with obsessive-compulsive disorder. J. Neurosurg. 104, 558–565.

Richard W Olsen and Timothy M DeLorey., 1999. GABA and glycine, in: Siegel GJ, Agranoff BW, Albers RW, et al. (Ed.), Basic Neurochemistry. Philadelphia: Lippincott-Raven;, New york, pp. 335–346.

Richter, M. a, de Jesus, D.R., Hoppenbrouwers, S., Daigle, M., Deluce, J., Ravindran, L.N., Fitzgerald, P.B., Daskalakis, Z.J., 2012. Evidence for cortical inhibitory and excitatory dysfunction in obsessive compulsive disorder. Neuropsychopharmacology 37, 1144–51.

Ronald F.Borne. Serotonin: The neurotransmitter for the ’90s. URL http://connection.ebscohost.com/c/articles/9411112292/serotonin-neurotransmitter-90s

Rowlett, J.K., Mattingly, B.A., Bardo, M.T., 1995. Repeated quinpirole treatment: Locomotor activity, dopamine synthesis, and effects of selective dopamine antagonists. Synapse 20, 209–216.

Russo, S.J., Nestler, E.J., 2013. The brain reward circuitry in mood disorders. Nat. Rev. Neurosci. 14, 609–25.

Saxena, S., Rauch, S.L., 2000. Functional neuroimaging and the neuroanatomy of obsessive-compulsive disorder. Psychiatr. Clin. North Am. 23, 563–586.

Sesia, T., Bulthuis, V., Tan, S., Lim, L.W., Vlamings, R., Blokland, A., Steinbusch, H.W.M., Sharp, T., Visser-Vandewalle, V., Temel, Y., 2010. Deep brain stimulation of the nucleus accumbens shell increases impulsive behavior and tissue levels of dopamine and serotonin. Exp. Neurol. 225, 302–9.

Shetreat, M.E., Lin, L., Wong, A.C., Rayport, S., 1996. Visualization of D1 dopamine receptors on living nucleus accumbens neurons and their colocalization with D2 receptors. J. Neurochem. 66, 1475–1482.

Shirayama, Y., Chaki, S., 2006. Neurochemistry of the Nucleus Accumbens and its Relevance to Depres- sion and Antidepressant Action in Rodents 277–291.

Shumyatsky, G.P., Tsvetkov, E., Malleret, G., Vronskaya, S., Hatton, M., Hampton, L., Battey, J.F., Dulac, C., Kandel, E.R., Bolshakov, V.Y., 2002. Identification of a signaling network in lateral nucleus of amygdala important for inhibiting memory specifically related to learned fear. Cell 111, 905–918.

Silva, L.R., Amitai, Y., Connors, B.W., 1991. Intrinsic oscillations of neocortex generated by layer 5 pyramidal neurons. Science 251, 432–435.

Sivilotti, L., Nistri, A., 1991. GABA receptor mechanisms in the central nervous system. Prog. Neurobiol. 36, 35–92.

Stefani, A., Fedele, E., Galati, S., Pepicelli, O., Frasca, S., Pierantozzi, M., Peppe, A., Brusa, L., Orlacchio, A., Hainsworth, A.H., Gattoni, G., Stanzione, P., Bernardi, G., Raiteri, M., Mazzone, P., 2005. Subthalamic stimulation activates internal pallidus: evidence from cGMP microdialysis in PD patients. Ann. Neurol. 57, 448–52.

Stern, O., 1924. Zur Theorie der Elektrolytischen Doppelschicht. Zeitschrift fur Elektrochemie 30, 508–516.

Stevens, J.R., 1997. Anatomy of schizophrenia revisited. Schizophr. Bull. 23, 373–383.

Sturm, V., Lenartz, D., Koulousakis, A., Treuer, H., Herholz, K., Klein, J.C., Klosterkötter, J., 2003a. The nucleus accumbens: a target for deep brain stimulation in obsessive–compulsive- and anxiety-disorders. J. Chem. Neuroanat. 26, 293–299.

Sturm, V., Lenartz, D., Koulousakis, A., Treuer, H., Herholz, K., Klein, J.C., Klosterkötter, J., 2003b. The nucleus accumbens: A target for deep brain stimulation in obsessive-compulsive- and anxiety-disorders. J. Chem. Neuroanat. 26, 293–299.

Sullivan, R.M., Talangbayan, H., Einat, H., Szechtman, H., 1998. Effects of quinpirole on central dopamine systems in sensitized and non-sensitized rats. Neuroscience 83, 781–9.

Svennilson, E., Torvik, A., Lowe, R., Leksell, L., 1960. Treatment of Parkinsonism by Stereotactic Thermolesions in the Pallidal Region. Acta Psychiatr Scand 35, 358–77.

Szechtman, H., Sulis, W., Eilam, D., 1998. Quinpirole induces compulsive checking behavior in rats: a potential animal model of obsessive-compulsive disorder (OCD). Behav. Neurosci. 112, 1475–1485.

Taira, T., Hori, T., 2003. Stereotactic Ventrooralis Thalamotomy for Task-Specific Focal Hand Dystonia (Writer's Cramp). Stereotact. Funct. Neurosurg. 80, 88–91.

Tass, P.A., Klosterkötter, J., Schneider, F., Lenartz, D., Koulousakis, A., Sturm, V., 2003. Obsessive-compulsive disorder: development of demand-controlled deep brain stimulation with methods from stochastic phase resetting. Neuropsychopharmacology 28 Suppl 1, S27–S34.

Tzschentke, T.M., 2001. Pharmacology and behavioral pharmacology of the mesocortical dopamine system. Prog. Neurobiol. 63, 241–320.

Van Dijk, A., Klompmakers, A. a., Feenstra, M.G.P., Denys, D., 2012. Deep brain stimulation of the accumbens increases dopamine, serotonin, and noradrenaline in the prefrontal cortex. J. Neurochem. 123, 897–903.

Van Dijk, A., Mason, O., Klompmakers, A. a, Feenstra, M.G.P., Denys, D., 2011. Unilateral deep brain stimulation in the nucleus accumbens core does not affect local monoamine release. J. Neurosci. Methods 202, 113–8.

Varatharajan, R., Joseph, K., Loeffler, S., Fuellgraf, H., Hofmann, U.G., Moser, A., 2014. N-Methyl-D-Aspartate Receptor Activation Interacts with Electrical High Frequency Stimulation in the Rat Caudate Nucleus in vitro and in vivo. Open J. Neurosci. 4, 1–7.

Wamsley, J.K., Gehlert, D.R., Filloux, F.M., Dawson, T.M.,. Comparison of the distribution of D-1 and D-2 dopamine receptors in the rat brain. J. Chem. Neuroanat. 2, 119–37.

Westerink, B.H., Damsma, G., De Vries, J.B., Koning, H., 1987. Dopamine re-uptake inhibitors show inconsistent effects on the in vivo release of dopamine as measured by intracerebral dialysis in the rat. Eur. J. Pharmacol. 135, 123–128.

White, F.J., Wang, R.Y., 1986. Electrophysiological evidence for the existence of both D-1 and D-2 dopamine receptors in the rat nucleus accumbens. J. Neurosci. 6, 274–280.

Wichmann, T., DeLong, M.R., 2006. Deep Brain Stimulation for Neurologic and Neuropsychiatric Disorders. Neuron 52, 197–204.

Williams, Z.M., 2015. Good vibrations with deep brain stimulation. Nat. Neurosci. 18, 618–9.

Windels, F., Carcenac, C., Poupard, A., Savasta, M., 2005. Pallidal origin of GABA release within the substantia nigra pars reticulata during high-frequency stimulation of the subthalamic nucleus. J. Neurosci. 25, 5079–5086.

Winter, C., Lemke, C., Sohr, R., Meissner, W., Harnack, D., Juckel, G., Morgenstern, R., Kupsch, A., 2008a. High frequency stimulation of the subthalamic nucleus modulates neurotransmission in limbic brain regions of the rat. Exp. Brain Res. 185, 497–507.

Winter, C., Mundt, A., Jalali, R., Joel, D., Harnack, D., Morgenstern, R., Juckel, G., Kupsch, a, 2008b. High frequency stimulation and temporary inactivation of the subthalamic nucleus reduce quinpirole-induced compulsive checking behavior in rats. Exp. Neurol. 210, 217–28.

Xie, Y., Heida, T., Stegenga, J., Zhao, Y., Moser, A., Tronnier, V., Feuerstein, T.J., Hofmann, U.G., 2014. High-frequency electrical stimulation suppresses cholinergic accumbens interneurons in acute rat brain slices through GABAB receptors. Eur. J. Neurosci. 1–10.

Yan, N., Chen, N., Zhu, H., Zhang, J., Sim, M., Yu, M., Wang, W., 2013. High-frequency stimulation of nucleus accumbens changes in dopaminergic reward circuit. PLoS One 8, e79318.

Yavich, L., Forsberg, M.M., Karayiorgou, M., Gogos, J.A., Männistö, P.T., 2007. Site-specific role of catechol-O-methyltransferase in dopamine overflow within prefrontal cortex and dorsal striatum. J. Neurosci. 27, 10196–10209.

Yoshida, M., Yokoo, H., Mizoguchi, K., Tanaka, T., Emoto, H., Tanaka, M., 1998. NMDA- and MK801-induced changes in dopamine release are attenuated in kainic acid-lesioned nucleus accumbens of conscious rats: an in vivo microdialysis study. Brain Res. 786, 226–9.

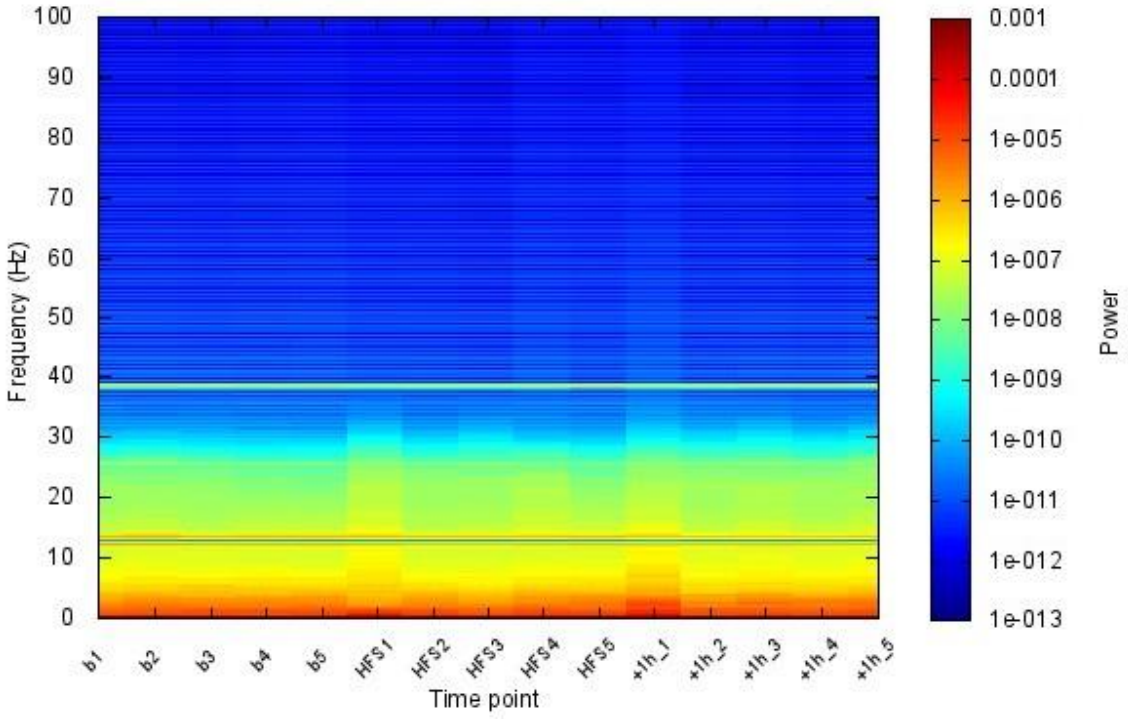
Appendix

Example of a spectrogram with all recorded time points.

b1-b2 – recordings before HFS (basal)

HFS1-HFS5- recordings after stimulation

+1h_1-+1h_5 – recordings 1 hour after stimulation



Abbreviations

5-HT	5-hydroxytryptamine; serotonin
aCSF	Artificial Cerebrospinal Fluid
c	Concentration
Cl	Chloride
CNS	Central Nervous System
DA	Dopamine
DBS	Deep Brain Stimulation
DOPAC	3, 4-dihydroxyphenylacetic acid
ECD	Electrochemical detection
ECF	Extracellular fluid
EDTA	Ethylendiamine tetraacetic acid
GABA	Gamma -amino-butyric acid
H	Hydrogen
HFS	High frequency stimulation
HIAA	5-hydroxyindole-3-acetic acid
HPLC	High pressure liquid chromatography
Hr	Hours
HVA	Homovanillic acid; 4-hydroxy-3-methoxy-phenylacetic acid
K	Potassium
L-Dopa	3, 4-dihydroxy-L-phenylalanine
LFPs	Local field potentials
MAO	Monoaminooxidase

Min	Minutes
NAc	Nucleus accumbens
Na	Sodium
OCD	Obsessive compulsive disorder
OFC	Orbitofrontal cortex
OPA	o-phthaldialdehyde
PCA	Perichloric acid
PD	Parkinson disease
S	Sulphur
SD	Standard Deviation
STN	Subthalamic Nucleus
V	Volume
VTA	Ventral Tegmental Area

Publications

- **Varatharajan**, **R** et.al., Electrical High Frequency Stimulation Modulates GABAergic Activity in the Nucleus Accumbens of Freely Moving Rats, *Neurochemistry International* (under review)
- Joseph, K , **Varatharajan**, **R** et.al., Modulation of extracellular levels of 5-HT in the caudate putamen of freely moving rats by high frequency stimulation of the subthalamic nucleus, *The Open Neuroscience Journal,* 2015, 14-20
- Prauss, K, **Varatharajan**, **R**, et.al., Transmitter self-regulation by extracellular glutamate in fresh human cortical slices, *Journal of Neurotransmission* 2014, 121(11):1321-7
- **Varatharajan R** et.al., N-Methyl-D-Aspartate Receptor Activation Interacts with Electrical High Frequency Stimulation in the Rat Caudate Nucleus in Vitro and in Vivo, *Open Journal of Neuroscience* 2014, 4:1

International Conferences

- **Varatharajan R**, et.al., The Effect of the NMDA receptor Antagonist Memantine on HFS in the Accumbens Nucleus of Freely Moving Rats, 9th fens forum of neuroscience, Milan, Italy, 2014

- **Varatharajan R**, et.al., Nucleus accumbens high frequency stimulation induces GABA release in freely moving rats. International Conference on DBS - 25 years, Duesseldorf, Germany, 2013 (Oral Presentation)
- **Varatharajan R**, et.al., Electrical High Frequency Stimulation Induces GABA Release in the Accumbens Nucleus of Freely Moving Rats, 8th fens forum of neuroscience, Barcelona, Spain, 2012
- Joseph K, Yijing, Xie, **Varatharajan R**, et.al., Striatal Serotonergic Activity is Modulated by High Frequency Stimulation of the Subthalamic Nucleus in Rats, 8th fens forum of neuroscience, Barcelona, Spain, 2012
- Choschzick I, Leppert J, Frahm L Loy M, Kerstein A, Willkomm S, **Varatharajan,R,** Tronnier V and Zechel C, subgroups of stem-like glioma cells, GSZ, Luebeck, Germany, 2010