Aus der Klinik für Psychiatrie und Psychotherapie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Promoting deep brain stimulation as a therapeutic alternative for treatment-resistant psychiatric disorders – an animal experimental study

zur Erlangung des akademischen Grades

Doctor of Philosophy (PhD)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

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Datum der Promotion: 8. Dezember 2017

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Abbreviations

AC	anterior cingulate
AM	amygdala
ANOVA	analysis of variance
AP	antero-posterior
ASR	acoustic startle response
BDNF	brain-derived neurotrophic factor
CER	conditioned emotional response
Cg25	subgenual cingulate, Brodman area 25
CPu	caudate putamen
DA	dopamine
DBS	deep brain stimulation
DM	dorsomedial thalamus
DOPAC	3,4-dihydroxyphenylacetic acid
dR	dorsal raphe nucleus
DR	discrimination reversal
DV	dorso-ventral
FSL	Flinders sensitive line
FST	forced swim test
GABA	gamma aminobutyric acid
GP	globus pallidus
Hipp	hippocampus
HPLC	high-performance liquid chromatography
ICSS	intracranial self-stimulation
IL	infralimbic
LH	learned helplessness
LI	latent inhibition
MD	major depression
MFB	medial forebrain bundle
MIS	maternal immune stimulation
ML	medio-lateral
mPFC	medial prefrontal cortex
Nacc	nucleus accumbens

NMDA	N-methyl-D-aspartic acid
NPE	non-pre-exposed
OCD	obsessive-compulsive disorder
PE	pre-exposed
PFC	prefrontal cortex
PL	prelimbic
PND	postnatal day
Poly-I:C	polyinosinic-polycytidylic acid
PPI	prepulse inhibition
REM	rapid eye movement
R/F	rate/frequency
SCT	sucrose consumption test
SPL	sound pressure level
STN	subthalamic nucleus
SZ	schizophrenia
tDCS	transcranial direct current stimulation
vmPFC	ventromedial prefrontal cortex
VTA	ventral tegmental area
5-HT	serotonin
5-HIAA	5-Hydroxyindoleacetic acid

Abstract

Currently available treatment methods for major depression (MD) and schizophrenia (SZ) yield unsatisfactory results and as a consequence, a considerable portion of patients remains therapy-resistant. Conceptualization of psychiatric disorders as representations of dysfunctional neural networks has led to investigating new causal therapeutic interventions such as deep brain stimulation (DBS), an approach that has been granted a humanitarian device exemption for severe obsessive-compulsive disorder and is tested for MD and SZ. Despite initial promising findings, recent trials for antidepressant DBS showed no efficacy warranting further research, including preclinical studies, to improve DBS settings. This thesis aimed to elucidate optimal DBS targets and parameters on anti-depressant efficacy and to test a preventive approach of DBS application in the context of SZ using valid rat models of both dysfunctions: i) Chronic-intermittent and chronic-continuous bilateral high-frequency DBS to different depression-associated brain regions were applied in the Flinders Sensitive Line rat model of MD and behavioral effects were assessed with a variety of tests. The intracranial self-stimulation paradigm was used to investigate neurobiological circuits mediating anti-depressant DBS effects. Results show symptom-specific anti-depressant effects with chronic-intermittent DBS to the ventromedial prefrontal cortex (vmPFC) and medial forebrain bundle (MFB) being more efficient in reducing depression-like behavior than nucleus accumbens-DBS. Effects could not be enhanced using prolonged DBS protocols. Anti-depressant responses of both areas seem to be mediated by different circuits as MFB- but not vmPFC-DBS was shown to interact with the reward system. ii) chronic-continuous vmPFC-DBS was applied prior to symptom manifestation to maternally immune stimulated rats constituting a neurodevelopmental SZ-model. The effects on behavioral and neurobiological SZ-associated deficits were tested in adulthood. Results show early neuromodulation to prevent behavioral SZ-like abnormalities in adulthood. In both models, effects of vmPFC-DBS on neurotransmission in pathology-relevant brain areas were tested and revealed pathology-specific normalization of disturbances in the serotonergic and dopaminergic systems. Findings of symptom- and target-specific anti-depressant DBS effects are of potential translational relevance suggesting that stimulation targets in the clinic should be selected on an individual basis considering the patient's symptom profile. They further indicate that continuous stimulation is not necessarily favorable to intermittent DBS protocols, pointing towards the usefulness of more temporally and spatially adaptive forms of DBS. The capacity of DBS to prevent SZ-like behavioral and neurobiological disruptions calls for research into non-invasive forms of neuromodulation to interfere with disease progression in high-risk indiviuals.

Zusammenfassung

Derzeit verfügbare Therapiemethoden der Depression (MD) und Schizophrenie (SZ) liefern unzureichenden Behandlungserfolg, weshalb ein beträchtlicher Anteil der Patienten therapieresistent bleibt. Die Auffassung psychiatrischer Störungen als Ausdruck dysfunktionaler neuraler Netzwerke führte zur Erforschung kausaltherapeutischer Methoden wie der tiefen Hirnstimulation (THS), die eine „humanitarian device exemption“ für die Therapie schwerer Zwangsstörung erhielt und für MD und SZ getestet wird. Trotz früherer positiver Resultate zeigten neuere Studien zu antidepressiver THS keine Effektivität. Daher sind weitere, vor allem präklinische, Studien zur Verbesserung der THS-Applikation nötig. Ziel dieser Arbeit war die Erforschung optimaler antidepressiver THS-Areale und -Parameter und die Untersuchung eines präventiven THS-Ansatzes im Kontext der SZ. Dafür wurden zwei valide Rattenmodelle beider Störungen verwendet. i) Im Flinders Sensitive Line Modell der Depression wurden Effekte chronisch-intermittierender und chronisch-kontinuierlicher THS verschiedener Areale auf depressionsähnliches Verhalten untersucht. Im Paradigma der intrakraniellen Selbststimulation wurden neurale Schaltkreise untersucht, die dem antidepressiven THS-Effekt zugrundeliegen. Resultate zeigen symptomspezifische Effekte und THS des ventromedialen präfrontalen Kortex (vmPFC) und des medialen Vorderhirnbündels (MFB) reduzieren MD-ähnliches Verhalten effektiver, als nucleus accumbens-THS. Effekte konnten durch verlängerte THS-Protokolle nicht gesteigert werden. Antidepressive Effekte beider Areale scheinen durch unterschiedliche Schaltkreise vermittelt zu werden, da MFB-THS, aber nicht vmPFC-THS mit dem Belohnungssystem interagiert. ii) Kontinuierliche vmPFC-THS wurde im prä-symptomatischen Stadium des maternalen Immunstimulationsmodells (MIS) der SZ angewandt. Resultate zeigen, dass frühe Neuromodulation SZ-ähnliche Verhaltens- und neurobiologische Abnormalitäten im Erwachsenenalter verhindert. In beiden Modellen wurden Effekte der vmPFC-THS auf Neurotransmission in relevanten Regionen untersucht und Normalisierungen pathologiespezifischer Störungen des Dopamin- und Serotoninsystems festgestellt. Regions- und symptomspezifische antidepressive Effekte der THS haben potentielle translationale Relevanz und suggerieren, dass THS-Areale unter Berücksichtigung des individuellen Symptomprofils ausgewählt werden sollten. Auch legen sie nahe, dass kontinuierliche der intermittierenden THS nicht überlegen ist, was auf die Nützlichkeit von zeitlich und räumlich angepassteren THS-Formen hindeutet. Die Fähigkeit der THS, SZ-ähnlichen Verhaltens- und neurobiologischen Abnormalitäten vorzubeugen, fordert die Erforschung nicht-invasiver Verfahren der Neuromodulation, um das Fortschreiten der Krankheit in Risikopatienten zu verhindern.

Introduction

One of the major challenges of modern medicine is the development of novel adequate treatment options for neuropsychiatric disorders. Two conditions are of particular interest: Major depression (MD) is counted among the leading causes of disability world-wide [1] and schizophrenia (SZ), estimated to affect 0.7% of the world's population, is generally considered the most severe and complex of psychiatric disorders [2]. Conventional biological treatment strategies involve chronic systemic drug administration, which affect entire neurotransmitter systems and accordingly lack local and temporal specificity. As for today, psycho-pharmacotherapy is considered unsatisfactory, yielding limited symptom reduction, unwanted side effects or even treatment resistance in a substantial number of patients [3,4]. Therefore, investigation into more rationale-driven therapeutic approaches that directly and causally interfere with pathophysiological mechanisms is needed.

This concept emerged due to growing understanding that psychiatric disorders are likely to originate from malfunctioning brain-circuitry with symptomatology arising as the clinical manifestation of particular activity- and neurochemical changes within relevant structural and functional networks [5] . This idea is supported by human imaging data, animal experimental studies, as well as theoretical knowledge that all suggest abnormal activity within particular key brain-structures to be associated with specific symptoms and to be normalized upon successful treatment [6,7]. In the context of MD, disturbances within the limbic-cortical loop have been often described, most notably hypermetabolism of the subgenual cingulum (Cg25), a region that is activated by acute sadness in healthy individuals and that is suggested to mediate symptoms of negative affect [8,9]. Further evidence suggests that the ventral striatum, primarily the nucleus accumbens (Nacc) but also the medial forebrain bundle (MFB), playing a central role in reward processing, is implicated in the occurrence of anhedonia, one of the core symptoms of MD [10,11]. In terms of SZ, the neurodevelopmental concept suggests that SZ pathology originates from complex interaction of genetic and environmental factors that induce abnormal neural development ultimately leading to behavioral symptoms in late adolescence/early adulthood [12,13]. The PFC has been attributed a major role in the disorder with environmental insults during critical periods prenatally and during adolescence interfering with maturational processes such as neuronal proliferation and synaptic pruning [14]. Further, fronto-striatal connections are found dysfunctional in SZ with frequent reports of striatal hyperdopaminergia, deficits in striatal dopamine regulation and abnormal prefrontal activity [15]. Moreover, aberrant metabolism along with reduced white matter volume has been described for the anterior limb of the internal

capsule, the white matter tract reciprocally connecting striatum, thalamus and frontal cortex [16].

Deep brain stimulation (DBS) constitutes a novel treatment approach that enables direct and specific modulations of pathological network activity through delivery of electrical current via stereotaxically implanted electrodes [17]. DBS has successfully been applied in movement disorders [17] and has recently gained approval in the form of a humanitarian device exemption for treatment of severe obsessive-compulsive disorder (OCD) [18]. In addition, DBS is being tested for other cases of treatment-refractory psychiatric disorders, including MD and recently SZ [19–22]. Clinical trials of DBS for MD include the Cg25 region, Nacc, ventral striatum, MFB, lateral habenula and inferior thalamic peduncle [21,23]. Trials have been performed for over 10 years and reported response rates of 40-70% [24]. However, recent randomized controlled trials for DBS in MD have failed to replicate earlier promising results [24,25], which demonstrates a clear demand for refined DBS protocols. It is undeniable that eventually, challenges within this line of research must be met clinically, yet, for redefining issues at the proof-of-concept level, performing controlled pre-clinical studies is inevitable. Thus, the use of validated animal models to study cross-species phenomena relevant to different aberrant behaviors is necessary [26].

The Flinders Sensitive Line (FSL) constitutes a genetic rat model of MD, rats which derive from a selective breeding program initially thought to promote resistance towards the cholinergic agonist diisopropyl fluorophosphate [27]. These animals turned out to display cholinergic hypersensitivity together with several behavioral and neurobiological characteristics resembling human patients suffering from MD. Abnormalities include behavioral despair, passive stress coping, anhedonia and rapid eye movement (REM) sleep disturbances that go along with disruptions in serotonergic, cholinergic and neuropeptide Y systems [27]. Depressive-like symptoms in FSL rats have been shown to decrease following all currently available anti-depressant drugs, electroconvulsive therapy, as well as DBS treatment [28,27,29]. The maternal immune stimulation (MIS) model of SZ was developed based on epidemiological studies showing an association between maternal infection during pregnancy and increased risk of SZ in the progeny. In this model, the injection of pregnant dams with the viral mimic polyriboinosinic-polyribocytidylic acid (poly-I:C) leads to a variety of SZ-relevant behavioral and neurobiological deficits in the offspring. Behavioral deficits in sensorimotor gating, selective attention and working memory as well as dopaminergic hypersensitivity all first appear in adult offspring, mirroring human SZ symptom manifestation [30–32]. Behavioral deficits in the MIS model are paralleled and largely preceded by brain strucural changes including enlarged lateral ventricles and reduced hippocampal volume [31], anatomical changes which are also observed in human SZ patients [33]. The administration of antipsychotic agents successfully normalizes behavioral deficits

in MIS rats [32] and recently, also DBS has demonstrated therapeutic effects [34–36]. Moreover, analogous to what has been observed in SZ patients [37], antipsychotics applied prior to symptom manifestation are able to prevent both the behavioral and structural abnormalities [38].

Objective

In a preclinical approach, the present work aims at developing optimal DBS protocols that can assist in improving DBS application in clinical trials for psychiatric disorders such as MD and SZ. To this end, the FSL and MIS rat models were used. The objectives of this thesis were threefold: 1) to identify optimal brain stimulation targets 2) to identify optimal stimulation duration, and 3) in the context of neurodevelopmental disorders such as SZ, to assess whether early intervention with DBS holds promise for disease prevention. For these purposes, 1) the effects on depressive-like behavior following bilateral intermittent high-frequency stimulation to the ventromedial prefrontal cortex (vmPFC), Nacc, medial forebrain bundle (MFB) and subthalamic nucleus (STN) were assessed in FSL rats (Rummel et al., 2016, Edemann-Callesen, Voget et al., 2015). Most of human clinical trials for MD have targeted the Cg25 and Nacc [23]. The vmPFC is considered the rodent analogue of the human Cg25 region due to its cytoarchitecture and anatomical connections [39]. Recently, DBS to the MFB, a fiber bundle connecting major parts of the brain reward center, has shown to rapidly improve depressive symptomatology in two clinical trials [21,40] and DBS to the STN was used as a negative control as it has shown to enhance depressive states in both human patients and rodents [41,42]. To determine whether anti-depressant effects can be regarded as genuine and not the result of a mere over-stimulation of the reward pathway, combined DBS with intracranial self-stimulation (ICSS) was further applied in these rats (Edemann-Callesen, Voget et al., 2015). 2) Since stimulation protocols in the clinic involve continuous rather than intermittent DBS as used in most animal experimental setups so far [29,39,43], the effects of both intermittent and continuous stimulation protocols were compared on anti-depressant responses in FSL rats (Rummel et al., 2016). 3) Neurodevelopmental disorders such as SZ show a developmental disease progression and maturational delay of symptom presentation [12]. This points to the possibility of a therapeutic window in which interference with pathophysiological processes might prevent disease progress, delay transition to psychosis or reduce severity of symptoms. Using the MIS rat model, continuous DBS to the vmPFC was applied during the pre-symptomatic phase of the disorder and its effects on the occurrence of SZ-relevant behavioral abnormalities in adulthood were assessed (Hadar et al., 2017). The vmPFC was chosen as a stimulation target based on previous studies showing vmPFC-DBS to effectively reverse behavioral deficits in MIS rats and for the involvement of the vmPFC in executive functions, impairments of which

are associated with SZ [34,36,44]. Finally, to complement the behavioral results and to shed light on the underlying neurobiological alterations induced by behaviorally effective DBS in both models, effects of vmPFC-DBS on neurotransmission were studied in brain areas relevant to the respective pathologies (Hadar et al., 2017, Rummel et al., 2016).

Methods

The following section summarizes the experimental designs and methods applied in this project. Relevant experimental groups as well as group sizes are depicted in Table 1. For detailed descriptions of the methods used please refer to the selected publications listed below.

Experimental design

Project 1 **(Rummel et al., 2016; Edemann-Callesen, Voget et al., 2015)**: To assess the anti-depressant potential of chronic-intermittent high-frequency DBS, adult male FSL and control rats were implanted with bilateral electrodes into either the vmPFC, Nacc, STN or MFB. Rats were randomly assigned to experimental groups and further assigned to receive either DBS or sham-stimulation. After one week of recovery, (sham)-DBS was started and rats underwent a behavioral test battery measuring core symptoms of depression: anhedonia, behavioral despair and learned helplessness. Rats rested for 2-3 days between test sessions. Stimulation was applied via an external current generator for 30 min every morning and for the entire length of each test. An additional 30 min session was applied on afternoons preceding a behavioral test day. Sham-stimulation rats underwent the same procedure yet no current was delivered.

The curve shift variant of the intracranial self-stimulation paradigm was applied to evaluate genuine anti-depressant effects of DBS as opposed to mere side effects caused by an over-stimulation of the brain reward center. Groups tested included rats that previously had undergone MFB (sham-)DBS as well as a second group of rats implanted with bilateral DBS electrodes into the vmPFC and a unilateral ICSS electrode into the left MFB. After two weeks of self-stimulation training, rats were tested with a within-subjects crossover design. The following conditions were applied: saline, amphetamine, haloperidol, sham-DBS, vmPFC/MFB-DBS, DBS+amph and DBS+halo. Amphetamine is a psychostimulant drug acting as a dopamine agonist, boosting extracellular dopamine concentrations [45] and is known to have reward-facilitating effects [45,46]. Haloperidol on the other hand acts as a D2 receptor antagonist and is used for its reward-attenuating effects in the ICSS paradigm [46,47]. Drugs were applied 15 min prior to testing. DBS sessions lasted 60 min and were performed right before ICSS testing. Animals

rested for 2-3 days in between tests.

Project 2 (Rummel et al., 2016): Based on the results obtained by intermittent DBS in project 1, project 2 was designed to assess how longer stimulation duration affects the anti-depressant effect in FSL rats. For that purpose, chronic-continuous DBS was applied to the vmPFC and Nacc over 16 days. Rats were implanted with bilateral electrodes into either vmPFC or Nacc and, after one week of recovery, connected to either a microstimulator (13.7g) [48] or dummy stimulator carried in a rodent jacket. Either type of stimulator was carried throughout the entire period of stimulation. Starting three days after stimulation onset, chronic-continuously treated animals were exposed to the same behavioral test battery as chronic-intermittent groups in project 1.

Project 3 (Hadar et al., 2017): To test the preventive efficacy of DBS, the neurodevelopmental MIS rat model of SZ was used: bilateral electrodes were implanted into the vmPFC of Wistar rats during adolescence on postnatal days (PND) 33-34 prior to symptom manifestation. Rats constituted the offspring of dams injected with poly-I:C (4 mg/kg, Sigma, Germany) dissolved in saline (MIS) or saline alone on gestational day 15. Starting on PND 35, MIS and saline rats received chronic-continuous DBS or sham-stimulation for 12 days, delivered via the same microstimulator devices used in project 2. Behavioral effects of DBS on sensorimotor gating, selective attention and behavioral flexibility were studied at PND>90.

After completion of all behavioral testing (project 1-3), long-term neurobiological effects of vmPFC-DBS were investigated to shed light on potential underlying mechanisms of behaviorally effective DBS (Rummel et al., 2016, Hadar et al., 2017). Groups tested included FSL rats exposed to chronic-continuous vmPFC-DBS in project 2 and MIS and saline rats previously undergoing preventive vmPFC-DBS in project 3. For neurobiological assessment, rats were sacrificed and their brains were dissected and processed for post-mortem high-performance liquid chromatography (HPLC) in relevant areas of the limbic-thalamo-cortical and striatal-thalamo-cortical circuit.

			vmPFC		Nacc		MFB		STN		**Neurobiology**	
			sham	DBS	sham	DBS	sham	DBS	sham	DBS	sham	DBS
Project 1	DBS	control					7	9				
		FSL	8	11	7	11	7	8	8	10		
	ICSS	control	7				4					
		FSL	5				4					
Project 2	DBS	FSL	10	11	10	10					6	6
Project 3	PPI	saline	8	10							8	10
		MIS	8	9							8	9
	LI	saline PE	12	8								
		saline NPE	8	7								
		MIS PE	10	9								
		MIS NPE	8	7								
	DR	saline	8	8								
		MIS	8	8								

Table 1: Experimental groups with respective animal numbers. Abbreviations: vmPFC: ventromedial prefrontal cortex, Nacc: nucleus accumbens, MFB: medial forebrain bundle, STN: subthalamic nucleus, DBS: deep brain stimulation, ICSS: intracranial self-stimulation, PPI: prepulse inhibition, LI: latent inhibition, DR: discrimination reversal, PE: pre-exposed, NPE: non-pre-exposed, FSL: Flinders sensitive line, MIS: maternally immune-stimulated.

Surgeries

All stereotactic implantations were performed under general anesthesia ((fentanyl (0.005 mg/kg), midazolam (2 mg/kg), medetomidine dihydrochloride (0.135 mg/kg)). Monopolar electrodes were bilaterally implanted at coordinates according to Paxinos and Watson with reference to bregma [49]: vmPFC (AP: +3.5 mm, ML: ±0.6 mm, DV: -3.6 mm), Nacc shell (AP: +1.2 mm; ML: ±1.8 mm; DV: -8.1 mm), MFB at the level of the lateral hypothalamus (AP: -2.5 mm, ML: ±1.7 mm, DV: -8 mm; bilaterally for MFB-DBS/ICSS group and unilaterally for vmPFC-DBS/ICSS group) or STN (AP: -3.6 mm; ML: ±2.5 mm; DV: -7.6 mm). vmPFC coordinates for juvenile MIS rats were slightly different to account for their smaller brain sizes with AP: +3.2 mm, ML: ±0.7 mm and DV: -3.3mm. For chronic-intermittent (sham)-DBS groups in project 1, anodes were wrapped around anchor screws in the skull, located in close proximity to respective electrodes, and the assembly was fixed using dental cement (Technovit, Heraeus Kulzer GmbH, Germany). Electrodes of chronic-continuous groups in projects 2 and 3 were plugged into a socket together with a screw ground electrode before fixing the assembly with dental cement. Upon completion, anesthesia was antagonized ((naloxone (0.12 mg/kg), flumazenil (0.2 mg/kg), atipamezole hydrochloride (0.75 mg/kg)) and MIS/saline rats were dressed with rodent jackets.

Deep brain stimulation

Chronic-intermittent DBS was controlled by a computer-interfaced constant current generator, delivered at 130 Hz, 100 µs and 300 µA, and monitored using an oscilloscope [20,21,50,43]. Chronic-continuous (sham-)DBS was applied at 130 Hz, 100 µs and 150 µA [48]. During the forced swim test and learned helplessness paradigm, the continuous stimulation assembly was removed due to technical reasons, instead electrodes were attached to wires connected to the external current generator.

Behavioral testing

In the **sucrose consumption test (SCT),** the quantity of a sweet solution consumed within a particular time frame serves as a measure for anhedonia [51]. Animals were habituated to sucrose solution (Milchmädchen, Nestlé, 1:3) 24h prior to the test session and food restricted thereafter until testing. During the test, rats had free access to the sucrose bottles for 15 min. Bottles were weighed before and after testing. The amounts of solution consumed over the 15 min period was normalized to the individual rat's body weight [29,51].

The **forced swim test (FST)** is designed to assess behavioral despair, as expressed by the disposition of an animal to give up after exposure to an unescapable, stressful stimulus [52]. 24h

before testing, rats were subjected to a conditioning swim session in water-filled glass cylinders for 15 min. During the test session, rats were placed into the same glass cylinders for 5 minutes and were video-taped. The rats' behavior was later analyzed for their time spent floating or initial latency to immobility [29,53].

The **learned helplessness paradigm (LH)** quantifies learned helpless behavior, i.e. the inability to acquire active avoidance in an aversive situation previously learned to be unescapable [54]. LH was performed in operant conditioning boxes and consisted of a conditioning and a testing session 24 h apart. Conditioning comprised 120 trials of inescapable shock (intensity: 0.8 mA) with alternating single shocks and resting phases. In the test session, rats underwent 15 single 60 sec shocks (intensity: 0.8 mA) with inter-shock-intervals of 24 sec [55]. Operant boxes were equipped with a lever, pressing of which would terminate the shock immediately. Animals that failed to press the lever within 60 s in more than 10 trials were considered helpless [54].

Prepulse inhibition (PPI) of the acoustic startle response (ASR) is a cross-species paradigm used to measure sensorimotor gating which is deficient in SZ patients and adult MIS offspring [56]. PPI was assessed in a sound-attenuated chamber using a movement-sensitive piezoelectric measuring platform connected to a PC with an analogue to digital (AD) converter and two loudspeakers for acoustic stimulation [34,35]. During test sessions, animals were placed in a wire mesh cage mounted on the transducer-platform. Test sessions started following a 5 min acclimatization phase with background noise (60dB sound pressure level (SPL), white noise) followed by 10 initial startle stimuli (100 dB SPL, white noise) lasting for 20 ms each. During the test, 7 different trial types were presented 10 times each in a pseudorandomized order and with inter-trial intervals of 20-30 sec: 1) startle-pulse alone (100dB SPL white noise, 20 ms); 2) control (no stimulus); 3+4) prepulse alone (72 or 68 dB, pure tone,10 kHz, 20 ms); 5-7) prepulse (72, 68, or 64 dB) each followed by a startle-pulse with an inter-stimulus interval of 100 ms. PPI was calculated using the formula 100 – 100% x (PPx/PA), in which PPx is the mean ASR of the 10 PPI trials (separate for prepulse intensities) and PA is the mean ASR to the pulse alone trials [34,35].

Latent inhibition (LI) is a cross-species paradigm measuring selective attention. Disrupted LI is seen in SZ patients and in adult MIS offspring [57]. A thirst-motivated conditioned emotional response (CER) procedure was used for LI assessment. Suppression of drinking to a tone previously paired with a foot shock was compared in rats undergoing non-reinforced exposure to the tone prior to conditioning (pre-exposed, PE) and in rats for which the tone was new (non-pre-exposed, NPE). Rats were first trained to drink in an experimental chamber. The LI procedure comprised of the following phases taking place 24 h apart: *Pre-exposure:* With the bottle

removed, PE rats were exposed to 40 tones (10 s, 80 dB, 2.8 kHz) with an inter-stimulus interval of 40 sec, whereas NPE rats did not receive tones. *Conditioning:* With the bottle removed, rats were subjected to 2 tone-shock pairings (0.5 mA, 1 s duration) presented 5 min apart, with shock immediately following tone termination. *Lick retraining Test:* Placed in the chamber, rats could drink from the bottle. After completion of 75 licks a tone was given for 5 min. Times to complete licks 51-75 (before tone onset) and licks 76-100 (after tone onset) were recorded with LI defined as shorter log times needed to finish licks 76-100 of PE than NPE rats [32].

Discrimination Reversal (DR) is a cross-species phenomenon that reflects an individual's capacity to adapt behavior according to changing stimulus/reinforcement contingencies with abnormally rapid DR considered relevant to the positive symptom pole in SZ [58]. To assess DR, a T-maze was immersed in a water pool with a platform hidden in one of the arms. On day 1 of DR testing, the platform was consistently placed in one of the arms and rats were trained to attain left-right position discrimination. Training lasted until criterion, i.e. five consecutive correct trials, was achieved. On the second day, rats were retrained to attain criterion followed by discrimination reversal training with the platform moved to the opposite arm. Again, training lasted until criterion on discrimination reversal was reached. For both position discrimination and discrimination reversal, the number of trials needed to achieve criterion was recorded [58].

Intracranial self-stimulation

The curve-shift variant of the ICSS-paradigm was applied [59]. Rats were placed in an operant chamber fitted with a lever, pressing of which elicited a 0.4 s train of electric pulses into the MFB with pulse frequency varying between trials of 1 min. The amount of presses performed per frequency was recorded. To initiate self-stimulation, stimulation was programmed to deliver 50 pulses per train at a current intensity of 200 μA. Subsequently, current intensity was adjusted to each individual animal to guarantee sufficient lever pressing (170–560 μA). After lever pressing was established, rats were subjected to rate/frequency (R/F) sessions with a descending range of frequencies (200–20 Hz, 80–8 pulses/train). Each frequency (one trial) was presented for 1 min, with lever pressing eliciting MFB-stimulation, followed by a time-out of 30 s where pressing had no effect. The sequence of descending frequencies was repeated four to five times (passes) per session. The range of stimulation frequencies applied to each rat was individually adjusted to obtain trials where stimulation was ineffective at the lower end and those where lever pressing reached a maximum rate at the high end. Once stimulation thresholds were stable (<10% variation over three consecutive days), rats continued to ICSS-testing. Here, animals underwent two R/F sessions (pre (baseline) and post-intervention) with interventions in between

sessions applied in randomized order: saline (0.9% saline), amphetamine (0.5 mg/kg d-amphetamine), haloperidol (0.05 mg/kg haloperidol), sham-DBS, vmPFC/MFB-DBS, DBS+amph and DBS+halo. All drugs were dissolved in 0.9% saline and applied intraperitoneally at a volume of 1.0 ml/kg. Frequency-response curves were fitted using a self-programmed MATLAB code [29,46]. Asymptote (maximum value) and threshold (frequency yielding 36.7% of the asymptotic response rate) were calculated for each pass as previously described [59]. Baseline and post-intervention R/F functions were compared to determine treatment-induced curve-shifts (as % change in thresholds from baseline).

Post-mortem HPLC

Micropunches were taken from brain slices (0.5 to 1 mm thick) of FSL rats previously undergoing chronic-continuous vmPFC-(sham-)DBS in project 2 and from MIS/saline rats that received preventive vmPFC-DBS in experiment 3. Punched areas for FSL rats included mPFC, orbitofrontal cortex, Nacc, hippocampus (Hipp), dorsomedial thalamus, amygdala and dorsal raphe; areas previously demonstrated relevant in FSL pathology [60,61]. In MIS/saline rat brains, punches were extracted from mPFC, Nacc, Hipp, caudate putamen (CPu) and globus pallidus (GP), all regions forming part of the cortico-striatal circuit. Monoamines (DA, 5-HT) and their metabolites (DOPAC, 5-HIAA) were separated on a column and electrochemically detected [60].

Analysis

Behavioral and biochemical data were analyzed using one-way and two-way analysis of variance (ANOVA) with treatment or phenotype and treatment as factors. Data from LI and DR were subjected to three-way ANOVA with phenotype, treatment and pre-exposure for LI/repeated factor of stage for DR as factors. Where required, multiple comparisons using post-hoc Holm-Sidak tests were conducted with an exception of LI and DR where LSD post-hoc testing was performed. For LH, data on ratios of helpless vs not-helpless rats were subjected to Fisher Exact test. Threshold and asymptote shifts before and after interventions in the ICSS paradigm were analyzed using two-way ANOVA with group (vmPFC-FSL, vmPFC-control, MFB-FSL, MFB-control) as between-subjects variable and intervention (saline, sham-DBS, DBS, amphetamine, haloperidol, DBS+amph and DBS+halo) as within-subjects variable.

Results

This section summarizes the key results obtained in this project. For a complete overview of all results please refer to the selected publications listed below.

1) Effects of chronic-intermittent DBS to the vmPFC, Nacc, MFB and STN on depressive-like behavior in the FSL rat model of MD – ***Rummel et al., 2016.***

Region-dependent behavioral effects were found when comparing vmPFC-, Nacc-, and STN-DBS on anhedonia as measured in the SCT ($F_{(3,51)}$=9.596, p<0.001), behavioral despair as measured in the FST ($F_{(3,47)}$=10.101, p=0.003) and on helplessness as measured in the LH paradigm. As for the SCT, post hoc comparisons showed decreased sucrose consumption in STN-stimulated FSL rats compared to all other groups (p<0.05 each). Although not significant, vmPFC-DBS showed a tendency to increase sucrose intake when compared to sham-stimulation (p=0.06). Nacc-DBS seemed to not affect consumption, yet taking into account the exact DBS target, a significant negative correlation between the distance to the intended implantation target and sucrose consumption suggests an anti-anhedonic potential also of Nacc-DBS ($R_{(8)}$= -0.698, p=0.0249). Following up on the treatment effect in the FST, post hoc tests showed both Nacc- and vmPFC-DBS to increase latency to float in comparison to sham-stimulation whereas STN-DBS decreased it compared to all other groups (p<0.05 each). In LH testing, none of the vmPFC-stimulated rats displayed helplessness. Helplessness was not affected under neither Nacc- nor STN-DBS in comparison to sham stimulation (p=0.713 and 0.354 respectively), but was significantly increased when compared to vmPFC-DBS for both Nacc (p=0.045) and STN (p=0.015). A separate experiment testing the anti-depressant potential of MFB DBS revealed symptom-specific effects of DBS to the MFB. In the SCT, a significant main effect for the factor phenotype ($F_{(1,26)}$=7.118, p=0.0130) showed that FSL rats consumed significantly less sucrose than controls. A significant phenotype x treatment interaction (F(1,26)=6.087, p=0.0205) led to post-hoc testing revealing a difference in consumption scores between FSL-sham and control-sham but not between DBS groups. Increased sucrose consumption under MFB-DBS in FSL rats compared to sham-stimulated controls was revealed by an independent-samples t-test after normalizing consumption scores to the means of the respective sham groups ($t_{(12)}$=2.238, p=0.045). No such effect was found between the control groups ($t_{(14)}$=1.472, p=0.1630). For the FST, we found a significant phenotype x treatment interaction ($F_{(1,23)}$=5.144, p=0.033). Post hoc tests indicate MFB-DBS to significantly reduce floating in FSL when compared to their sham counterparts (p<0.05) but not in control rats. LH was not affected by MFB-DBS.

Effects of DBS on reward-seeking behavior in FSL rats – ***Edemann-Callesen, Voget et al., 2015.***
Regarding post-intervention threshold shifts in the ICSS paradigm, significant main effects for both factors group ($F_{(3,90)}$=7.461, p=0.0028) and intervention ($F_{(6,90)}$=60.15, p<0.0001) and a significant interaction between the two ($F_{(18,90)}$=1.872, p=0.0283) were found. Post hoc analyses revealed a leftward curve-shift of the R/F function after application of amphetamine and DBS+amph, and a rightward shift with haloperidol and DBS+halo in both FSL and control groups when compared to both saline and sham-DBS (p<0.05 each). DBS alone, just as the application of saline, had no effect on the R/F function when compared to sham-DBS. Post hoc analyses further showed that in control but not in FSL rats, MFB-DBS+halo produced a threshold shift that was significantly greater than curve-shifts obtained by haloperidol and MFB-DBS alone (p<0.05 each). MFB-DBS+amph did not produce any R/F curve shift exceeding the threshold shifts obtained under both interventions alone in neither FSL nor control groups. Analyzing changes in asymptote, a significant main effect for the factor intervention ($F_{(6,90)}$=12.43, p<0.0001) was found; the asymptote was decreased across all groups after the application of haloperidol alone and DBS+halo.

2) Anti-depressant effects of chronic-continuous DBS to vmPFC and Nacc in FSL rats – ***Rummel et al., 2016.***
As in project 1, target-specific effects were found. For chronic continuous DBS, one-way ANOVA revealed a significant effect in the FST ($F_{(1,20)}$=5.779, p<0.005) with post hoc tests showing increased latency to immobility in vmPFC- when compared to sham-stimulated FSL rats (p<0.05). DBS to the Nacc was ineffective (p=0.301). In the LH paradigm, rats undergoing Nacc-DBS showed significantly more helpless behavior than mPFC-treated rats, none of which displayed helplessness (p=0.033). No effect of DBS to either target was observed in the SCT.

3) Effects of vmPFC-DBS during adolescence on schizophrenia-like abnormalities in adult MIS rats – ***Hadar et al., 2017.***
For PPI, a significant phenotype x treatment interaction ($F_{(1,31)}$=14.84, p<0.001) was found; subsequent post hoc tests confirmed significantly reduced PPI in sham-stimulated MIS offspring in comparison to sham-stimulated saline rats and revealed significantly higher PPI levels in DBS-treated MIS rats compared to their sham-stimulated counterparts (p<0.05 each). Three-way ANOVA for LI analysis revealed significant main effects for the factors treatment ($F_{(1,61)}$=5.26, p=0.025) and pre-exposure ($F_{(1,61)}$=36.74, p<0.001), as well as a significant interaction between the factors phenotype x treatment x pre-exposure ($F_{(1,61)}$=4.21, p=0.045). Significant differences

between PE and NPE groups in saline-sham ($p<0.01$), saline-DBS ($p<0.01$) and MIS-DBS groups ($p<0.01$) but not in MIS-sham groups were revealed by post hoc tests. For DR, no difference on position discrimination was found between groups. In discrimination reversal trials significant main effects were found for the factors phenotype ($F_{(1,56)}=8.84$, $p=0.006$), treatment ($F_{(1,56)}=6.96$, $p=0.013$) and stage ($F_{(1,56)}=87.06$, $p<0.001$), as well as a significant interaction between the factors ($F_{(1,56)}=3.23$, $p=0.047$). Sham-stimulated MIS rats showed enhanced reversal which was prevented by DBS as indicated by post-hoc testing ($p<0.0001$).

Biochemical mechanisms of behaviorally effective DBS – ***Rummel et al., 2016, Hadar et al., 2017.***
Chronic-continuous anti-depressant vmPFC-DBS in FSL rats significantly reduced 5-HT contents in the prelimbic portion of the vmPFC ($t=3.200$, $p=0.009$) and in the dorsal raphe ($t=2.820$, $p=0.022$) when compared to sham groups. vmPFC-DBS did not affect DA, DOPAC or 5-HIAA contents in any of the regions tested. In MIS rats, neurobiological abnormalities as expressed in elevated contents of DA in the Nacc and GP, reduced hippocampal levels of DA and DOPAC, reduced contents of 5-HT in the mPFC, Hipp, CPu and GP as well as reduced levels of 5-HIAA in the mPFC and Hipp were found [35,62]. For DOPAC a significant main effect for the factor treatment ($F_{(1,24)}= 4.20$, $p=0.05$) indicates elevated prefrontal levels in both saline and MIS offspring treated with vmPFC-DBS as opposed to sham-stimulation. Both 5-HT and 5-HIAA were reduced in the mPFC of sham-treated but not vmPFC-stimulated MIS rats as revealed by a significant phenotype x treatment interaction ($F_{(1,23)}=4.48$, $p=0.045$ for 5-HT and $F_{(1,23)}=5.46$, $p=0.029$ for 5-HIAA) and subsequent post hoc tests ($p<0.05$ each). The same pattern was found in the hippocampus (5-HT: $F_{(1,31)}=5.22$, $p=0.029$; 5-HIAA: $F_{(1,31)}=4.57$, $p=0.041$). Further, vmPFC- when compared to sham-DBS increased DA contents in the CPu ($F_{(1,31)}=14.68$, $p=0.011$) and elevated 5-HT in the GP ($F_{(1,25)}=12.27$, $p=0.002$) of saline as well as MIS rats. Finally, pathologically elevated DA contents in the GP of MIS rats were normalized by vmPFC-DBS as revealed by a significant phenotype x treatment interaction ($F_{(1,24)}=4.71$, $p=0.04$) and subsequent post-hoc testing ($p<0.05$).

Discussion

This thesis aimed at testing behavioral and neurobiological effects of DBS using two validated animal models of neuropsychiatric disorders. In an effort to shed light on optimal DBS application in the context of MD and SZ, both temporal and spatial aspects of DBS were tested. To this end different brain targets and DBS durations were studied. Moreover, a preventive DBS

approach was investigated in the neurodevelopmental MIS model of SZ. Our results suggest that in the FSL rat model of MD, vmPFC serves as the most promising stimulation-target and that chronic-intermittent DBS mode is favorable to chronic-continuous DBS application. Further, DBS applied in the early, pre-symptomatic stage of disease in the poly-I:C-induced neurodevelopmental rodent model of SZ prevented the emergence of SZ-like behavioral deficits in adulthood. Neurobiologically, anti-depressant vmPFC-DBS reduced 5-HT tissue contents in FSL rats and preventive vmPFC-stimulation in MIS rats specifically reversed some of the poly-I:C-induced abnormalities in serotonergic as well as DAergic neurotransmission.

1) Anti-depressant effects of chronic-intermittent DBS are target- and symptom specific: DBS to the vmPFC and MFB seems to be more effective than Nacc-DBS in FSL rats – ***Rummel et al., 2016, Edemann-Callesen, Voget et al., 2015.***

DBS to the STN was used as a negative control and showed depressogenic effects as reflected by decreased sucrose consumption and a shorter latency to swim-test immobility together with increased helplessness when compared to vmPFC-DBS. These results agree with previous pre-clinical and clinical studies [41,42] and approve the usefulness of the behavioral paradigms applied here in testing anti-depressant effects of DBS in rats. Earlier studies report similar anti-depressant effects of DBS to the vmPFC and Nacc when tested in the FST [63]. In line with these findings, we observed vmPFC- and Nacc-DBS to equally reduce swim-test immobility. The FST has been developed as a tool for screening of potential new antidepressants and is one of the most widely used tests of depressive-like behavior in rodent models [64]. However, one test can obviously not portray the heterogeneous nature of MD with its diverse symptomatology. In this study a wide battery of paradigms was applied to assess several depression-associated symptoms and to detect differential effects of various stimulation targets on these. Testing for anhedonia, vmPFC seems to outperform Nacc as a DBS-target. For Nacc-DBS, reduced anhedonia was only seen in animals whose electrode tips were located in closer proximity to the intended Nacc shell coordinate. As discussed in Rummel et al., 2016, this is in accordance with previous studies reporting Nacc-DBS effects to depend on the particular subregion stimulated [65,66]. While the Nacc core is associated with behavioral responses to conditioned stimuli, the shell mediates behavior in response to unconditioned, natural stimuli such as food and hedonic responses to stimuli like sucrose [67]. This functional differentiation is likely mediated by discrete anatomical afferent and efferent connections of both subregions [68,69]. With regard to learned helplessness, whereas vmPFC-stimulated rats displayed no helpless behavior, no change in learned helplessness was observed following Nacc-DBS. For further discussion please refer to Rummel

et al., 2016. Taken together, these findings suggest a greater anti-depressant potential of vmPFC-DBS as compared to Nacc-DBS in FSL rats. This is consistent with another study comparing a variety of DBS targets for their anti-depressant capacity in rats [70].

In an additional experiment, DBS to the MFB was applied. Based on its central role in the reward circuitry and its connections to other DBS targets, the MFB has been proposed as an effective new target for DBS and has already been shown to yield rapid anti-depressant effects in two clinical trials [21,40]. FSL rats consumed less sucrose than control rats regardless of intervention, confirming their depressive-like phenotype. This strengthens the validity of the FSL model and opposes earlier studies claiming that exposure to stress is necessary for FSL rats to display anhedonia [27]. Anhedonia was reduced under MFB-DBS, an effect which was restricted to FSL rats and did not occur in controls. A similar phenotype-specific antidepressant effect has been reported under ventral tegmental area (VTA)-DBS [71]. With vmPFC-DBS in contrast, our group found elevated sucrose consumption in control rats [29]. As highlighted in Edemann-Callesen et al., 2015, this differential effect of subcortical reward-associated and cortical DBS targets indicates that stimulation of the midbrain DAergic system comprising of both VTA and MFB might interact more directly with the underlying FSL pathology than vmPFC-DBS. Further, a reduction in behavioral despair under MFB-DBS as reflected by decreased time spent immobile in the FST was observed in FSL rats. These data are in accordance with other preclinical research testing the anti-depressant potential of MFB-DBS [72]. Helpless behavior, as already observed for the Nacc, remained unchanged under MFB-stimulation.

The present data suggest that in FSL rats, DBS to the Nacc is less beneficial in terms of antidepressant effects as opposed to vmPFC- and MFB-DBS. The vmPFC plays a major role in emotion regulation and memory among others [20,73]. It has been suggested that the mPFC mediates learning of associations between events and contexts on one hand and associated adaptive behaviors such as emotional responses on the other [73]. Further, an involvement of the vmPFC in the modulation of stress responses through the HPA axis has been proposed [70]. This might account for the effectiveness of vmPFC-DBS in reducing behavioral despair and in eliminating helplessness, aspects of depression-like behavior that involve stress coping [54,74]. MFB-DBS on the other hand, increasing hedonic state but not affecting learned helpless behavior, seems to be more effective in treating reward-related aspects of MD.

In consideration of the target-specific effects of DBS on depression-associated symptoms, the questions arise as to which neurobiological circuits are involved in mediating the anti-depressant response and whether the same or distinct circuitries are operated by different stimulation targets. DBS to vmPFC and Nacc for instance have been reported to induce different

patterns of regional activity and functional connectivity in rats [63]. For the MFB, there is a debate on whether anti-depressant effects of MFB-stimulation occur due to an overruling of the reward circuit [75] or whether the MFB rather presents a quick and efficient entry to the pathological depression network, by activating connections with the other DBS targets [21,76,77]. These illustrate important points of consideration as we strive to identify targets stimulation of which would yield genuine anti-depressant effects instead of side effects from an over-activated reward center.

Anti-depressant effects of vmPFC-DBS seem not to be mediated by the reward system – ***Edemann-Callesen, Voget et al., 2015.***

To investigate the involvement of the reward circuit in the anti-depressant effects of vmPFC and MFB-DBS, the intracranical self-stimulation paradigm was applied. At first, reward-facilitating effects under amphetamine and reward-attenuating effects with haloperidol were observed. These are expected effects confirming the validity of the experimental setup [29,46]. In a former study, we stated that the anti-anhedonic effect of DBS to the vmPFC in the FSL model is independent of the involvement of the reward circuit as vmPFC-DBS had no effect on reward-seeking behavior in FSL rats and their controls [29]. This finding is replicated here. Notably, in contrast to what was presumed, MFB-DBS did not change reward-seeking behavior either. This is surprising given the MFB's central anatomical position and functional role in the reward circuitry [78]. However, as discussed in Edemann-Callesen et al., 2015, the ICSS paradigm investigates the ability of an intervention to affect reward-seeking behavior in relation to maximal response rates of self-stimulation into the MFB. DA release in reward-associated areas has been suggested to be a functional mechanism of stimulation to the MFB [79,80]. Considering that DBS to the MFB was delivered through the same electrodes as subsequent self-stimulation, a ceiling effect of dopamine release with MFB self-stimulation is a plausible explanation for the unchanged response rate following DBS. We therefore added two more conditions to our test-paradigm that involve the combination of DBS with haloperidol and amphetamine, drugs directly interacting with the DA system and having clear dose-dependent effects on reward-seeking in rats [45,47]. We speculated that in this way, more subtle effects of DBS on reward-seeking behavior could be detected. In combination with amphetamine, DBS did not produce a threshold shift exceeding the shift produced by amphetamine alone. This is most likely due to the ceiling effect as suggested above; as amphetamine is a DA releasing agent [78] and lever presses increase with elevations in DA as reflected by higher dosages of amphetamine [45,47], MFB-DBS cannot further increase already maximum response rates produced by amphetamine. Interestingly, we found DBS applied in combination with haloperidol to reduce reward-seeking to a greater extent than

did haloperidol alone, showing that MFB-DBS does interact with the reward system. Of note, this was seen in control but not FSL rats. We speculated that this differential effect is related to the interaction of mesolimbic DA and 5-HT, which, along with abnormalities in the 5-HT system, has been proposed to be defective in FSL rats [27,81]. Particular 5-HT receptor subtypes regulate accumbal DA release [82] and 5-HT injections into the Nacc increase DA levels in controls but not in FSL rats [81]. Further, activation of the serotonergic system has been described under MFB-stimulation [83,84]. All of these findings give rise to our hypothesis that interaction of midbrain DA and 5-HT might underlie the combined reward-attenuating effect of both MFB-DBS and haloperidol which would explain why it was observed in control but not in FSL rats. In addition, we observed an asymptote shift with the combination of MFB-DBS and haloperidol. However, as this was seen across groups, we rule out the possibility that this shift might have confounded the results. For further details please refer to Edemann-Callesen et al., 2015.

2) Chronic-intermittent DBS seems more beneficial in terms of anti-depressant efficacy than chronic-continuous DBS in FSL rats – ***Rummel et al., 2016.***

As opposed to most pre-clinical studies, DBS in clinical trials of MD is applied in a continuous manner. The amount of treatment responders increases with prolonged stimulation durations [85] while interruption of DBS has been reported to result in relapse to depression [86]. For that reason, we hypothesized that anti-depressant effects in the FSL model would increase with longer stimulation times. However, while chronic-continuous vmPFC-DBS still reversed behavioral despair and helplessness, without surpassing previous effects obtained under intermittent DBS, no anti-depressant response was found under continuous DBS to the Nacc. These results disagree with another study investigating the effects of increasing DBS duration on depressive-like behavior, reporting better anti-depressant effects after 21 and 28 days than after 7 and 14 days of DBS to the lateral habenula [87]. However, in this chronic stimulation form, animals received stimulation for 30 min per day, which in fact resembles the chronic-intermittent protocol applied here. This suggests that positive behavioral effects may increase when extending the time-period in which intermittent DBS is applied, yet sustained stimulation is not necessarily favorable in animal experimental preparations. It is likely that continuous DBS programmed to deliver constant stimulation might simply override the neural network and that stimulation performed in a more physiological-related fashion yields better behavioral outcomes [88]. Improving stimulation settings to mimic physiological neuronal activity has already been shown to be preferable to tonic invariable stimulation in a rat model of PD [89]. In FSL rats, it was shown that DBS programmed to mimic the natural firing pattern of VTA neurons integrating burst firing and low

frequency activity reduced depression-like behavior [71,90]. Further, adaptive forms of DBS that take into account pathology-specific spatial and temporal disruptions are currently investigated as a means to directly modulate pathological firing patterns[91]. Such closed-loop DBS has been reported to yield greater benefits as compared to conventional stimulation protocols in human Tourette and PD patients and in primate models of PD [92–94]. It seems reasonable to assume that adaptive ways of delivering DBS might also be advantageous in the context of depression.

3) Chronic-continuous DBS to the vmPFC in adolescent MIS rats prevents the occurrence of SZ-related behavioral abnormalities in adulthood – ***Hadar et al., 2017.***

Unlike DBS in preclinical studies of MD, reports on the application of DBS in neurodevelopmental animal models are scarce. Few studies have been performed reporting behavioral effects of acute DBS on PPI in adult offspring of poly-I:C-treated dams. Our group previously reported DBS to the vmPFC, Nacc and dorsomedial thalamus to normalize PPI and LI [34,36]. To the present, this is the first study to investigate preventive effectiveness of DBS applied in a sensitive phase during adolescence on behavioral and neurochemical SZ-like abnormalities in adult MIS rats. We found vmPFC-DBS to prevent disruption of sensorimotor gating, selective attention and executive function in all three paradigms tested. The vmPFC regulates behavioral flexibility [44] and performance in PPI, LI and DR tasks in rodents has been reported to depend on normal vmPFC function [95–97]. Disruption of vmFPC glutamatergic signaling by injection of the NMDA antagonist MK-801 reduces PPI [95] whereas LI has been shown to rely on GABAergic vmPFC-neurotransmission with infusions of the GABA antagonist bicuculline into the vmPFC impairing LI [96]. Further, excitotoxic vmPFC lesions enhanced reversal learning which could be normalized by BDNF infusions into the vmPFC [97]. We previously found prefrontal glucose metabolism to be reduced in adolescent as well as adult MIS rats [35] which is in accordance with the frequently reported hypofrontality seen in SZ patients [98]. Along with this, decreased PFC volumes including the subgenual cingulate cortex have been reported in SZ patients compared to healthy controls [99], an abnormality which could also be demonstrated in poly I:C offspring with volume reductions being present from late adolescence [31]. Not a part of this thesis but adding to the behavioral results, in Hadar et al., 2017 we further reported early vmPFC-DBS to prevent the enlargement of lateral ventricles, one of the hallmark neurobiological abnormalities in schizophrenia [33]. Altogether, these data point to an early involvement of the vmPFC in the mediation of behavioral and brain structural disturbances emerging in adult MIS rats that can be interfered with by neuromodulation of vmPFC activity in the adolescent stage.

DBS to the vmPFC selectively normalizes pathology-specific neurobiological alterations in two different rat models of neuropsychiatric disorders – ***Rummel et al., 2016, Hadar et al., 2017.***

In FSL rats, cortical as well as subcortical 5-HT tissue contents have been shown to be elevated [60,61] and normalized under behaviorally effective anti-depressant treatment [61]. Similarly, here we show reduced 5-HT tissue contents after chronic-continuous anti-depressant vmPFC-stimulation. This DBS effect was seen specifically for the stimulation target – the prelimbic portion of the vmPFC – and the dorsal raphe nucleus (dR), a region being reciprocally connected to the vmPFC containing the majority of serotonergic neurons [100]. This targeted effect contrasts with more widespread effects produced by systemic drug treatments. Unlike those treatments, DBS in clinical trials of MD is generally not associated with considerable side effects [23], suggesting that vmPFC-DBS – or stimulation of Cg25 in the clinical context – might yield favorable therapeutical benefit by directly targeting and interfering with aberrant circuits in depression.

SZ is associated with a dysbalance of cortical and subcortical DAergic signaling: there is ample evidence for the involvement of a hyperactive subcortical DA system along with pathologically enhanced D2 receptor activation in the positive symptoms of the disorder [101,102] , while the negative symptoms and cognitive deficits are related to cortical DAergic hypofunction and reduced D1 receptor binding [101]. In line with this, in a previous study we found increased accumbal DA contents along with reduced levels of DOPAC in the mPFC of poly I:C offspring as compared to healthy control rats [35]. Here, we show increased DA tissue contents in the Nacc and globus pallidus (GP) of MIS rats, with the latter being normalized by DBS to the vmPFC. This complements our behavioral data as PPI, LI and DR are paradigms depicting the positive pole in SZ, associated with subcortical hyperdopaminergia [103]. In combination with the evidence for striatal DA dysregulation in MIS rodents [104] it is surprising that accumbal DA was not affected by DBS. Studies have shown deficits of the PFC to modulate the DA system throughout development in SZ [7]. As we have discussed in Hadar et al., 2017, it is conceivable that increased mesolimbic DA signaling alone is not sufficient to induce behavioral deficits but rather a combination with additional impairments like PFC dysfunction may be necessary. Also, a variety of studies points to the serotonergic system which is known to exert inhibitory control on DA function to be dysfunctional and implied in negative symptoms such as affective impairments in SZ [105]. It has been shown that depletion of tryptophan enhances and SSRI treatment reduces negative symptoms of the disorder [106,107]. Here, we found reduced tissue levels of 5-HT in the mPFC, hippocampus (hipp), caudate putamen (CPu) and GP in adult MIS rats, replicating results obtained by earlier studies [35,62]. Pathologically low 5-HT contents in the MIS mPFC and hipp were normalized by DBS. Second generation antipsychotics were developed

based on the dopamine-serotonin hypothesis of schizophrenia suggesting a dysbalance of these monoamines to produce the wide spectrum of SZ symptoms [108]. It seems conceivable that the behavioral effectiveness of DBS is due to normalization of this interaction rather than pure restoration of monoamine contents. Taken together, our data suggest that emergence of behavioral schizophrenia-like abnormalities might be prevented by interfering with the development of DA- and serotonergic dysregulation at an early stage. Interestingly, DBS to the vmPFC in two different animal models of neuropsychiatric disorders shows discrete effects: in FSL rats, for which we previously described elevated 5-HT levels and no alterations in DA contents [60], vmPFC-DBS reduced 5-HT tissue contents in relevant areas with no effect on DA. DBS applied to the same target using the same parameters in MIS rats partially normalized abnormally high DA levels and increased pathologically reduced levels of 5-HT. This implies that vmPFC-DBS effects are disease-specific, improving behavioral symptoms by directly and specifically interfering with the underlying pathophysiology characteristic for each phenotype.

Limitations

Results of preclinical behavioral outcomes are averaged across groups for effects to become obvious, whereas in clinical trials, patients get individually classified as responders, in the case of MD with a reduction in depression rating scales of >50%. This discrepancy constitutes a general problem when seeking to compare animal experimental and clinical studies. The averaging of behavioral outcomes in preclinical research renders a distinction into responders/non-responders impossible thereby potentially blunting treatment effects. Furthermore, a patient population is by definition more heterogeneous than rats of a same strain, thereby increasing the complexity as to which aspects/symptoms of MD/SZ can be evaluated in the patient situation. Also, based on the stimulation parameters that are clinically applied, we used invariable parameters for all DBS-targets tested. However, it is conceivable that due to factors such as target size and structural configuration, different stimulation amplitudes might be more suitable for different stimulation targets [63]. This should be taken into consideration by future studies.

Conclusion/ Outlook

The results regarding anti-depressant DBS effects in the FSL rat model of depression are in partial contrast to results obtained in recent clinical trials with MD patients [24,25]. One explanation could be that negative outcomes are due to problems involving patient- and target selection [109]. We found DBS of different stimulation targets to exert symptom-specific anti-depressant effects.

This is of potential translational relevance, implying that target selection in clinical trials should occur on an individual basis, taking into consideration the individual patient's symptom profile [109]. This is further supported by the finding of vmPFC-DBS effects to be independent of the reward circuit, as opposed to effects of MFB-DBS. We might speculate that vmPFC in rodents and Cg25 in human patients might be a safer target in terms of undesired reward-related side effects. To strengthen this, it has been shown that DBS of the Nacc and ventral striatum can induce symptoms of mania in patients treated for OCD and bipolar disorder [110,111]. Additionally, we demonstrate that chronic-continuous DBS is not superior to intermittent stimulation in terms of anti-depressant effectiveness. It is even conceivable that constant stimulation might override the neural network, thereby reducing treatment success [112]. We therefore suggest that adaptive forms of DBS in the form of closed-loop stimulation as described above might yield better treatment outcome in MD. For that, we need to shed light on specific spatial and temporal disruptions that are associated with symptom manifestation. While for PD, elevated beta band oscillations constitute the most widely described network abnormality [113], investigation into oscillatory irregularities that could serve as potential biomarkers for depression is in the early stages with very few and inconclusive data reported in rats as well as in human patients [28,60,114].

For SZ and other neurodevelopmental disorders, our results show that interfering with maturational processes in an early, pre-symptomatic stage can halt disease progression, reverse neurobiological abnormalities and prevent the emergence of behavioral symptoms. This agrees with studies showing that preventively administered anti-psychotic treatment can preclude symptom manifestation in individuals at high risk for psychosis as well as in animal models [37,38]. Application of pre-symptomatic DBS has also been found to prevent enlargement of lateral ventricles as reported in Hadar et al., 2017, which further strengthen the notion that DBS is potent in stopping disease progression. All together, these results are of high translational relevance. However, applying DBS to at-risk individuals would obviously be far too invasive. In this context, instead of promoting DBS as a preventive tool in SZ, it rather serves as an investigative tool that helps us understand underlying mechanisms and allows us to interfere with the developmental course of the disease. Instead of DBS, more non-invasive forms of neuromodulation can be developed that are feasible in prodromal, high-risk adolescents. Transcranial direct current stimulation (tDCS) is a non-invasive neuromodulatory technique involving application of direct current to the brain via scalp electrodes [115] which is already under investigation in SZ [116]. Based on the present results, future research should focus on the role of the vmPFC in disease progression in SZ and on developing tDCS paradigms interfering with disease-promoting impairments of that region.

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Affidavit

I, Mareike Voget certify under penalty of perjury by my own signature that I have submitted the thesis on the topic “Promoting deep brain stimulation as a therapeutic alternative for treatment-resistant psychiatric disorders – an animal experimental study”. I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (s.o) and are answered by me. My contributions in the selected publications for this dissertation correspond to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author of correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

Date _____ Signature

<u>Declaration of any eventual publications</u>

Mareike Voget had the following share in the following publications:

Publication 1:

Rummel, J., **Voget, M.**, Hadar, R., Ewing, S., Sohr, R., Klein, J., Sartorius, A., Heinz, A., Mathé, A.A., Vollmayr, B. & Winter, C. (2016). Testing different paradigms to optimize antidepressant deep brain stimulation in different rat models of depression. *Journal of Psychiatric Research*, 81, 36-45.

Contribution in detail: Built the micro stimulators used for continuous DBS, performed surgeries, behavioral, and biochemical HPLC testing of animals in experiment 2, performed data analysis and interpretation for experiment 2, contributed to writing and revising the manuscript.

Publication 2:

Edemann-Callesen, H.*, **Voget, M.***, Empl, L., Vogel, M., Wieske, F., Rummel, J., Heinz, A., Mathé, A.A., Hadar, R., & Winter, C. (2015). Medial forebrain bundle deep brain stimulation has symptom-specific anti-depressant effects in rats and as opposed to ventromedial prefrontal cortex stimulation interacts with the reward system. *Brain stimulation*, 8(4), 714-723. 2015.

Contribution in detail: Contributed to surgeries and behavioral testing, took part in data analysis and interpretation and in drafting and revising the manuscript. * H. Edemann-Callesen and M. Voget contributed equally to this study.

Publication 3:

Hadar, R., Bikovski, L., Soto-Montenegro, M.L., Schimke, J., Maier, P., Ewing, S., **Voget, M.**, Wieske, F., Götz, T., Desco, M., Hamani, C., Pascau, J., Weier, I. & Winter, C. (2017). Early neuromodulation prevents the development of brain and behavioral abnormalities in a rodent model of schizophrenia. *Molecular Psychiatry.*

Contribution in detail: Built the micro stimulators for DBS, performed parts of the surgeries, contributed to behavioral testing, conducted biochemical HPLC analyses, revised the manuscript

Signature of the doctoral candidate

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Selected Publications

Rummel, J., **Voget, M.**, Hadar, R., Ewing, S., Sohr, R., Klein, J., Sartorius, A., Heinz, A., Mathé, A.A., Vollmayr, B. & Winter, C. (2016). Testing different paradigms to optimize antidepressant deep brain stimulation in different rat models of depression. *Journal of Psychiatric Research*, 81, 36-45. IF 4.465
https://doi.org/10.1016/j.jpsychires.2016.06.016

Edemann-Callesen, H.*, **Voget, M.***, Empl, L., Vogel, M., Wieske, F., Rummel, J., Heinz, A., Mathé, A.A., Hadar, R., & Winter, C. (2015). Medial forebrain bundle deep brain stimulation has symptom-specific anti-depressant effects in rats and as opposed to ventromedial prefrontal cortex stimulation interacts with the reward system. *Brain stimulation*, 8(4), 714-723. IF 4.793
https://doi.org/10.1016/j.brs.2015.02.009

Hadar, R., Bikovski, L., Soto-Montenegro, M.L., Schimke, J., Maier, P., Ewing, S., **Voget, M.**, Wieske, F., Götz, T., Desco, M., Hamani, C., Pascau, J., Weiner, I. & Winter, C. (2017). Early neuromodulation prevents the developm. ent of brain and behavioral abnormalities in a rodent model of schizophrenia. *Molecular Psychiatry*. IF 13.314
https://doi.org/10.1038/mp.2017.52

Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

For reasons of data protection, my CV is not published in the online version of my thesis.

Publications

Hadar, R., Bikovski, L., Soto-Montenegro, M.L., Schimke, J., Maier, P., Ewing, S., **Voget, M.**, Wieske, F., Götz, T., Desco, M., Hamani, C., Pascau, J., Weiner, I. & Winter, C. (2017). Early neuromodulation prevents the development of brain and behavioral abnormalities in a rodent model of schizophrenia. *Molecular Psychiatry.* IF 13.314

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Acknowledgments

First of all I would like to thank my direct supervisor, Prof. Dr. Christine Winter, for letting me be part of the lab and for giving me the opportunity to work on this project, for your constant support, encouragement in so many circumstances, and for your trust. Second, I am very grateful to Prof. Dr. Dr. Andreas Heinz for acting as the formal supervisor of my thesis.

Thank you so much, Dr. Ravit Hadar, for always having an open door and for all the scientific discussions and long conversations we had.

I would further like to thank Christiane Kölske and Renate Winter for your excellent technical assistance.

Thank you Julia, for making me join the lab in the first place, for sharing everything with me and for a lot of fun we had. I am especially thankful to Franziska for all the incredible help you offered me, for your painting and tin foil-wallpapering efforts and for every smoking break you forced on me. Also, thank you Henriette for being such a great colleague and for having made so many endless recording days more bearable.

I am very grateful to Gülçin for your invaluable company, for countless cheer-ups and for your never-ending supply of PG tips.

Thank you so much Giuseppe for sharing my struggles throughout all this time, for giving me confidence when most needed and for never stopping to believe in me.

A very big thanks is to Nicolai, for your endless disposition and patience, for hours you spent dealing with my questions and for the bouncy balls.

Finally, I would like to thank my parents Charlotte & Julius for all the freedom and support you gave to me and for having created this incredible bunch of characters that I am lucky to be part of.