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DISSERTATION

"Deep Brain Stimulation as a potential treatment for Major Depressive Disorder – an animal experimental study"

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List of Abbreviations

5-HIAA	5-Hydroxyindoleacetic acid
5-HT	Serotonin
AC	Anterior cingulate cortex
AM	Amygdala
ANOVA	Analysis of variance
AS	Activated synchronization states
a.u.	arbitrary units
Cg25	Subgenual cingulate
cLH	congenital learned helpless
cNLH	congenital non-learned helpless
CPu	Caudate putamen
DA	Dopamine
D-amph	D-amphetamine
DBS	Deep Brain Stimulation
dl	Dorsolateral orbitofrontal cortex
DM	Dorsomedial thalamus
dO	Dorsolateral orbitofrontal cortex
DOPAC	3,4-Dihydroxyphenylacetic acid
dR	Dorsal raphe nucleus
EP	Entopeduncular nucleus
EPM	Elevated plus maze
FRL	Flinders Resistant Line
FSL	Flinders Sensitive Line
FST	Forced swim test
GABA	γ-Aminobutyric acid
Glu	Glutamate
GP	Globus pallidus internus
Hipp	Hippocampus
HPA	Hypothalamic-pituitary-adrenal
HPLC	High performance liquid chromatography
ICSS	Intracranical self-stimulation

IL	Infralimbic prefrontal cortex
LFP	Local field potential
LH	Learned helplessness
MDD	Major depressive disorder
MFB	Medial forebrain bundle
mPFC	medial prefrontal cortex
NAcc	Nucleus accumbens
OF	Open field
PL	Prelimbic cortex
REM	Rapid eye movement
R/F	Rate/frequency
SCT	Sucrose consumption test
STN	Subthalamic nucleus
TRD	Treatment-resistant depression
vl	Ventrolateral orbitofrontal cortex
vmPFC	Ventromedial prefrontal cortex
vO	Ventrolateral orbitofrontal cortex
VTA	Ventral tegmental area

Abstract

Introduction: Deep brain stimulation (DBS) is an effective tool to therapeutically modulate pathological neural activity and has recently been promoted as potential treatment for major depressive disorders (MDD). The concept of MDD as a dysfunction of neuronal networks rather than of distinct brain areas has led to the clinical investigation of a number of DBS targets, including the subgenual cingulate (Cg25), nucleus accumbens (NAcc) and medial forebrain bundle (MFB). Despite initial promising results, approx. 40% of all patients treated with DBS continue to suffer from treatment-resistant depression (TRD). This indicates that there is not the one ideal stimulation target for all patients. Rather, the individual symptom profile should be considered for target selection. The aim of the present thesis was to investigate symptom-specific DBS effects of different stimulation targets, at different stimulation parameter in animal models of different expression levels of the disease. *Methods:* Flinders Sensitive Line (FSL) and congenitally learned helpless (cLH) rats as well as their respective controls received chronic-intermittent or chronic-continuous DBS to the ventromedial prefrontal cortex (vmPFC, rodent analog to the Cg25 area), NAcc, MFB, or subthalamic nucleus (STN) and were subjected to a battery of behavioral tests investigating depression- and reward-associated behavior. Additional neurobiological investigations focused on neurochemistry and neural population activity as measured via local field potential recording. *Results:* i) FSL rats had increased serotonin (5-HT) contents in several cortical and subcortical regions and alpha, beta as well as low gamma oscillatory activity was decreased in the medial prefrontal cortex (mPFC) and NAcc, ii) vmPFC-DBS was more effective than NAcc-DBS, iii) STN-DBS induced depressiogenic effects, iv) chronic-continuous DBS did not improve effects observed with chronic-intermittent DBS, instead chronic-intermittent DBS outperformed chronic-continuous DBS, v) DBS effects depended on the disease stage modeled, vi) antidepressant vmPFC-DBS effects came with reduced 5-HT contents and increased 5-HT turnover rates, and vii) effective MFB-, but not vmPFC-DBS operated via the mesolimbic brain reward system. *Conclusions:* In FSL rats, antidepressant DBS effects were symptom-, parameter- and target-specific and likely mediated via the 5-HT system and normalized gamma activity. Differently effective DBS targets interacted with different circuits, supporting the notion that individual symptom profiles should be considered when selecting a stimulation target.

Abstrakt

Einleitung: Die Tiefe Hirnstimulation (THS) ist eine wirksame Methode um pathologische neurale Aktivität therapeutisch zu verändern. Neuerdings wurde sie auch als Behandlungsmöglichkeit für Majore Depression (MD) vorangebracht. Da MD als eine Dysfunktion neuronaler Netzwerke statt bestimmter Hirnareale verstanden wird, wurde die THS diverser Zielregionen klinisch untersucht, u.a. die des subgenualen Cingulums (Cg25), Nucleus Accumbens (NAcc) sowie des Medialen Vorderhirnbündels (MFB). Trotz vielversprechender Erstergebnisse leiden etwa 40% der Patienten mit THS-Behandlung weiterhin unter therapieresistenter Depression (TRD), was darauf hindeutet, dass es nicht das eine optimale Stimulationstarget für alle TRD-Patienten gibt. Eher sollte das individuelle Symptomprofil bei der Bestimmung des Stimulationsareals berücksichtigt werden. Ziel dieser Dissertation war es, symptom-spezifische THS-Effekte unterschiedlicher Areale unter unterschiedlichen Parametern in Tiermodellen unterschiedlicher Schweregrade der Krankheit zu testen. *Methoden:* Ratten der Flinders Sensitive Line (FSL) und kongenital hilflose (cLH) Ratten sowie deren Kontrollen erhielten chronisch-intermittierende oder chronisch-kontinuierliche THS des ventromedialen prefrontalen Cortex (vmPFC; Äquivalent des humanen Cg25), NAcc, MFB oder Nucleus Subthalamicus (STN) und wurden einer Reihe Verhaltenstests unterzogen um depressions- und belohnungsrelevantes Verhalten zu untersuchen. Ergänzende neurobiologische Charakterisierung basierte auf neurochemischen Untersuchungen sowie der Messung neuraler Aktivität mittels Feldpotentialableitungen. *Ergebnisse:* i) FSL Ratten hatten einen erhöhten Serotonin(5-HT)-Gehalt in kortikalen und subkortikalen Regionen und reduzierte Alpha-, Beta- sowie niedrige Gammaaktivität im medialen Präfrontalen Cortex (mPFC) und NAcc, ii) vmPFC-THS war wirksamer als NAcc-THS, iii) STN-THS wirkte depressiogen, iv) chronisch-kontinuierliche THS verstärkte nicht die Wirkung chronisch-intermittierender THS, stattdessen übertraf chronisch-intermittierende THS die chronisch-kontinuierliche v) THS-Effekte hingen von dem dargestellten Krankheitsstadium ab, vi) antidepressiv wirksame vmPFC-THS ging einher mit reduziertem Gehalt an 5-HT und erhöhtem 5-HT-Umsatz und vii) wirksame MFB-THS aber nicht vmPFC-THS interagierte mit dem mesolimbischen Belohnungssystem. *Schlussfolgerungen:* In FSL Ratten waren antidepressive THS-Effekte symptom-, parameter- und targetspezifisch und vermutlich über das 5-HT System und normalisierter Gammaaktivität vermittelt. Unterschiedlich wirksame THS-targets agierten mit unterschiedlichen Schaltkreisen, was die Idee einer auf dem Symptomprofil basierten Targetwahl stützt.

Introduction

Major Depressive Disorder (MDD) is among the leading causes of disability worldwide. Despite established behavioral, pharmacological and electroconvulsive treatment options, approximately one third of MDD patients remain therapy-resistant (1). The existence of such treatment-resistant depression (TRD) is not surprising, considering that the exact neuropathology of MDD has not yet been elucidated prohibiting the development of treatment strategies directly interacting with the underlying pathophysiology. Accumulating data suggests that MDD is a multifactorial clinical representation of a dysfunctional limbic-cortical network (2).

Deep brain stimulation (DBS) is an effective method to modulate pathological network activity (3). It involves the stereotactic implantation of stimulation electrodes into brain structures associated with the respective pathology. Electric current is locally administered via a stimulator device. The application of DBS for MDD is being investigated and up to now numerous targets have been tested in TRD patients: subgenual cingulate (Cg25; 29-63% responders) (2,4–6), anterior limb of the capsula interna (53%) (7), medial forebrain bundle (MFB; 85%) (8), and nucleus accumbens (NAcc)/ ventral striatum (25% (9) and 45% (11–13)). Case reports further point to the lateral habenula (14) and the inferior thalamic peduncle (15) as potential DBS targets (for review see (16)). However, promising results first reported for NAcc/ventral striatum- and Cg25-DBS in open-label trials were not replicable in multicenter, prospective, randomized trials, supporting the notion that high-quality data for DBS in MDD is still lacking (17). Further, approx. 40% of all patients treated with DBS continued to suffer from TRD. This indicates that there is not the one ideal stimulation target for all patients. Rather, the individual symptom profile of each patient should be the basis when deciding what area to stimulate. The present thesis aimed at investigating the antidepressant efficacy of DBS of various targets. As therapeutic DBS effects may best be studied in aberrant systems and depend on the underlying pathology, this thesis, in contrast to most preclinical depression research using naïve animals (18–22), used two genetic animal models mimicking different expression levels of the disease: the Flinders Sensitive Line (FSL) and congenitally learned helpless (cLH) rats. FSL display several depressive-like symptoms, such as passive stress coping, reduced appetite, elevated REM sleep, stress-induced anhedonia, as well as HPA axis and neuropeptide Y system dysregulations (23–25). As these symptoms are sensitive to common antidepressant treatment (26–30), the FSL is considered a valid model for translational depression research bearing strong face, construct and predictive validity (25,31,32). cLH present with a congenitally learned helpless phenotype

(33). Learned helplessness is the inability of an individual (rodent or human) to control future aversive stimuli upon exposure to uncontrollable stress (34). This effect has been proposed as a psychopathological mechanism in development and maintenance of MDD (35,36). cLH animals show helpless behavior even without prior stress exposure and as neither most of the available anti-depressive agents nor electroconvulsive therapy antagonize their behavioral peculiarities cLH haven been suggested to model TRD (37).

Detailed knowledge about the animal models used is the basis for assessing pathophysiological and therapeutic mechanisms as well as potential new treatments. Therefore *Study I* (35) characterized neurochemical properties and neural population activity in several brain regions of the FSL rat model of depression. As mentioned above, meanwhile there are several targets that were clinically investigated and cumulative data suggest that there might not be just one optimal stimulation target for all TRD patients, but that DBS site and stimulation parameters should be selected based on the patients' individual symptom profile. To investigate which site and parameters would be ideal for which depression symptom one would require large homogenous patient groups, which, due to the heterogeneity of TRD patients would be challenging, if not impossible. Using animal models, however, allow controlled settings and thus enable such an approach. In the major study of the thesis (*Study II* (39)) we aimed at investigating symptom-specific stimulation effects i) of different brain sites relevant to depression (ventromedial prefrontal cortex (vmPFC, rodent analog to the Cg25 area (18,19)/ NAcc/ nucleus subthalamicus (STN)) ii) at different stimulation parameters (chronic-intermittent/ chronic-continuous (the latter being investigated by the co Ph.D. student Mareike Voget), and iii) at different expressions of the disease (therapy-responsive FSL/ therapy-resistant cLH rats). To assess DBS effects animals were subjected to a test battery of depression-like behavior, namely anhedonia, which is the inability to experience pleasure, behavioral despair/ immobility and learned helplessness. Further, biochemical substrates of behaviorally effective versus ineffective DBS were analyzed using in vivo microdialysis and post-mortem high performance liquid chromatography (HPLC).
As anhedonia is a core symptom of depression (40) in *Study III* (41) we focused on the anti-anhedonic effect observed under vmPFC-DBS and aimed at testing whether it is mediated via the mesolimbic dopaminergic brain reward system by subjecting FSL rats and controls that received vmPFC-DBS to the intracranial self-stimulation (ICSS) paradigm. In this operant paradigm rats self-administer rewarding stimulation to the MFB via an implanted electrode. The performance in the ICSS paradigm is suggested to reflect the hedonic state of an animal and reward-

facilitating or –attenuating effects of any intervention, such as drug treatment or DBS, can be quantified (42).

Recently, clinical studies reported promising antidepressant effects of DBS to the MFB and it has been argued to be effective especially on anhedonia (8,43). In *Study IV* (44) we hence tested the antidepressant efficacy of MFB-DBS in FSL and control rats in the same behavioral paradigms as in *Study II* and anxiety-like behavior in addition. We further compared its effect on ICSS behavior with that of vmPFC-DBS to conclude on whether both targets act via the same neurobiological circuits.

Methods

All materials and methods used in the present thesis are detailed in the publications listed in the appendix.

Animals:

Male Flinders Sensitive Line (FSL) and congenital learned helpless (cLH) rats and corresponding controls (Flinders Resistant Line (FRL)/ congenital non-learned helpless (cNLH)) were housed in standard conditions with food and water available ad libitum and single housed after electrode implantations. All studies were carried out in accordance with the European Communities Council Directive of 24th November 1986 (86/609/EEC) for care of laboratory animals and after approval of the Local Ethics Committee (Senate of Berlin). All efforts were made to reduce animal suffering and the numbers of animals used.

Experimental Design:

Study I comprised two experiments. In experiment 1, naïve FSL and FRL (each n=10) rats were decapitated and content levels of dopamine (DA), serotonin (5-HT), their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA), glutamate and γ-aminobutyric acid (GABA) of several brain areas were detected using post-mortem HPLC. In the second experiment electrophysiological properties of the vmPFC, NAcc and STN were assessed by recording local field potentials (LFPs) in anesthetized FSL (n=7) and control (n=5) rats.

Study II comprised three experiments. In experiment 1, effects of chronic-intermittent DBS to the vmPFC, NAcc and STN were tested. FSL and controls were divided into the following groups: sham-DBS (with electrodes in the STN (FRL: n=6; FSL: n=8), NAcc (each n=7) or

vmPFC (FRL: n=7; FSL: n=8)), STN-DBS (FRL: n=9; FSL: n=10), NAcc-DBS (FRL: n=8; FSL: n=11), and vmPFC-DBS (FRL: n=8; FSL: n=11). Each animal received DBS or sham-DBS for 30 min each morning. A further sham-/DBS session was applied on afternoons before a behavioral testing day and DBS was performed during testing. Behavioral testing was conducted with 2-3 days in between tests and included locomotion in the Open Field (OF), anhedonia-like behavior in the Sucrose Consumption Test (SCT), depressive-like behavior in the Forced Swim Test (FST), and helpless behavior in the Learned Helplessness Paradigm (LH). After completion a subgroup of FSL rats (sham-DBS (n=10), STN-DBS (n=9), vmPFC-DBS (n=10)) were subjected to accumbal in vivo microdialysis to test acute neurochemical DBS effects. Experiment 2 aimed at testing whether chronic-continuous adds benefits to chronic-intermittent DBS. FSL and controls were divided into the groups: sham-DBS (NAcc (FRL: n=12; FSL: n=13), or vmPFC (each n=10)), NAcc-DBS (FRL: n=14; FSL: n=11), and vmPFC-DBS (FRL: n=14; FSL: n=10). Animals received chronic-continuous DBS for 16 consecutive days. Behavioral testing was conducted as in experiment 1. Brains of FSL animals were partly processed for post-mortem HPLC to test longterm DBS effects. In experiment 3, the most effective DBS protocol of experiment 1 and 2 was applied in cLH and controls. Sham-DBS (each n=9) or vmPFC-DBS (each n=11) and behavioral testing was conducted according to experiment 1.

Study III focused on the reward-manipulating effect of vmPFC-DBS. Animals were subdivided into two groups: FRL sham (n=4; electrode implantation but no vmPFC-DBS until the ICSS testing phase), FRL vmPFC-DBS (n=8), FSL sham (n=4) and FSL vmPFC-DBS (n=8). Animals received intermittent vmPFC-DBS or sham-DBS according to *Study II* for two weeks, during which depressive- and anhedonia-like behavior was assessed using FST and SCT. Subsequently, rats of the DBS groups were tested in the intracranial self-stimulation (ICSS) procedure to assess reward-manipulating effects. This procedure comprised two phases lasting a total of approx. six weeks: 1) induction of self-stimulation and training and 2) testing under the influence of different interventions, namely sham-DBS, low frequency vmPFC-DBS, high frequency vmPFC-DBS, saline, fluoxetine and D-amphetamine (D-amph).

In *Study IV* antidepressant and reward-manipulating effects of DBS to the MFB were assessed. FSL and controls were divided into two groups: MFB-groups (FRL: n=16; FSL: n=15, with electrodes bilaterally implanted into the MFB only) and vmPFC-group (each n=7, with electrodes bilaterally implanted into the vmPFC and unilaterally into the MFB). The MFB-group rats were further subdivided into MFB-sham (each n=7), and MFB-DBS (FRL: n=9; FSL: n=8), receiving chronic-intermittent DBS treatment as in *Study II*. Behavioral testing included the

same test battery as *Study II* and the Elevated Plus Maze to evaluate anxiety-like behavior. After behavioral testing, the MFB- and vmPFC-group rats were subjected to the ICSS-paradigm according to *Study III*. Interventions included sham-DBS, MFB-DBS, vmPFC-DBS, saline, D-amph, haloperidol, vmPFC-DBS+D-amph, MFB-DBS+D-amph and vmPFC-DBS+haloperidol or MFB-DBS+haloperidol

When applicable, animals were decapitated and brains snap frozen in 2-methylbutane (Sigma Aldrich, Germany) at -60 °C, sliced into 20 µm coronal sections and Nissl-stained for electrode and probe localization using light microscopy.

Surgeries:

Stereotactic implantations were performed under general anesthesia. Monopolar platinum electrodes (0.25 mm, MS303-6-AIU, PlasticsOne Inc., USA) were implanted and anodes were wrapped around anchor srews in the skull. For animals subjected to microdialysis a guide cannula was implanted unilaterally into the NAcc. The assembly was fixed using dental cement (Technovit, Heraeus Kulzer GmbH, Germany). In *Study IV* monopolar recording electrodes (0.125 mm, Plastics One, USA) were implanted ipsilateral into the left vmPFC, NAcc and STN under urethane anesthesia (1.2 g/kg i.p., Sigma Aldrich, Germany).

Deep Brain Stimulation:

Stimulation was controlled by a computer-interfaced constant current generator (STG4008, MultiChannelSystems GmbH, Reutlingen, Germany) at 130 Hz, 100 µs and 300 µA. Chronic-continuous sham-/DBS was applied by using a portable microstimulator (45). Animals in sham groups were connected to the stimulator, yet did not receive any stimulation.

Behavioral testing:

Open Field Test (OF): Rats were placed in the center of an open field arena (1.2x1.2 m) for 10 min. Distance traveled was scored using a video tracking software (EthoVision XT 8.5, Noldus IT, Netherlands) (46). *Sucrose Consumption Test (SCT):* Rats were allowed to consume sucrose solution (Milchmädchen, Nestlé, 1:3) for 15 min after food restriction. Total intake was normalized to the individual body weight (47). *Forced Swim Test (FST):* Rats were conditioned to water-filled glass cylinders (depth: 33 cm, 25°C) for 15 min. After 24 h a 5 min test session was conducted. The rats' behavior was analyzed for time spent immobile and initial latency to immobility in *Study II* (48). *Learned Helplessness Paradigm (LH):* Rats were exposed to operant boxes (Operant Behavior System Mannheim Type 259900, TSE, Bad Hamburg, Germany). The

paradigm consisted of a conditioning and a testing phase 24 h apart. Animals predisposed to helplessness (cLH) were omitted to conditioning, which consisted of 120 trials of inescapable shock (0.8 mA) with single shocks and resting phases lasting 10 s +/- 50%. Test sessions, for which operant boxes were equipped with a lever, consisted of 15 single shocks lasting 60 s and inter-shock-intervals of 24 s. Pressing the lever terminated the shock. Animals that failed to terminate the shock within 60 s in >10 trials were classified as helpless (*Study II*) (49). In *Study IV* time to terminate the shock was analyzed and expressed as the summed latency to escape from shock. *Intracranial Self-Stimulation (ICSS):* In the ICSS paradigm, consisting of a training and a test session, rodents self-administer rewarding electrical stimulation via a MFB electrode (42). Rats were placed into an operant chamber equipped with a lever (TSE Systems, Bad Homburg, Germany) once per day for a session of approx. 45 min. Lever pressing triggered a train of electric stimulation pulses into the MFB, with pulse frequency varying between 1-min trials. During such a so called response rate /frequency (R/F) session, with response rate being the number of lever presses, rats were exposed to a descending range of frequencies (200-20 Hz) with each frequency (trial) lasting 1 min. This sequence of descending frequencies ranges was repeated four to five times (passes) per R/F session. Stimulation frequency applied was individually adjusted to each animal to obtain trials in which stimulation was ineffective and trials in which lever pressing reached a maximum rate. Once the stimulation threshold had stabilized over 3 consecutive days ICSS-testing began, which consisted of two R/F sessions (baseline and post-intervention). Interventions are stated in the section 'Experimental design'. Frequency-response curves were fitted using a code programmed in MATLAB. The maximum response rate (asymptote) and threshold (frequency yielding 36.7% of the asymptotic response rate) were calculated for each pass (42). To measure intervention-induced curve-shifts R/F functions from pre- and post-interventions were compared as a percentage change from baseline. *Elevated Plus Maze (EPM):* The maze consisted of two open and two closed arms (42×42 cm). Animals were allowed to explore for 5 min. Mean time spent in open arms, which negatively correlates with the attributed level of anxiety, was determined using a video tracking software (EthoVision XT 8.5, Noldus IT, Netherlands).

In vivo microdialysis:

A microdialysis probe (CMA 12, 2 mm membrane, CMA Microdialysis, Kista, Sweden) was introduced into the implanted guide cannula of re-anesthetized animals and perfused with artificial cerebrospinal fluid (Sigma Aldrich, Germany). Samples were collected in 20 min-intervals and monoamines (DA, 5-HT) and their metabolites (DOPAC, 5-HIAA) were

immediately analyzed by using HPLC (LC-10 AD, Shimadzu, Kyoto, Japan) with electrochemical detection (DECADE II, Antec, Leyden, Netherlands). Samples were collected until four consecutive values were stable. Subsequently, two samples were collected under sham-/DBS and five thereafter (50).

Post-mortem HPLC:

Micropunches were taken from 0.5-1 mm thick brain slices from various cortical and subcortical brain sites: in *Study I* from the medial prefrontal cortex (mPFC, subdivided into anterior cingulate (AC), prelimbic (PL) and infralimbic (IL) cortices), netrolateral (vl) and dorsolateral (dl) orbitofrontal cortex (OFC), thalamus (Thal), hippocampus (Hipp), NAcc, caudate putamen (CPu), entopeduncular nucleus (EP, equivalent to human globus pallidus (GP) internus), GP externus and STN, and in *Study II* from all cortical areas as in *Study I,* Hipp, NAcc, dorsomedial thalamus (DM), amygdala (AM) and dorsal raphe (DR). DA, 5-HT, DOPAC, and 5-HIAA were separated on a column (ProntoSil 120-3-C18-SH; Bischoff Analysentechnik und -geräte GmbH, Germany) and electrochemically detected (41 000, Chromsystems Instruments & Chemicals GmbH, Germany). In *Study I* glutamate and GABA were precolumn-derivatized with o-phthalaldehyde-2-mercaptoethanol, separated on a column (ProntoSil C18 ace-EPS) and detected by their fluorescence at 450 nm after excitation at 330 nm (51).

Electrophysiological recordings:

Local Field Potentials (LFP) were recorded in anesthetized animals unilateral from the vmPFC, NAcc shell and STN over a period of 5 h and made against ground screws affixed close to each recording electrode. Signals were bandpass filtered (0.05 Hz- 300 Hz), amplified, sampled at 1 kHz and digitized using a programmable neuronal data acquisition system (Omniplex, Plexon, Texas, USA). Epochs (20-50 s) of robust activated network states (AS), in which LFP show a peak frequency similar to the awake and behaving state (52), were identified via visual inspection of offline data of vmPFC recordings. The time segments identified to show such epochs of AS were also used for signal analysis from the NAcc shell and STN. Power spectral densities of LFP data segments were calculated by employing the Fast Fourier Transform function (Spike 2 Version 6 data analysis software; Hanning Window, 1.024 Hz resolution). The frequency spectrum was divided into five EEG bands (53): theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz), low gamma (30-45 Hz) and high gamma (60-100 Hz). Power spectra were normalized to the total power between the range 103-147 Hz and 153-197 Hz, then averaged across all frequencies within each range and expressed in arbitrary units (a.u.).

Data analysis:

Behavioral and biochemical data were analyzed using one- and two-way analysis of variance (ANOVA). Data gathered from ICSS tests were curve-fitted, threshold and asymptote shifts between pre- and post-treatment R/F sessions were statistically evaluated with 2×6 (*Study III*) and 4×7 (*Study IV*) ANOVAs. Pearson product-moment correlations were performed on sucrose intake and electrode placement. Ratios of helpless vs. not helpless animals were analyzed by Fisher Exact Test. Data sets of two sample groups and electrophysiological data were analyzed using independent Student's t-tests. If applicable, multiple comparisons were corrected using post hoc Holm-Sidak tests. All statistical analyses were completed with SigmaStat 4.0 (Systat Softare, Chicago, USA) and were performed at a significance level of $p<0.05$.

Results

Results from the present thesis are detailed in the publications listed in the appendix. I here summarize the main findings of each study. Figures refer to those in the regarding publication.

Study I: "Altered local field potential activity and serotonergic neurotransmission are further characteristics of the Flinders sensitive line rat model of depression."

The FSL rat model of depression was characterized on a biochemical and electrophysiological level. In FSL rats, levels of 5-HT and its metabolite 5-HIAA were increased compared to control rats in all investigated medial prefrontal and orbitofrontal cortical regions, in the thalamus and the rodent analog of the pallidal globe (EP and GP). In the NAcc, levels of 5-HT were increased but not of 5-HIAA, while in the hippocampus 5-HIAA but not 5-HT was elevated. There were no differences in tissue levels of 5-HT or 5-HIAA in the caudate putamen and STN between FSL and control rats. DA and DOPAC levels did not differ in any region between FSL and control rats. Levels of glutamate were increased in the NAcc and levels of GABA were increased in the GP of FSL (Table 1; Fig. 1).

Electrophysiological recordings of LFP showed decreased oscillatory activity in FSL when compared to control rats in every region in the alpha (vmPFC: $t=2.347$, $p=0.047$; NAcc: $t=3.436$, $p=0.009$, STN: $t=4.574$, $p=0.002$) and beta band (vmPFC: $t=3.574$, $p=0.001$; NAcc: $t=4.628$, $p<0.001$; STN: $t=4.224$, $p<0.001$). There was no difference between FSL and controls in the theta (vmPFC: $t=0.382$, $p=0.716$; NAcc: $t=-0.884$, $p=0.411$; STN: $t=-0.042$, $p=0.968$) and high gamma band oscillatory activity (vmPFC: $t=-0.795$, $p=0.429$, NAcc: $t=0.311$, $p=0.756$; STN: t=-

0.490, p=0.626). In the low gamma band FSL showed decreased activity when compared to control rats in the vmPFC (t=2.297, p=0.028) and NAcc (t=3.137, p=0.004) while in the STN oscillatory activity was increased (t=-2.192, p=0.036) (Fig. 2).

Study II: "Testing different paradigms to optimize antidepressant deep brain stimulation in different rat models of depression."

The primary aim of this study was to investigate antidepressant efficacy of different stimulation settings and hence two animal models of depression (FSL and cLH rats) were used. Control animals (FRL and cNLH) allowed testing DBS effects independent of the underlying pathology. To reduce complexity the study focused on data presentation of the pathologic phenotypes, while data of controls can be found in the supplementary section (Table S1).
Experiment 1: Symptom-specific effects of chronic-intermittent DBS to the vmPFC, NAcc and STN were analyzed. Chronic-intermittent DBS induced target-specific effects in FSL rats in all paradigms tested: SCT ($F_{(3,31)}$=9.596, p<0.001), FST ($F_{(3,47)}$=10.101, p=0.003), and LH (Fig. 1). Post hoc tests showed STN-DBS to decrease sucrose intake when compared to all other treatment groups (each p<0.05). NAcc-DBS was anti-anhedonic in a site-specific manner with the more centered the electrodes targeted the center of the NAcc shell the more the animals consumed ($R_{(8)}$=-0.698, p=0.025). Compared to the sham-DBS group, vmPFC-DBS almost significantly increased sucrose consumption (p=0.06). In the FST, animals in the STN-DBS group ceased active movements considerably earlier compared to all other groups (each p<0.05). Rats in the NAcc- and vmPFC-DBS group started immobile behavior later than sham-treated animals (each p<0.05). In the LH test, Fisher exact test revealed STN-DBS to increase helplessness when compared to vmPFC-DBS (p=0.015) but not when compared to sham-conditions (p=0.354). NAcc-DBS did not affect helplessness when compared to sham-conditions (p=0.713) but in comparison to vmPFC-DBS (p=0.045). Only vmPFC-DBS showed, if at all, a trend towards a significant effect when compared to sham-treatment (p=0.07). There was no difference between NAcc- and STN-DBS groups (p=0.620).

Experiment 2: To investigate whether stimulation parameters resembling clinical DBS application enhance antidepressant effects obtained under chronic-intermittent DBS as usually applied in animal experiments, effects of chronic-continuous NAcc- and vmPFC-DBS were tested in the same behavioral paradigms as in experiment 1. Data revealed target-specific DBS effects in the FST ($F_{(1,20)}$=5.779, p<0.05), and LH paradigm but not in the SCT ($F_{(2,38)}$=0.0172,

p=0.983) (Fig. 2). As such, post hoc tests showed NAcc-DBS to not affect latency to immobility (p=0.301), while vmPFC-DBS increased it when compared to sham-DBS (p<0.05). None of the animals of the vmPFC-DBS group showed helpless behavior in the LH paradigm, which was significantly less when compared to NAcc-DBS treated rats (p=0.033). Fisher exact test showed no further difference between treatment groups (vmPFC-DBS vs. sham: p=0.534; NAcc-DBS vs. sham: p=0.072).

Experiment 3: Based on the promising effects of chronic-intermittent vmPFC-DBS in FSL we tested its potential in cLH rats. Here, chronic-intermittent vmPFC-DBS had no antidepressant effect in any of the behaviors tested: SCT (t=1.448, p=0.167), FST (t=-1.185, p=0.263), LH (p=1.00) (Fig. 3).

Acute neurochemical effects of antidepressant (vmPFC-DBS) and ineffective (STN-DBS) stimulation were analyzed via in vivo microdialysis with sample probes positioned in the NAcc of FSL animals (Fig. S2B, 4). Two-way repeated measures ANOVA with treatment and time as independent variables showed that there was no effect of any treatment on extracellular DA levels (treatment: $F_{(2,71)}$=0.611, p=0.549; time: $F_{(7,71)}$=1.414, p=0.213; treatment × time: $F_{(14,71)}$=0.723, p=0.745). As for DOPAC there was an effect for the factor treatment ($F_{(2,176)}$=11.185, p<0.001) and time ($F_{(7,176)}$=4.507, p<0.001) and an interaction between both factors ($F_{(14,176)}$=3.828, p<0.001). Post hoc tests showed an increase of DOPAC with STN-DBS compared to baseline, sham-DBS and vmPFC-DBS (each p<0.05). No effects were found on 5-HT levels, neither by treatment ($F_{(2,55)}$=0.183, p=0.834) nor time ($F_{(7,55)}$=0.0796, p=0.999) and no interaction between both factors ($F_{(14,55)}$=0.0966, p=1.000). There was an effect on levels of 5-HIAA by treatment ($F_{(2,182)}$=12.848, p<0.001) and time ($F_{(7,182)}$=11.167, p<0.001) and there was an interaction between factors ($F_{(14,182)}$=2.645, p=0.002), as levels increased with STN-DBS when compared to baseline levels and both other treatment groups (each p<0.05) and with vmPFC-DBS when compared to baseline (p<0.05).

Longterm neurochemical effects of antidepressant effective chronic-continuous vmPFC-DBS in FSL rats were compared to sham-DBS via post-mortem HPLC (Fig. 5). vmPFC-DBS decreased 5-HT contents in the stimulation target (t=3.200, p=0.009), and in the dorsal raphe (t=2.820, p=0.022) compared to sham conditions. Tissue contents of DA, DOPAC and 5-HIAA were not affected (all p's>0.06) (Table S2).

Study III: "Anti-anhedonic effect of deep brain stimulation of the prefrontal cortex and the dopaminergic reward system in a genetic rat model of depression: an intracranial self-stimulation paradigm study."

The efficacy of vmPFC-DBS to affect depressive- and anhedonia-like behavior was tested, as well as its reward-manipulating potential. In the FST, 2-way ANOVA showed an effect for treatment ($F_{(1,20)}$=7.448, p<0.05), while phenotype had no effect ($F_{(1,20)}$=2.8712, p=0.106) and phenotype × treatment interaction did not reach significance ($F_{(1,20)}$=3.324, p=0.086). As for the SCT, there was a main effect for stimulation ($F_{(1,20)}$=10.75, p<0.05). Phenotype had no effect ($F_{(1,20)}$=0.166, p=0.688) and no interaction was found between both factors ($F_{(1,20)}$=0.11, p=0.743) (Fig. 3). A 2x6 ANOVA on threshold shift data showed a main effect for treatment ($F_{(5,35)}$=36.483, p<0.001) but not for phenotype ($F_{(5,35)}$=2.073, p=0.193) and there was no interaction between factors ($F_{(5,35)}$=1.191, p=0.334) (Fig. 4). Holm-Sidak post hoc analysis revealed a left shift of the R/F function after D-amph treatment in FSL and FRL when compared to all other treatments (p<0.05). There was no significant curve shift with any other treatment.

Study IV: "Medial Forebrain Bundle Deep Brain Stimulation has Symptom-specific Antidepressant Effects in Rats and as Opposed to Ventromedial Prefrontal Cortex Stimulation Interacts With the Reward System."

The efficacy of MFB-DBS to affect depressive-associated behavior as well as its reward-manipulating potential were assessed. Two-way ANOVA showed in the SCT a difference for the factor phenotype ($F_{(1,26)}$=7.118, p=0.013) but not for the factor treatment ($F_{(1,26)}$=0.028, p=0.868). There was an interaction between phenotype and treatment ($F_{(1,26)}$=6.087, p=0.021). Post hoc tests revealed that the FSL-sham group consumed less sucrose than the control-sham group (p<0.05). A t-test on data normalized to the respective sham-group demonstrated a significant difference between MFB-stimulated and sham-stimulated FSL ($t_{(12)}$=2.238, p=0.045), while no difference was found between MFB-DBS and sham-DBS treated controls ($t_{(14)}$=1.472, p=0.163). As for the time spent immobile in the FST, there was an effect for the factor phenotype ($F_{(1,23)}$=51.306, p<0.001). There was no effect for treatment ($F_{(1,23)}$=2.695, p=0.114) but an interaction effect between both factors ($F_{(1,23)}$=5.144, p=0.033). Post hoc tests showed DBS to decrease time spent immobile in FSL rats (p<0.05). Anxiety-like behavior as measured in the EPM differed between treatment groups ($F_{(1,24)}$=4.411, p=0.046), but not between phenotypes ($F_{(1,24)}$=2.457, p=0.130). There was no interaction between both factors

($F_{(1,24)}$=0.395, p=0.536). In the LH-test no effects were found on the summed latency to escape from shock for the factors phenotype ($F_{(1,23)}$=3.354, p=0.080) and treatment ($F_{(1,23)}$=0.117, p=0.735) and no interaction between factors ($F_{(1,23)}$=0.087, p=0.771) (Fig. 3).

Regarding the reward-related effects as measured in the ICSS (Fig. 4), there were changes in thresholds for the factors intervention ($F_{(6,90)}$=60.15, p<0.0001) and group ($F_{(3,90)}$=7.461, p=0.003) and an interaction between both factors ($F_{(18,90)}$=1.872, p=0.028). Post hoc tests showed a right shift of the R/F function after haloperidol and DBS+haloperidol when compared to saline and sham-DBS in FSL and control rats (each p<0.05). DBS alone did not induce an effect on R/F function compared to sham-DBS. Further post hoc tests analysis revealed that in control but not in FSL rats, MFB-DBS+haloperidol induced an increase in the threshold (p<0.05), while each intervention alone remained ineffective.

Discussion

This thesis aimed at investigating i) biochemical and electrophysiological characteristics of the FSL animal model of depression, ii) symptom-specific stimulation effects of different brain sites at different stimulation parameter and at different expression levels of depression, iii) whether the anti-anhedonic effect of vmPFC-DBS is mediated via the mesolimbic brain reward system, iv) the antidepressant effectiveness of MFB-DBS, and v) whether vmPFC-DBS and MFB-DBS operate via the same neurobiological circuits.

FSL rats show elevated 5-HT levels in most cortical and subcortical brain regions and decreased alpha, beta and low gamma oscillatory activity in the mPFC and NAcc.

Detailed knowledge about the underlying pathophysiology of the animal model used in preclinical research is fundamental for correct data interpretation. Therefore, in *Study I* biochemical traits and neural population activity of the FSL rats were further characterized.

FSL rats were reported to have increased levels of 5-HT and 5-HIAA in the hippocampus, hypothalamus, NAcc and PFC and this is possibly due to decreased 5-HT receptor availability (54). In line with this we found 5-HT and 5-HIAA levels of FSL rats to be increased in the cortical areas investigated (mPFC, OFC) and the subcortical regions thalamus, EP and GP when compared to control rats. In MDD patients an increase of the 5-HT system has been reported, as well as no alteration of the system and decreased activity (for review (55)). Such diverging findings demonstrate the multifaceted nature of MDD with different neurobiological states to possibly cause different behavioral symptom profiles (56). The FSL rats might hence represent a

model of one such MDD subgroup.

Parallel to neurochemical characteristics that may underlie certain pathologies, specific patterns of synchronized neuronal activity might reflect pathological states (57). One method to measure such oscillatory activity is the recording of local field potentials (LFP), which represent the summed synchronized activity of multiple neurons (58). We here found decreased oscillatory activity in the low gamma band in the vmPFC and NAcc of FSL when compared to control rats. In humans, gamma band activity has been associated with emotion regulation (59) and found to be reduced in prefrontal areas of MDD patients (60,61). In the FSL rats decreased gamma band activity has also been shown in the VTA (53). DBS of the VTA normalized gamma band activity and reduced depressive-like behavior of FSL rats (53,62,63). In combination with our data of *Study II* this shows that gamma band activity is reduced in areas in which DBS is antidepressant effective (vmPFC, NAcc, VTA). We therefore hypothesize that reduced gamma band activity may be a characteristic pathophysiological trait of FSL rats and that normalization of these activity patterns may mediate antidepressant effects. Supporting this hypothesis we found increased gamma band activity in the STN of FSL and in *Study II* depressiogenic effects of STN-DBS.

Antidepressant DBS effects are symptom-, parameter-, and target-specific.

Given the still limited effectiveness of antidepressant DBS it is important to improve the outcome of todays stimulation treatments. An approach to ameliorate DBS efficacy might be the consideration of the individual symptom profile when selecting the DBS target (64), as the symptom profiles of MDD patients likely reflect different underlying pathological mechanisms or MDD subgroups (56,65). Therefore in *Study II*, the major study of this thesis, we investigated antidepressant efficacy of different stimulation sites and parameters in different symptoms relevant for depression. We first applied chronic-intermittent DBS as commonly applied in animal experiments (41,44,66–68) to the vmPFC, NAcc or STN in the FSL animal model of depression. STN-DBS served as a negative control for our setup as STN-DBS was found to induce depressive-like behavior in rodents (69) and 8% of Parkinson patients with STN-DBS treatment experience depression (70). Accordingly, FSL rats consumed less sucrose, became immobile earlier and displayed a higher tendency towards learned helplessness compared to animals receiving vmPFC-DBS. vmPFC-DBS increased sucrose consumption in FSL and control rats, while NAcc-DBS effects on anhedonia became only evident when looking at the individual electrode placement, reflecting the subregion-specific NAcc-DBS effects as previously reported by others (71). Both stimulation sites had equivalent beneficial effects in the

FST as latency to immobility increased in both treatment groups, which is congruent with previous reports of comparable antidepressant effects of vmPFC- and NAcc-DBS in naïve rats (20). In the LH paradigm, however, only vmPFC-DBS showed a tendency to reduce helplessness. Taken together, these findings suggest that antidepressant DBS-effects are symptom-specific and support the notion that patients with different symptom profiles could benefit from different stimulation targets (64,65).

Meng et al. (2011) found increased antidepressant DBS effects with increased DBS duration (67) and further, chronic-continuous stimulation is the protocol applied in clinical settings (72,73). In our next experiment we therefore tested whether the effects observed under chronic-intermittent vmPFC- and NAcc-DBS in experiment 1 would increase with administration of chronic-continuous stimulation. Surprisingly, we found that chronic-intermittent DBS was more beneficial and thus outperformed chronic-continuous DBS. In this context, application of scheduled, intermittent DBS instead of continuous DBS was recently found beneficial for treating Tourette syndrome (74). Of interest for future preclinical research is our finding that chronic-intermittent DBS does suffice to induce behavioral effects and thus intermittent stimulation does not necessarily needs to be replaced by continuous DBS as applied in the clinic.

Due to its invasiveness DBS is only applied in patients suffering from therapy-resistant depression. However, most preclinical research investigating the antidepressant potential of DBS use naïve animals (18–22). Similar to the fact that antidepressant drugs are ineffective in healthy humans (75), some antidepressant DBS effects might "need" a pathologocial system to work (76,77). The FSL rat is a model of depression pathology, yet it is responsive to all common antidepressant therapies (26–30). In experiment 3 we used the cLH animal model of TRD, which neither responds to electroconvulsive stimuli nor standard antidepressant drugs (37,78), and tested whether the most promising stimulation setting from experiment 1 and 2 would trigger antidepressant effects. Our data showed chronic-intermittent vmPFC-DBS however to be ineffective in cLH animals. Off note, the cLH rat line is not resistant per se: pharmacological inhibition of the habenula (79), enriched environment during young age (80), and application of a monoamine oxidase-B inhibitor (81) effectively reduced depressive-like behavior in cLH rats. In this context our data suggests vmPFC-DBS to not suffice for all expression levels of depression. This rather sobering finding is in line with recent reports of lacking Cg25-DBS efficacy in the clinic (17).

Biochemical mechanisms of DBS.

As presented above, FSL animals are characterized by elevated intracellular 5-HT levels. Zangen et al. found that increased 5-HT content levels in FSL rats were reduced upon antidepressant treatment (82). In line with this we found chronic-continuous vmPFC-DBS to decrease 5-HT tissue content in the mPFC and dorsal raphe of FSL animals. Zangen et al. later found that mere basal extracellular levels of monoamines do not correlate with depressive-like behavior in FSL rats. Instead depressive-like behavior was related to an interaction between serotonin and dopamine within the NAcc, with an injection of 5-HT to induce an increase of DA levels in control animals. This 5-HT/ DA interaction was found absent in FSL animals. Application of chronic antidepressant treatment normalized 5-HT/DA interactions and depressive-like behavior in FSL rats (83). Accordingly, when measuring acute DBS effects, we found both antidepressant vmPFC-DBS and depressiogenic STN-DBS to increase 5-HT turnover in the NAcc of FSL rats. However, solely STN-DBS also increased DA turnover indicating that STN-DBS may not only disturb 5-HT activity (69) but also 5-HT/DA interactions.

Antidepressant vmPFC-DBS does not operate via the mesolimbic brain reward system.

In *Study III* we replicated the antidepressant and anti-anhedonic efficacy of vmPFC-DBS found in the preceding study using the SCT and FST. With anhedonia being a cardinal symptom of depression we aimed at further investigating the anti-anhedonic effect of vmPFC-DBS. Anhedonia suggestively reflects disruptions within the brain reward system (84), which has the two primary components ventral tegmental area (VTA) and NAcc interconnected by the medial forebrain bundle (MFB). We tested whether the anti-anhedonic effect of vmPFC-DBS is mediated via the brain reward system by subjecting animals to the curve-shift variant of the ICSS paradigm (42). In this test paradigm animals receive rewarding electric pulses of varying frequencies to the MFB when pressing a lever. The threshold frequency at which an individual rat is willing to press the lever in order to receive MFB stimulation is stable once the animal is trained. The performance of an animal can be depicted by relating its response rates (=lever presses) to the stimulation frequency in a sigmoidal response/frequency (R/F) curve. The threshold is defined as the stimulation frequency yielding 36.7% of the asymptotic response rate (i.e. maximum rate of lever pressing). An intervention that has reward-facilitating effects induces a decrease of the threshold as the stimulation is rewarding already at lower frequencies due to a hyperactive brain reward system (85). Such a reward-facilitating effect is reflected by a curve-shift of the R/F curve to the left compared to the baseline curve. Accordingly, reward-attenuating interventions induce a rightward shift (42). Neither low nor high frequency vmPFC-DBS

induced a curve-shift, indicating the absence of manipulating efficacy within the mesolimbic brain reward system.

Antidepressant MFB-DBS does operate via the mesolimbic brain reward system.

Based on recently published beneficial effects of MFB-DBS in the clinic (for review see (86)), in *Study IV* we assessed the antidepressant potential of MFB-DBS in the FSL rat model.

MFB-DBS decreased time spent immobile in FSL rats, reflecting its anti-depressant efficacy as described in the clinic, and, as recently published, in naïve Sprague-Dawleys (22). Further, MFB-DBS treated rats consumed more sucrose solution in the SCT than sham-controls.

Based on the latter effects we further aimed at testing whether MFB-DBS as opposed to vmPFC-DBS operates via the mesolimbic brain reward system using the ICSS paradigm similar to *Study III*. To obtain even more specific results we added an intervention that combined MFB-DBS with drugs that interact with the DA system, as stimulation to the MFB was the baseline that was compared with and hence the combination of MFB-DBS and ICSS might not suffice to conclude on the impact of MFB-DBS on the brain reward system. Here we used the DA receptor blocker haloperidol, which, when applied alone, induced a reward-attenuating effect. Application of haloperidol plus MFB-DBS induced a larger threshold shift than haloperidol alone in control rats, suggesting that effects of MFB-DBS are mediated via the reward system. This finding only became visible in control rats which might be due to the disrupted accumbal 5-HT/DA interactions of FSL animals mentioned above (83). Reward-related MFB-DBS effects were associated with the activation of the 5-HT system (87–90) and hence our data suggests that the response of MFB-DBS plus haloperidol is mediated via functional 5-HT/DA interactions. Congruently, a recent publication showed that a mere increase of accumbal monoamines do not mediate antidepressant MFB-DBS efficacy, as antidepressant MFB-DBS in naïve rats did not induce acute increases of DA or 5-HT in the NAcc (22).

In contrast, application of haloperidol plus vmPFC-DBS did not change the threshold shift beyond the shift induced by the drugs alone. Congruent with *Study III*, this indicates that vmPFC-DBS does not act via the brain reward system. Likewise this indicates that MFB-DBS and vmPFC-DBS act via different neurobiological circuits. Recent publications support this notion: expression levels of the immediate early gene *zif268*, which indicates neuronal activity, increased in cortical regions only upon vmPFC-DBS (20). MFB-DBS also affected subcortical regions, with VTA and NAcc shell to increase and dentate gyrus to decrease *zif268* signals (22). These findings are interesting in the context of considering the individual symptom profiles/ affected underlying circuit of a patient when selecting the stimulation target.

In conclusion, this thesis demonstrated that targeting the vmPFC results in stronger antidepressant effects than NAcc-DBS in the FSL rat model of depression. Antidepressant effects in FSL rats are likely mediated via the 5-HT system and normalized gamma band activity pattern. Increased duration of DBS continuity and prolonged application does not add beneficial effects, which is interesting considering the predominance of continuous stimulation in the clinic. Together with the sobering findings of failed efficacy in cLH animals and in the clinic, this thesis highlights the need to further improve DBS settings as applied today. Further, effective MFB-DBS and vmPFC-DBS operate via different neurobiological circuits, which is important when considering individual symptom profiles for selecting the stimulation target.

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Affidavit

I, Julia Rummel, certify under penalty of perjury by my own signature that I have submitted the thesis on the topic “Deep Brain Stimulation as a potential treatment for Major Depressive Disorder – 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

Declaration of any eventual publications

Julia Rummel had the following share in the following publications:

Publication 1: Voget M, **Rummel J**, Avchalumov Y, Sohr R, Haumesser JK, Rea E, Mathé AA, Hadar R, van Riesen C, Winter C. Altered local field potential activity and serotonergic neurotransmission are further characteristics of the Flinders sensitive line rat model of depression. *Behav Brain Res*. 2015
Contribution in detail: Conducted biochemical analyses and critically revised the manuscript.

Publication 2: **Rummel J**, Voget M, Hadar R, Ewing S, Sohr R, Klein J, Sartorius A, Heinz A, Mathé AA, Vollmayr B, Winter C. Testing different paradigms to optimize antidepressant Deep Brain Stimulation in different rat models of depression. *J Psychiatr Res.* 2016
Contribution in detail: Established behavioural test paradigms, conducted all surgeries, behavioural and biochemical testing of exp. 1 and 3 and some of exp. 2, performed data analyses and interpretation, drafted the manuscript.

Publication 3: Rea E*, **Rummel J***, Schmidt TT, Hadar R, Heinz A, Mathé AA, Winter C. Anti-Anhedonic Effect of Deep Brain Stimulation of the Prefrontal Cortex and the Dopaminergic Reward System in a Genetic Rat Model of Depression: An Intracranial Self-Stimulation Paradigm Study. *Brain Stimul*. 2014. *shared 1st authorship.
Contribution in detail: Established behavioural test paradigms (except ICSS), partly conducted surgeries, testing, data analyses and interpretation, drafting of the manuscript.

Publication 4: Edemann-Callesen H, Voget M, Empl L, Vogel M, Wieske F, **Rummel J**, Heinz A, Mathé AA, Hadar R, Winter C. 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 Stimul*. 2015
Contribution in detail: Established behavioural test paradigms (except ICSS and EPM), partly conducted surgeries and data analyses and critically revised the manuscript.

Signature of the doctoral candidate

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Copies of Selected Publications

Voget M, **Rummel J**, Avchalumov Y, Sohr R, Haumesser JK, Rea E, Mathé AA, Hadar R, van Riesen C, Winter C (2015). Altered local field potential activity and serotonergic neurotransmission are further characteristics of the Flinders sensitive line rat model of depression. Behav. Brain Res. *291*, 299–305. http://dx.doi.org/10.1016/j.bbr.2015.05.027

Rummel J, Voget M, Hadar R, Ewing S, Sohr R, Klein J, Sartorius A, Heinz A, Mathé AA, Vollmayr B, Winter C (2016). Testing different paradigms to optimize antidepressant deep brain stimulation in different rat models of depression. J Psychiatr Res *81*, 36–45. http://dx.doi.org/10.1016/j.jpsychires.2016.06.016

Rea E, **Rummel J**, Schmidt TT, Hadar R, Heinz A, Mathé AA, Winter C (2014). Anti-anhedonic effect of deep brain stimulation of the prefrontal cortex and the dopaminergic reward system in a genetic rat model of depression: an intracranial self-stimulation paradigm study. Brain Stimul *7*, 21–28. http://dx.doi.org/10.1016/j.brs.2013.09.002

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Curriculum Vitae

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

Due to data privacy my CV will not be published in the electronic version of my PhD thesis.

List of publications

Hadar R, Vengeliene V, Barroeta Hlusicke E, Canals S, Noori HR, Wieske F, Rummel J, Harnack D, Heinz A, Spanagel R, Winter C (2016). Paradoxical augmented relapse in alcohol-dependent rats during deep-brain stimulation in the nucleus accumbens. Transl Psychiatry *6*, e840.

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

Voget M, Rummel J, Avchalumov Y, Sohr R, Haumesser JK, Rea E, Mathé AA, Hadar R, van Riesen C, Winter C (2015). Altered local field potential activity and serotonergic neurotransmission are further characteristics of the Flinders sensitive line rat model of depression. Behav. Brain Res. *291*, 299–305.

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