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Milaine Roet

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MODULATING MICROCIRCUITS IN DEPRESSION

PROEFSCHRIFT

Ter verkrijging van de graad van doctor aan de Universiteit Maastricht,
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Volgens het besluit van het college van Decanen,
In het openbaar te verdedigen op vrijdag
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Milaine Roet
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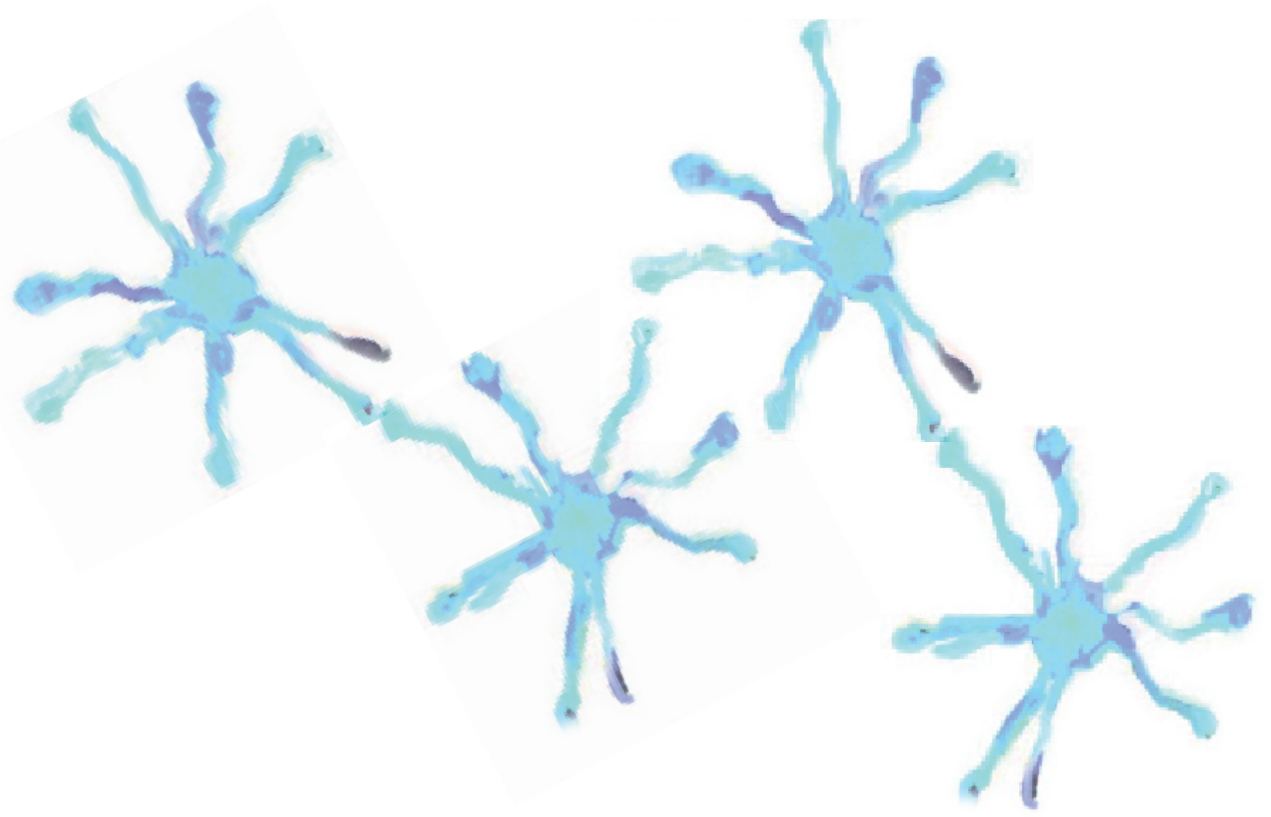
The research described in this thesis was performed at the School for Mental Health and Neuroscience, Department of Neurosurgery, Maastricht University, Maastricht, the Netherlands.

‘’There is no scientific study more vital to man than the study of his own brain. Our entire view of the universe depends on it”

Francis Crick

List of contents



1 General introduction

Major depressive disorder

Major depressive disorder (MDD) is a mental condition characterized by a depressed mood and a loss of interest in otherwise enjoyable daily activities for more than two weeks. For the diagnosis of MDD according to the Diagnostic and Statistical Manual of Mental Disorders number 5 (DSM-5), five of the following symptoms need to be present: a depressed mood, anhedonia, insomnia or hypersomnia, psychomotor retardation or agitation, loss of energy or fatigue, worthlessness or guilt, change in weight or appetite, impaired concentration or indecisiveness, and thoughts of death or suicidal ideation or an attempt [1]. Globally, MDD is the leading cause of disability with a worldwide prevalence of 4.4 %, affecting 322 million people in 2015 (Fig. 1). Almost half of the affected people live in South-East Asia or the Western Pacific region. A majority of MDD is seen in older adulthood, and females (5.1%) are affected more than males (3.6%) (Fig. 2). Its prevalence increased by 18.4% between the years of 2005 and 2015, indicating the relative growth of the age groups in which MDD mostly occurs [2, 3]. Because of its high and increasing prevalence, an appropriate treatment for MDD is important.

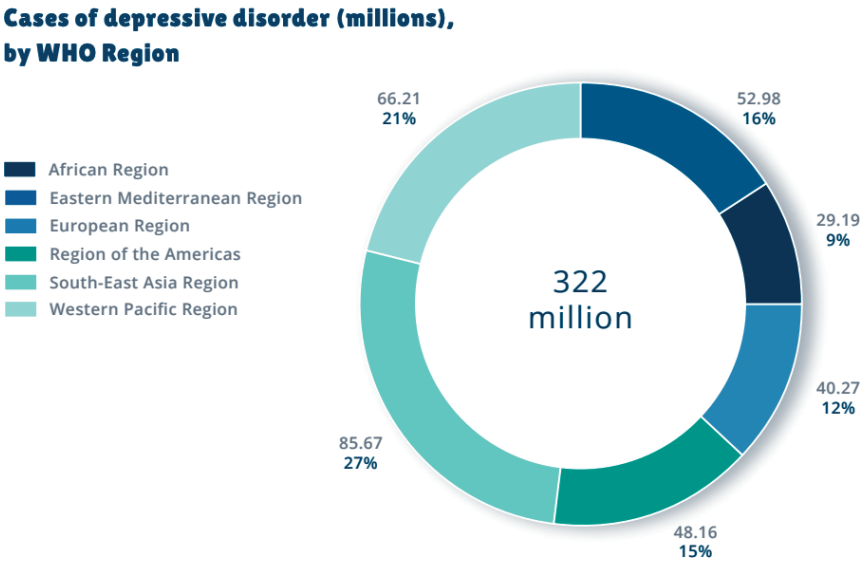


Figure 1. Regional distribution of depressive disorder cases. Adopted from WHO, Depression and Other Common Disorders 2017 [2].

The treatment

MDD can be treated using antidepressant medication and psychological therapies [4]. However, approximately one-third of treated patients do not respond adequately to these treatments [5]. These patients suffer from treatment-resistant depression (TRD), which is associated with more mental-health disorders, hospitalizations, past suicide attempts and consequently higher treatment costs compared to non-TRD [6, 7]

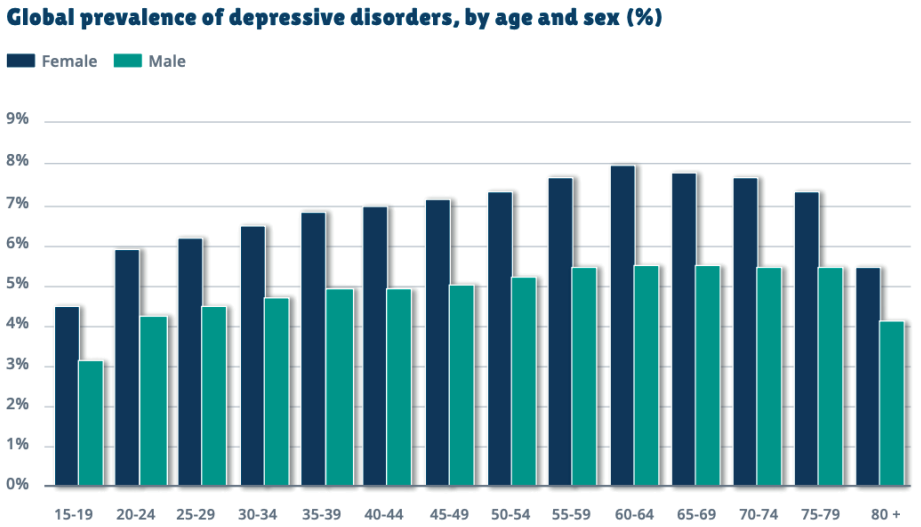


Figure 2. Global prevalence of depressive disorder, by age and gender. Adopted from WHO, Depression and Other Common Disorders 2017 [2].

For TRD, different therapies modalities can be given, such as, electroconvulsive therapy (ECT), vagal nerve stimulation (VNS), transcranial magnetic stimulation (TMS) and deep brain stimulation (DBS).

When immediate medical care for TRD is needed due to worsening of depressive symptoms, ECT can be given. In ECT, a small electrical current is passed through the brain under general anesthesia causing a series of seizures. Generally 70% of patients obtain a significant release of symptoms after multiple sessions, however, the relapse rate after 6 to 12 months is around 50% [8, 9].Therefore, ECT is not considered a cure, but rather a tool to overcome an acute phase of depression with severe symptoms. Its side-effects can include both short- and long-term memory loss.

VNS is the first invasive neurostimulation technique used for the long-term treatment of TRD and was approved by the FDA in 2005. Research has shown that after two years of VNS stimulation, 53.1% of TRD patients fulfilled the response criteria of a 50% reduction in depression scores [10]. Furthermore, it has been shown that adjunctive VNS to antidepressant treatment can significantly improve the quality of life in TRD [11]. Side-effects can include voice alteration, cough and pain [10].

A less invasive form of TRD therapy is repetitive transcranial magnetic stimulation (rTMS). In TMS, a magnetic field is used to modulate mostly superficial neuronal cells in the brain [12]. Meta-analysis for rTMS randomized controlled trials (RCTs) showed a pooled remission and response rate of 16.0% and 25.1% for unilateral rTMS and 5.7% and 11.0% for sham treatment, respectively. The remission rate is ranged from depression scores of 7 or less to 10 or less, while the response rate is defined as a 50% or more reduction in depression scores. The pooled remis-

sion and response rates for bilateral rTMS were 16.6% and 25.4% for rTMS and 2.0% and 6.8% for sham treatment, respectively [13].

Both ECT and TMS generate wide disperse modulation signals, making the technique not circuit or cell group specific and mostly suitable for more superficial brain regions, although certain deep TMS coils for stimulation of deeper brain regions in TRD exist with a response- and remission rate of 38.4% and 32.6% for deep TMS and 21.4% and 14.6% for the sham group, respectively [14].

DBS is another stimulation method, evolved in parallel, not having the major limitation of such a wide disperse signal as evoked with ECT and TMS, with the additional possibility to reach deeper brain regions.

Deep brain stimulation

DBS is an invasive treatment modality that is widely investigated for TRD. In DBS, an electric current is given through implanted electrodes in the brain using stereotaxis [15]. DBS has shown to be of great therapeutic value in Parkinson's disease, refractory epilepsy, Tourette's syndrome and obsessive compulsion disorder (OCD) [16-19]. Motivated by this success and since DBS in OCD patients consistently showed improvement in mood, DBS has been applied for TRD [19, 20]. Different brain regions for DBS for TRD have been targeted based on the imbalance of the limbic cortico-striatal-thalamic-cortico (CSTC) mood circuit and the involved brain regions [21, 22]. So far the subgenual cingulate cortex (SCC/SCG) which is showed to be hyperactive in untreated MDD patients [23], the nucleus accumbens (NAcc) which is involved in different cognitive functions such motivation and reward [24], the ventral capsule/ventral striatum (VC/VS) which is also showed to be hyperactive in untreated MDD and OCD patients [25], the ventral part of the anterior limb of the internal capsule (vALIC) initially studied in OCD patients showed additional anti-depressant effects [26], the lateral habenula (LHb) which is negatively associated with reward and presumably hyperactive in untreated MDD patients, the inferior thalamic peduncle (ITP) which is thought to play a role in non-reward tractor theory of depression where the non-reward system is more easily triggered in depression leading to negative emotional states [27], the bed nucleus of the stria terminalis (BNST) responsible for integrating a proper response to environmental and social setting changes [28] and the medial forebrain bundle (MFB) important in reward-seeking behavior [29], have been stimulated with DBS in TRD[30-37](Fig. 3).

Although case reports and open-label trials have shown promising results for DBS in TRD, several RCTs showed inconsistent results [38-42]. In summary, Dougherty et al. 2015 demonstrated that DBS in the VC/VS of thirty TRD patients did not significantly improve the response rate in depression rating scales compared to the control group after a 16-weeks trial period [38]. However, Bergfeld et al 2016 has shown that when stimulating the ventral anterior limb of the ventral capsule (vALIC), 10 out of 25 patients showed a significant decrease in depressive symptoms after a 12-week, double blind, cross-over study [39]. A third RCT named the BROADEN-trial has

been aborted preliminary due to a poor futility analysis. No significant decrease in depression rating scales after a 6 months trial period was found [40]. A fourth DBS study, investigating VC/VS stimulation in eight TRD patients was also discontinued due to a futility analysis showing no

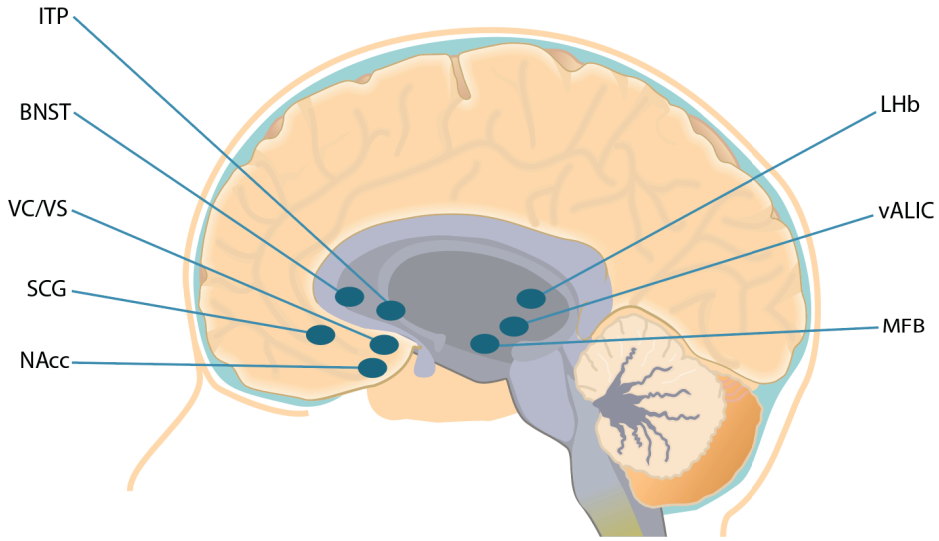


Figure 3. Deep brain stimulation targets for treatment resistant depression. The different brain regions research for DBS in TRD. SCG; subgenual cingulate cortex, NAcc; nucleus accumbens, VC/VS; ventral capsule/ventral striatum, vALIC; ventral part of the anterior limb of the internal capsule, LHb; lateral habenula, ITP; inferior thalamic peduncle, BNST; bed nucleus of the stria terminalis, MFB; medial forebrain bundle.

significant difference between active versus sham stimulation after a 16 weeks trial period [41].

Altogether, these results imply that DBS of the VC/VS, SCC, or vALIC does not cure TRD in all patients, but showed to significantly improve depression rating scales in certain subgroups of patients. Since the primary outcome of RCTs is an average decrease in depression rating scales for all patients, individual effects might be overshadowed which implicates that we are in need for a more personalized DBS approach.

Depression; subdividing its heterogeneity into subgroups

The inconsistent results of open-label trials and RCTs raised discussions concerning the correct interpretation of trial results, patient selection criteria, optimal stimulation parameters, the indication of different stimulation targets and the underlying pathological circuits modulated by DBS.

Recent research has revealed 4 connectivity-based biotypes of depression when analyzing resting-state connectivity. Their results show that the four biotypes are defined by either increased anxiety (biotype 1 and 4), increased anhedonia and psychomotor retardation (biotypes 3 and 4)

or increased anergia and fatigue (biotypes 1 and 2) (Fig. 4)[42]. Such complexity of TRD should be taken into account when evaluating the results of DBS treatment approaches and for future treatment of TRD. Study outcomes should be interpreted using subgroups of depression which creates the possibility to make future treatments more individualized proven to be beneficial in other psychiatric diseases such as OCD [43].

Furthermore, we need to better understand the various underlying pathological circuits in TRD creating multiple subtypes of TRD and integrate them with our present diagnostic categories to reach a better nosology and treatment.

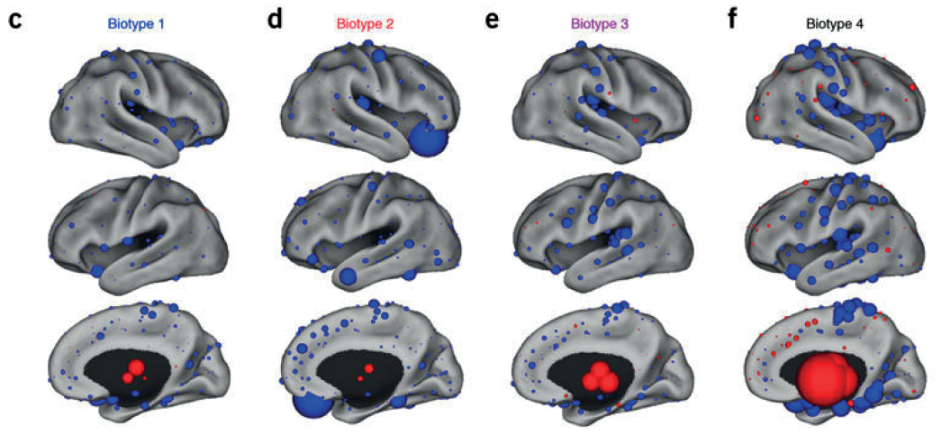


Figure 4. Functional connectivity biomarkers for diagnosing neurophysiological biotypes of depression. (c–f) The neuroanatomical locations of the nodes with the most discriminating connectivity features are illustrated for each biotype for the four-cluster solution, colored and scaled by summing the results of Wilcoxon rank–sum tests of patients as compared to controls across all connectivity features associated with that node. Red represents increased and blue decreased functional connectivity in depression. Adopted from Drysdale et al. 2017 [36].

Animal models of depression

To study depression, different clinical and pre-clinical approaches are available. To investigate and modulate the neuronal microcircuits that underlie depression, animal models are valuable tools. To mimic depression, various animal models have been developed. Among those, the 'Chronic Unpredictable Stress' (CUS) model is a well-validated and widely used model for depression [44]. This CUS model is based on the loss of responsiveness to a reward when rodents are subjected to a schedule of minor stressors over a long period of time. It aims to model a chronic depressive-like state that develops gradually over time in response to stress and unfortunate events, mimicking the natural induction of depression, thereby improving its translational potential [45-46].

Since previous research done in our laboratory has shown anti-depressant effects when stimulating the ventromedial prefrontal cortex (vmPFC), homologous to the SCG in humans, with DBS in rats exposed to CUS, I continued using this model [47].

We need more information regarding the responsible circuits for depressive behavior in humans, therefore I will investigate if the depressive traits seen in depressive rats can be clustered as well, researching possible microcircuits for different depressive behavior. With this research, I hope to get a better understanding of the involved microcircuits in depression and possible confounding factors for results which are seen in human research thus far.

I hypothesize that different microcircuits are responsible for different modalities seen in the depressive-like behavior, and that stimulating these different microcircuits with DBS will alleviate different depressive-like behavior in modalities such as; anxiety, behavioral despair, motivation or (an)hedonia. This will help us further in identifying microcircuits responsible for different subtypes of depression and future DBS treatment for TRD.

Exciting microcircuits

Standard electrical DBS works on a macro-scale and might not be precise enough to stimulate microcircuits, since the electrical currents spreads widely across multiple neurons. For this reason, we introduce a novel technique called 'magnetothermal DBS' (mDBS) in this thesis. This technique communicates with neurons in a more delicate way, on a nano-scale and wirelessly. MDBS works through magnetic nanoparticles (MNPs) which heat when exposed to an alternating magnetic field (AMF). This heat signal can activate heat-sensitive cation channels in close proximity which consecutively induce neural excitation. The AMF leaves the rest of the body unaffected which limits the occurrence of unwanted side-effects. MDBS allows for a wireless, remote control of neural activity [48]. In this thesis we investigated if this novel technique works in naïve mice, to be further explored for microcircuit activation and as treatment for disorders in the future.

Personalized approach in treating TRD

By combining the knowledge of subtypes in depression and possible microcircuits responsible for different behavioral traits in TRD, I hope to motivate a more personalized approach in the treatment of TRD with DBS. To my knowledge, this will improve DBS trial results and more importantly, help patients suffering from this disorder.

AIM OF THE THESIS

Improving DBS for TRD by investigating depression subtypes

The overall aim of this thesis is to study if depression with its various depressive-traits can be subdivided into different subtypes with different pathologically disturbed microcircuits. To study this, I focus on the prefrontal cortex and investigate if DBS of subregions of the prefrontal cortex alleviate particular symptoms of depression in the CUS animal model of depression. I have stimulated the infralimbic- (IL), prelimbic- (PreL) and dorsal peduncular (DP) cortex.

Stimulating microcircuits and refining the interface of neuronal modulation

The second aim of this thesis is to explore the feasibility of modulating microcircuits with a more advanced wireless technique of neuromodulation called mDBS.

The following five research question are formulated and addressed in this thesis.

Research questions of the thesis

- How can we further improve deep brain stimulation outcomes for treatment-resistant depression?
- Can we disentangle depression into multiple microcircuits responsible for different modalities seen is this disorder using an animal model of depression?
- Can the current method of deep brain stimulation be improved with the usage of nanoparticles, so that deep brain stimulation could potentially stimulate microcircuits and work wirelessly?
- Does magnetothermal deep brain stimulation, which operates with nanoparticles, work in animal models?
- Is it possible to apply magnetothermal deep brain stimulation in humans?

OUTLINE OF THE THESIS

MDD is the leading cause of disability worldwide with a prevalence of 4.4 %, affecting 322 million people in 2015 [2]. Approximately 30% of MDD patients do not responds adequately to treatment, causing TRD. For TRD, different treatment modalities such as DBS in various brain regions has been researched. In **chapter 2,** I will elaborate on the different brain regions stimulated in TRD, the results of open-label and placebo-controlled trials. Furthermore, I will go into detail about possible subtypes of depression and a personalized treatment approach for DBS in TRD in the future. In **chapter 3,** I research the possibility of subgroups in depression and depressive-traits in an animal model of depression. I dissected the anti-depressant effects of DBS in the vmPFC cortex in 'depressed' rats by subdividing the vmPFC into the PreL-, IL- and DP cortex. I investigated if high frequency (HF) DBS in these different subregions alleviated different depressive-like behavior in modalities such as; (an)hedonia, anxiety, behavioral despair and lack in motivation. This will pave the way to a better understanding of previous DBS trial results and a better understanding of a future personalized treatment. When investigating the different subregions of the prefrontal cortex, the DP cortex seemed non suitable for electrical stimulation and caused overt seizures in the stimulated animals. This unexpected side-effect is extensively described in **chapter 4**. To more precisely stimulate micro-circuitries on a nano-scale, I provide an overview of the established forms of advanced neuromodulation, and integrate the possible usage of MNPs to further advance their applications in **chapter 5**. I extensively describe the mechanism of optogenetics, designer receptors exclusively activated by designer drugs (DREADD), focused ultrasound and magnetic neuronal modulation with emphasis on mDBS. In **chapter 6**, we introduce a proof of principle of the new advanced technique mDBS. This research was done in collaboration with the department of Material Science and Engineering (DMSE) of the Massachusetts Institute of Technology (MIT, USA). We investigated mDBS in naïve mice and were able to control their rotation movements. We hope that future research incorporates this technique in other disease models such as our depression model in rats, so that it may pave the way to a non-invasive but highly specific method of neuromodulation. In **chapter 7,** I report on endogenously expressed TRPV1 in the human brain since this protein is needed for mDBS to function. Using endogenously expressed TRPV1 might overcome the disadvantage of lentiviral delivery, which to date is needed in rodents experiments, making the technique more clinically applicable. Finally, I provide a general discussion and conclusion in **chapter 8,** in which I answer the research questions formulated in this introduction, address the limitations of my studies and future perspectives, and will end with my conclusion.

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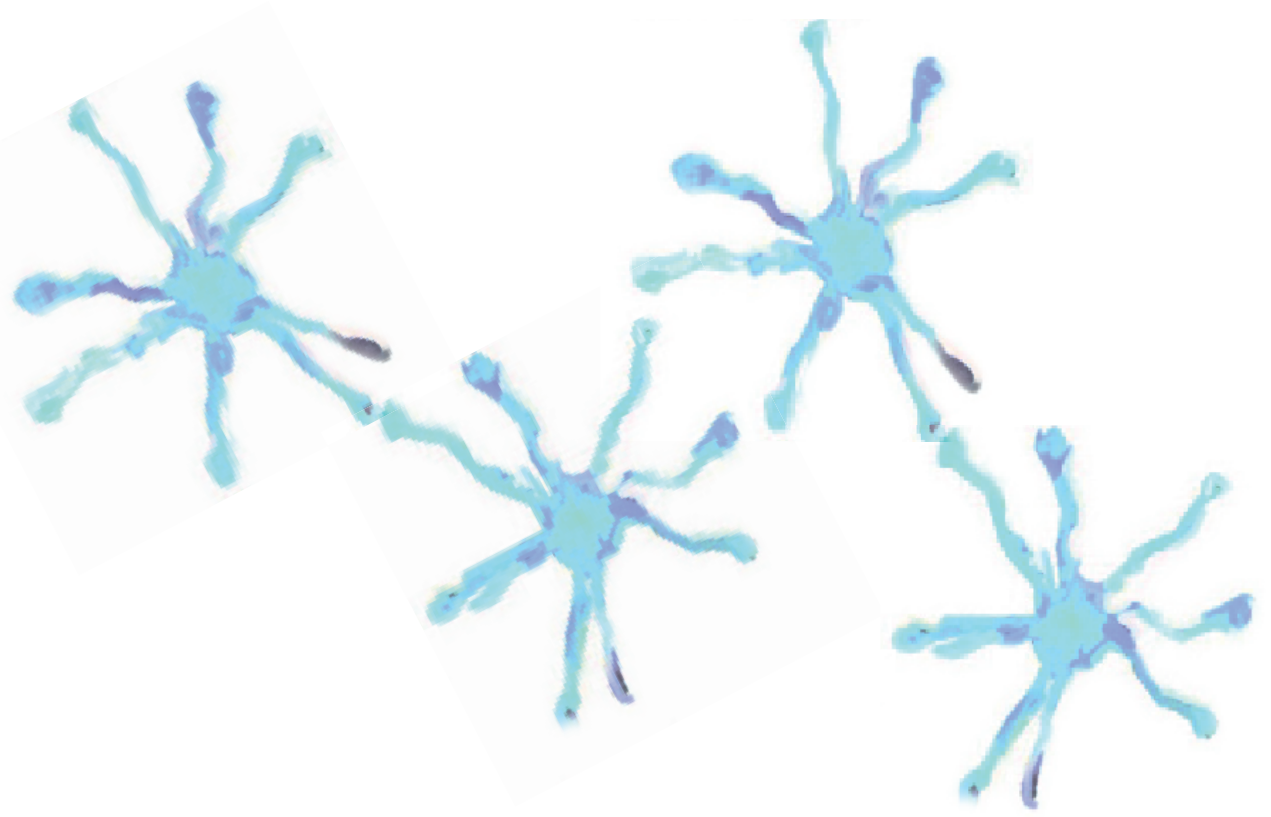

2 Deep Brain Stimulation for Treatment-Resistant Depression: Towards a More Personalized Treatment Approach

Milaine Roet, Jackson Boonstra, Erdi Sahin, Anne E.P. Mulders, Albert F.G. Leentjens and Ali Jahanshahi

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ABSTRACT

Major depressive disorder (MDD) affects approximately 4.4% of the world's population. One third of MDD patients do not respond to routine psychotherapeutic and pharmacotherapeutic treatment and are said to suffer from treatment-resistant depression (TRD). Deep brain stimulation (DBS) is increasingly being investigated as a treatment modality for TRD. Although early case studies showed promising results of DBS, open-label trials and placebo-controlled studies have reported inconsistent outcomes. This has raised discussion about the correct interpretation of trial results as well as the criteria for patient selection, the choice of stimulation target, and the optimal stimulation parameters. In this narrative review, we summarize recent studies of the effectiveness of DBS in TRD and address the relation between the targeted brain structures and clinical outcomes. Elaborating upon that, we hypothesize that the effectiveness of DBS in TRD can be increased by a more personalized and symptom-based approach. This may be achieved by using resting-state connectivity mapping for neurophysiological subtyping of TRD, by using individualized tractography to help decisions about stimulation target and electrode placement, and by using a more detailed registration of symptomatic improvements during DBS, for instance by using 'experience sampling' methods (ESM).

Keywords: major depressive disorder; treatment resistant depression; deep brain stimulation; neuropsychological subtypes; personalized treatment approach

1. INTRODUCTION

Major depressive disorder (MDD) is a common mood disorder that affects one's feelings, thoughts, and behavior. According to the Diagnostic and Statistical Manual of Mental Disorders number 5 (DSM-5), for a diagnosis of MDD, five of the following symptoms need to be present for at least two weeks: depressed mood, reduced interest or pleasure, weight loss or reduced appetite, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, worthlessness or excessive guilt, impaired concentration or indecisiveness, and recurrent thoughts of death or suicidal ideation or attempts. Either 'depressed mood' or 'loss of interest or pleasure' is essential for a diagnosis [1]. The total number of people suffering from MDD worldwide was estimated to be 322 million in 2015 and its prevalence increased by 18.4% between 2005 and 2015 [2]. Therefore, effective treatment of MDD merits intense consideration.

Whereas psychotherapy and antidepressant medication are effective in the majority of patients, approximately one third of patients do not respond to these therapies. In the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial, the cumulative remission rate of MDD patients after four successive treatments was 67% [3]. In line with this, a meta-analysis of 92 studies of the effectiveness of psychotherapy showed that 62% of patients no longer met the criteria of depression after treatment [4]. Failure to respond to a treatment algorithm of several steps is commonly referred to as treatment resistance, although there is still discussion about the exact definition of treatment refractoriness [5]. Treatment-resistant depression (TRD) is associated with more (comorbid) mental health disorders, a higher number of hospitalizations, and more suicide attempts, leading to higher treatment costs compared to non-TRD [6]. In addition, patients with TRD show a higher demand of healthcare resources and costs of health care compared to non-TRD patients [7]. Various alternative treatment options for TRD are currently being investigated, including vagal nerve stimulation (VNS) [8], repetitive transcranial magnetic stimulation (rTMS) [9], and deep brain stimulation (DBS) [10].

The aim of this narrative review is to provide an overview of recent studies of the effectiveness of DBS in TRD with a special focus on the relationship between the targeted brain structures and clinical outcomes. Based on these findings, we discuss the importance of distinguishing between different clinical phenotypes of depression that would allow for more personalized symptom-based treatment approaches, which may be a key factor in improving treatment outcomes.

2. RECENT INSIGHTS ON THE PATHOPHYSIOLOGY OF DEPRESSION

It is hypothesized that in depression, there is an imbalance in the limbic cortico-striatal-thalamic-cortico (CSTC) mood circuits [11,12], yet many aspects of circuitopathy in MDD remain largely unknown. Based on different models [11,12], three main components of the CSTC mood circuits

have been proposed (Fig. 1). First, the ventral component is essential for recognizing emotions and initiating an adequate emotional and behavioral response. In this circuit, the amygdala, ventral striatum, ventral part of the anterior cingulate cortex, orbitofrontal cortex, ventrolateral prefrontal cortex, and downstream structures such as the hypothalamus and locus coeruleus are involved. Second, the dorsal component that regulates the emotional responses and requires cognitive processing. Here, the dorsolateral and dorsomedial prefrontal cortex, the dorsal part of the anterior cingulate cortex, and the hippocampus are involved. Third, a modulating region is present, although no consensus has been made about its precise anatomical organization and function. Some have suggested that this component consists of the thalamus and the rostral part of the anterior cingulate cortex [11–13]. As implied by Mayberg et al., the model of depression indicates that depression is associated with a decreased activity in dorsal limbic and neocortical regions and a relative increase in ventral paralimbic regions. Treatment of depression therefore requires the inhibition of the overactive ventral regions, resulting in the disinhibition of the underactive dorsal regions. To mediate this process, proper functioning of the rostral cingulate cortex is required [12]. These mood circuits overlap with the circuitry involved in compulsive traits; DBS of the ventral capsule/ventral striatum (VC/VS) in treatment resistant obsessive-compulsive disorder (OCD) patients has led to improvements in mood which prompted studying the application of DBS in TRD patients [14,15].

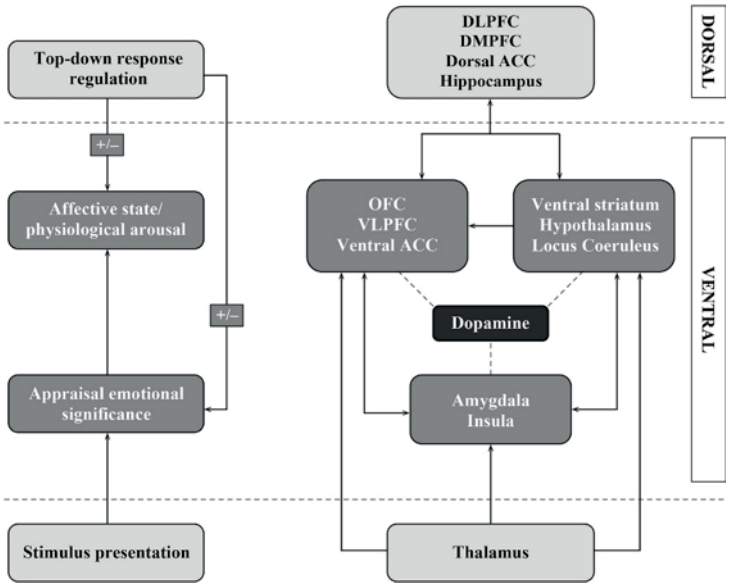

Figure 1. Schematic representation of emotional processing and its neurobiological base. Figure from Moonen et al. (2017) [82] with permission.

Expanding the Cortico-Striatal-Thalamic-Cortico Mood Circuits

One region that is not included in the CSTC mood circuits and yet has been a region of interest for DBS targeting in TRD for over a decade is the subgenual cingulate gyrus/cortex (SCG/SCC) [10]. This region has shown hyperactivity in untreated depressed patients [16], is part of the ventral component, and has projections to the amygdala, hippocampus, superior and medial temporal gyri, ventral striatum, mid- and posterior cingulate cortex, thalamus, hypothalamus, periaqueductal gray, and lateral habenula [17,18]. Furthermore, in recent years, it has become known that several other brain areas all belonging to the ventral component play a role in the pathophysiology of depression. Among these are the thalamic peduncles (THp) that interconnects with the prefrontal cortex including the orbitofrontal cortex (OFC) [19], the medial forebrain bundle (MFB) that projects to the frontal cortex, the nucleus accumbens (NAcc) and ventral striatum [20], and the ventral part of the anterior limb of the internal capsule (vALIC) which forms a homeostatic system with the MFB and the bed nucleus of the stria terminalis (BNST) [21] (Fig. 2).

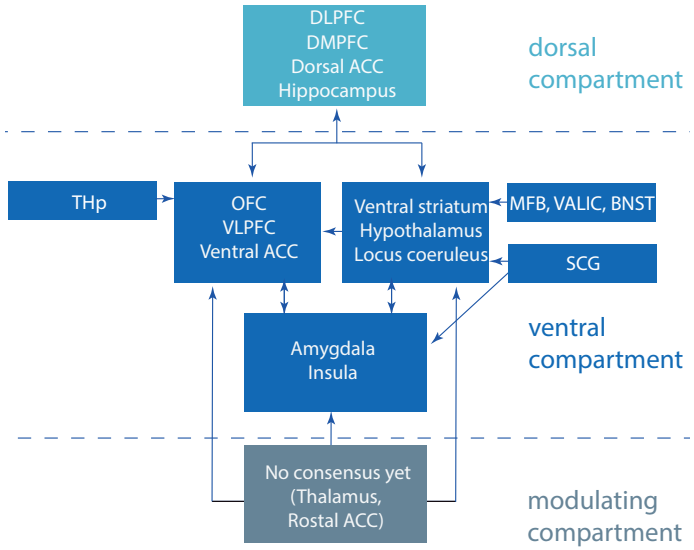

Figure 2. Cortico-striatal-thalamic-cortico mood circuits divided in a dorsal, ventral, and modulating compartment based on Alexander et al. [11], Mayberg et al. [12], and Moonen et al. [82] expanded with regions researched with deep brain stimulation (DBS) for treatment-resistant depression (TRD). DLPFC; dorsolateral prefrontal cortex, DMPFC; dorsomedial prefrontal cortex, ACC; anterior cingulate cortex, THp; thalamic peduncles, OFC; orbitofrontal cortex, VLPFC; ventrolateral prefrontal cortex, MFB; medial forebrain bundle, vALIC, ventral part of the anterior limb of the internal capsule, BNST; bed nucleus of the stria terminalis, SCG; subgenual cingulate gyrus, HPA axis; hypothalamic pituitary adrenal axis.

3. DEEP BRAIN STIMULATION FOR TREATMENT-RESISTANT DEPRESSION

DBS is an invasive neuromodulation technique that is effective in managing clinical symptoms of neurological and psychiatric disorders, such as Parkinson's disease (PD) [22,23] and OCD [24]. At stimulation settings commonly used in clinical practice, DBS decreases the spontaneous firing of neuronal populations and activates axonal projections near the electrode [25]. This modulates pathological activity and replaces it with regular patterns of discharge with intervals of burst activity [26,27]. More recent theories suggest that DBS destabilizes abnormal synchronous oscillatory activity within the basal ganglia circuitry improving hyperkinetic symptomology [23]. However, the exact mechanism(s) by which DBS normalizes electrical activity in the basal ganglia and exerts beneficial effects on PD symptoms remain unknown. In DBS for TRD, target selection has mostly been based on either neuroimaging studies or clinical observations of mood improvement following DBS in OCD [10,15,28]. For these reasons, the underlying mechanisms of action are poorly studied. DBS studies for TRD (Table 1) and the outcomes for selected brain targets (Table 2) are described below.

3.1. Subgenual Cingulate Gyrus/Cortex

The first clinical trial of DBS of the SCG for TRD was performed in 2005 and included six patients with MDD [10]. The severity of depression was measured using the Hamilton Depression Rating Scale (HDRS) and the Montgomory Asberg Depression Rating Scale (MADRS). The HDRS has been the gold standard for the assessment of depression for years [29]. A clinical response is commonly defined as a decrease in the HDRS score of more than 50% compared to baseline, and clinical remission is defined as a decrease in the HDRS score to eight or less. After one month, two out of six patients met the criteria for response. At the end of the sixth month, a response was seen in four out of six patients, with three of the patients reaching remission or near remission. Preliminary observations with positron emission tomography (PET) showed a metabolic hyperactive SCG (Brodmann area 25, Cg25) during depressive states. It was speculated that DBS would reduce this hyperactivity [16] (Table 2). The improvement in depression scores after DBS was thought to be due to effectively disrupting focal pathological activity in limbic-cortical circuits. After 3 months of stimulation of the subgenual cingulate region (Cg25) in patients suffering from TRD, local cerebral blood flow (CBF) was decreased in Cg25 and the adjacent orbitofrontal cortex (Brodmann area 11). Moreover, after three and six months of stimulation, CBF was decreased in the hypothalamus, anterior insula, and medial frontal cortex of long-term responders, while CBF increased in the dorsolateral prefrontal cortex (dlPFC), dorsal anterior, posterior cingulate, and premotor and parietal regions (Table 2) [10]. In the different open-label trials, response rates varied from 20 to 57% after 1 month, 33.3 to 87.5% after 6 months, and 29 to 62.5% after 12 months (Table 1) [10,30–41]. In a long term follow-up, Kennedy et al. (2011) reported response rates at 1, 2, and 3 years after DBS implantation in the SCC of TRD patients

of 62.5%, 46,2%, and 75%, respectively [30] (Table 1). In a case series of DBS of the SCG in five TRD patients, a decrease in the score of the depression rating scale was only found in one of the five TRD patients. This patient turned out to be stimulated in the posterior gyrus rectus (PGR) based on single subject tractography results rather than the initially targeted Cg25 [42]. A recent exploratory meta-analysis of four observational studies investigating DBS for TRD (Holtzheimer et al. 2012, Lozano et al. 2012, Puigdemont et al. 2012, and Kennedy et al. 2011) reported relatively large response and remission rates following DBS treatment: the twelve-month response and remission rates were 39.9% (95% CI = 28.4% to 52.8%) and 26.3% (95% CI = 13% to 45.9%). The included studies reported a significant decrease in depression scores between 3 and 6 months (Hedges' g = −0.27, $p = 0.003$), while no additional decrease was found between 6 and 12 months, suggesting that maximal antidepressant effects occur mostly within the first 6 months of treatment [43]. However, adverse events can occur, including worsening of depression, suicidal ideation, and seizures (Table 1). A study consisting of a double-blind active vs. sham stimulation phase of four weeks, followed by an open-label stimulation for up to 24 months, reported no significant differences between the active and sham stimulation of the SCG and no reduction in HDRS scores in the first four weeks. In the open-label phase, response rates were 37.5%, 43% and 23% after 6, 12 and 28 months, respectively. Remission rates were 12.5% and 14.2% at 6 and 12 months, respectively, and 33.3% at 24 and 28 months [44].

A randomized controlled trial (RCT) investigating DBS of the subcallosal cingulate, known as the BROADEN trial, was aborted prematurely. The study lasted six months, during which all patients should have received SCC implantation surgery. After six months, blinding would have been uncovered and both groups would have been offered open-label DBS for another six months. At the end of the first six months, responses of the treatment group and control group were predicted to be 40% and 18.5%, respectively. In this trial, the response rate was defined as more than or equal to a 40% decrease in MADRS scores from baseline. However, after six months, only 20% of patients ($n = 12$) in the treatment group showed a response versus 17% of patients ($n = 5$) in the control group. At that time, a futility analysis predicted the probability of a successful study outcome to be 17% or less leading to the funding for DBS electrodes for this study to be discontinued. The actual study was never published, but results were published and mentioned in Morishita et al. (2014) [45,46]. It has been postulated that the patients enrolled in the BROADEN trial had extreme and chronic depression with a mean duration of the current depressive episode of 12 years, nearly twice that of previous open-label studies. Therefore, these patients could have required a longer treatment period before significant results emerge. Long-term outcomes of SCG DBS in TRD patients for up to 8 years show that most patients have a sustained antidepressant response [40]. However, these results need to be interpreted carefully as the patient group consisted of both MDD and bipolar type-II disorder patients. Further comparison between high- and low frequency DBS in the SCG in TRD showed no significant difference in effectiveness between the two groups and a 44.44% response rate at 13 months of stimulation [47].

3.2. Nucleus Accumbens

Another brain region involved in MDD is the NAcc, part of the mesolimbic dopaminergic circuit involved in different cognitive functions such as motivation and reward [31] (Table 2). DBS of the NAcc exerts immediate and long-term positive clinical effects in TRD and has been shown to significantly improve depression scores within one week [31]. Visualized with PET–computed tomography (PET-CT or PET/CT), NAcc-DBS increased metabolic activity in the ventral striatum, dlPFC, dorsomedial PFC (dmPFC), cingulate cortex, and the amygdala. Furthermore, metabolic activity in the vmPFC, ventrolateral prefrontal cortex (vlPFC), dorsal caudate nucleus, and part of the thalamus were decreased. Targeting the NAcc was essential for the effect of DBS on anhedonia (i.e., the inability to feel pleasure) in patients suffering from TRD. However, when Schlaepfer et al. (2008) looked at single items of depression rating scales, capturing aspects of anhedonia such as 'work and activities', 'apparent sadness', and the 'inability to feel', no significant improvements were found following NAcc-DBS. A follow-up study showed a 50% response rate in 10 patients suffering from TRD undergoing NAcc-DBS after 10 months [32]. In a more recent study reporting the long term effects of NAcc-DBS, 45% of TRD patients (n = 11) were classified as responders with a 50% reduction in HDRS scores after 12 months of stimulation, which remained until the last follow-up of 4 years [33] (Table 1). Several side effects were reported, such as seizure, agitation, and a transient increase in anxiety. In addition, one attempted suicide and one completed suicide were reported, for which the relation with the DBS treatment is uncertain.

3.3. Ventral Capsule/Ventral Striatum

The VC/VS is thought to be hyperactive in MDD [48] (Table 2). Capsulotomy (i.e., lesioning) of the VC/VS improved not only OCD symptoms but also depressive symptoms, inspiring stimulation of the VC/VS for TRD [15]. In an open-label trial that stimulated the VC/VS in 15 TRD patients, responder rates at three months, six months, and 12 months were 53.3%, 46.7%, and 53.3%, respectively, using the MADRS as an outcome measure, and were 46.7%, 40%, and 53.3%, respectively, using the HDRS as an outcome measure [34]. Adverse events ranged from pain or discomfort at the incision site, to hypomania, mixed bipolar state, and increased depression due to battery depletion.

The first RCT of DBS of the VC/VS for TRD was performed by Dougherty et al. (2015) who stimulated 30 patients for 16 weeks. There were no significant differences in response rates between the intervention and sham group in the double-blind phase [49,50]. Another RCT of VC/VS DBS in eight TRD patients was discontinued after an interim futility analysis of active vs. sham stimulation showed no difference in effects between the two groups after 16 weeks. These results were never published but were discussed by Rezai et al. [51].

3.4. The Ventral Part of the Anterior Limb of the Internal Capsule

The anterior limb of the internal capsule (ALIC) is another brain region that was initially studied for DBS in OCD. One study aimed at stimulating the NAcc and discovered that most treated

OCD patients (9 out of 16) actually received DBS in the ventral part of the ALIC (vALIC), which improved obsessive compulsive scale scores, showed anti-depressive effects, and led to the clinical implementation of vALIC-DBS in TRD [28]. DBS of the vALIC has also been associated with a decreased metabolism in the OFC, subgenual anterior cingulate cortex, and right dlPFC [52–54] (Table 2).

The first RCT of DBS of the vALIC for TRD was conducted by Bergfeld et al. (2016), investigating 25 TRD patients during a 52 week open-label trial, which resulted in a significant decrease in HDRS scores in the whole group during the optimization phase, although overall HDRS scores were still in the depression range (22.2 at baseline vs. 15.9 after optimization phase). Ten of the 25 patients could be classified as responders, with a more than 50% decrease on the HDRS. After the optimization phase, a RCT with a cross-over design including nine responders and seven non-responders ensued and showed a significantly lower score in the active DBS phase compared with the sham DBS phase (mean HDRS score of 13.6 (95% CI; 9.8–17.4 vs. 23.1 (95% CI; 20.6–25.6)) (HDRS < 0.001). However, the scores on the HDRS in the active treatment group were still within the mild to moderate depression range [55]. Both crossover phases lasted approximately 21 and 18 days, respectively.

3.5. Lateral Habenula

The activity of the LHb is negatively associated with reward, meaning its neurons increase their firing rate in a non-reward situation or in the omission of a reward. LHb hyperactivity could therefore explain the lower reward-seeking behavior in TRD [56] (Table 2). Speculation that DBS of the LHb could lead to the inhibition of hyperactivity prompted the first case study of LHb-DBS in TRD, which notably led to full remission of the patients' depressive symptoms [57]. A clinical non-randomized study in six patients suffering from TRD is currently being held, investigating the safety, tolerability, and benefit of LHb DBS in TRD. Patients that respond at 12 months of stimulation will enter a randomized, staggered withdrawal phase. During this phase, a double-blind discontinuation will be attempted at month 12 or 13, decreasing the stimulation by 50% and then completely discontinuing it during the following two weeks. Evaluation will take place at 15 months, where, in the meantime, escape criteria are included, and if met, will stop the blinded phase and will be continued with an open treatment [58].

3.6. Thalamic Peduncles

The inferior thalamic peduncle (ITP) is a bundle of fibers connecting the OFC to the thalamus. The OFC is thought to play a role in the non-reward attractor theory of depression, where the orbitofrontal non-reward system is more easily triggered in depression, causing negative emotional states [59] (Table 2). Stimulating the ITP could disrupt this enhanced triggering and lead to less depressive symptoms. ITP stimulation for OCD has already shown improvements of the score on the Yale-Brown Obsessive-Compulsive scale in five OCD patients [60]. A case study in one TRD

patient reported that DBS of the ITP decreased depressive symptoms [61]. However, within this study, two brain regions were investigated, the second being the BNST.

3.7. Bed Nucleus of the Stria Terminalis

The BNST is involved in a range of behaviors, such as stress response, social behavior, and extended duration of fear states. This nucleus assesses sensory information from the environment, coupled together with the subjects current mood and arousal, integrating a proper response to environmental and social setting changes [21] (Table 2). Raymaekers et al. (2017) indicated that both BNST and ITP stimulation could alleviate depressive symptoms; however, due to a small sample size, no statistical analyses were conducted [61].

3.8. Medial Forebrain Bundle

The MFB is a fiber tract connected to various parts of the limbic system thought to play a role in reward-seeking systems [20] (Table 2). In one trial, DBS of the superolateral branch of the MFB resulted in more than a 50% decrease in depressive symptoms in six out of seven TRD patients within seven days [62]. An additional interim analysis of MFB-DBS in TRD confirmed these findings, showing more than a 50% decrease in depressive symptoms in three out of four patients within seven days of stimulation. At 26 weeks follow-up, two patients showed more than an 80% decrease in depression rating scales [35] (Table 1).

Taken together, the results of the aforementioned studies of DBS for TRD imply that stimulation at a number of different brain areas can alleviate depressive symptoms, which is in line with the view that MDD is a circuitopathy involving various brain regions and networks mainly within the limbic CSTC mood circuits [12,63]. However, how DBS of those targets improves the depressive symptoms is not completely clear. Moreover, stimulation parameters vary between studies due to a need to adjust and balance therapeutic effects to side effects.

MDD is a circuitopathy that involves a wide range of brain structures and exhibits diverse clinical manifestations. Therefore, a one-size-fits-all approach to the DBS targeting may not be beneficial in all patients, whereas a patient-centric selection based on individually disrupted neurocircuits could improve therapeutic outcomes. In evaluating the effects of DBS, one needs to focus on overall improvement on depression rating scales as well as individual scores and symptom-specific improvements. This will enhance the understanding of the effects of DBS and eventually contribute to the development of more personalized treatment approaches. Seemingly, this also applies in other psychiatric disorders such as OCD, where personalized approaches with content-specific DBS targets have already proven to be beneficial [64].

Table 1. DBS in treatment-resistant depression (TRD); published open-label and randomized controlled trials.

Region (DBS)	Study	Open-Labeled, RCT or Case-Report	N	Follow-Up	Age (Mean)	Length of Current Depressive Episode, Years (Mean)	Response Rate (%) in HDRS or MADRS Scores	Remission Rate (%)	Serious adverse events (N)
SCG	**Mayberg et al., 2005**	Open-label	6	6 months	46	5.58	33.3 (1 month), # 83 (2 months), # 66.6 (6 months), #	0 (1 month) 33.3 (3 months) 33.3 (6 months)	Suicidal ideation: 2 Syncope: 1 Lead problem: 1
	Lozano et al., 2008	Open-label	20	12 months	47.4	6.9	60 (6 months), # 55 (12 months), #	35 (6 months) 35 (12 months)	Seizure: 1 Lead problem: 3
	Kennedy et al., 2011	Open-label	20	1, 2 and 3 years, last follow-up (3–6 years)	47.4	6.9	62.5 (1 year), # 46.2 (2 years), # 75 (3 years), # 64.3 (last follow-up), #	18.8 (1 year) 15.4 (2 years) 50 (3 years)	Worsening depression:3 Suicidal ideation:3
	Puigdemont et al., 2012	Open-label	8	12 months	47.4	6.3	87.5 (1 week), # 37.5 (1 month), # 87.5 (6 months), # 62.5 (12 months), #	50 (1 week) 37.5 (6 months) 50 (12 months)	Suicide attempt: 1
	Lozano et al., 2012	Open-label	21	12 months	47.3	5.0	57 (1 month), # 48 (6 months), # 29 (12 months), #	-	Suicide: 1 Suicide attempt: 1
	Holtzheimer et al., 2012	Open-label	17	24 months	42	5.34	41 (6 months), # 36 (12 months), # 92 (24 months), #	18 (6 months) 36 (12 months) 58 (24 months)	Suicidal ideation: 1 Suicide attempt: 2
	Merkl et al., 2013	Open-label	6	24 h Last follow up (24–36 weeks)	50.66	2.13	33.33 (36 weeks), #	33.33 (36 weeks)	Headaches: 6 Tenseness in neck region: 1
	Holtzheimer et al., 2017	*RCT*	60 (52)	6 months (24 months)	50.53	12.62	22 (6 months), ‡ 54 (12 months), ‡ 48 (24 months), ‡	10 (6 months) 17 (12 months) 25 (24 months)	Suicide attempt: 2 Suicidal ideation: 2 Seizure: 2

Table 1. DBS in treatment-resistant depression (TRD); published open-label and randomized clinical trials. (continued)

Region (DBS)	Study	Open-Labeled, RCT or Case-Report	N	Follow-Up	Age (Mean)	Length of Current Depressive Episode, Years (Mean)	Response Rate (%) in HDRS or MADRS Scores	Remission Rate (%)	Serious adverse events (N)
	Eitan et al., 2018	*RCT HF* vs. *LF DBS*	9	13 months	46	-	44.44 (13 months), ‡	-	-
	Merkl et al., 2018	RCT	8	28 months (n = 6) 4 years (n = 2)	48.25	2	37.5 (6 months), # 43.0 (12 months), # 23.0 (28 months), #	12.5 (6 months) 14.2 (12 months) 33.0 (24 months) 33.3 (28 months)	Manic episode: 1
	Crowell et al., 2019	Open-label	28	4 (n = 14) 8 (n = 11) years	44.9 (45.9)	45.1 (46.6)	18 #	21	Suicide attempt: 6 Suicidal ideation: 8 Anxiety: 6 Worsening depression: 2
PGR	**Accolla et al., 2016**	Open-label	5 (1)	6 months (24 months)	45.20	-	-	-	-
NAcc	**Schlaepfer et al., 2008**	Open-label	3	6–24 weeks	46.7	7.2	-	-	None
	Bewernick et al., 2010	Open-label	10	10 months	48.6	10.8	50 (1 month), # 50 (6 months), # 50 (12 months), #	30 (1 month)	Suicide: 1 Suicide attempt: 1
	Bewernick et al., 2012	Open-label	11	12 months 24 months Last follow up (max 4 years)	48.36	9.26	45 (12 months), #	9.1 (24 months)	Pain: 4 Seizure: 1 Agitation: 3 Suicide:1 Suicide attempt: 1
VC/VS	**Malone et al., 2009**	Open-label	15	6 months (n = 15) 12 months (n = 11)	46.3	21	20 (1 month), # 40 (6 months), # 53.3 (last follow-up) #	20 (6 months) 40 (last follow-up)	Suicidal ideation: 2 Syncope: 1 Lead problem: 1

Table 1. DBS in treatment-resistant depression (TRD); published open-label and randomized clinical trials. (continued)

Region (DBS)	Study	Open-Labeled, RCT or Case-Report	N	Follow-Up	Age (Mean)	Length of Current Depressive Episode, Years (Mean)	Response Rate (%) in HDRS or MADRS Scores	Remission Rate (%)	Serious adverse events (N)
	Dougherty et al., 2015	RCT	30	24 months	47.7	11.4	20 (16 weeks), ¥ 20 (12 months), ¥ 23.3 (24 months), ¥	13 (12 months) 20 (24 months)	Suicide: 1 (stimulation off) Suicide attempt: 4 Suicidal ideation: 5 Lead revision: 3
vALIC	**Van der Wal et al., 2020** (follow-up of the RCT Bergfeld et al. 2016)	Open-label	25	2 years	52.5	7.42	32.0 (2 years, ITT) #	20.0 (2 years, ITT)	Pain: 1 Agitation: 3 Suicidal ideation: 6 Fatigue: 4
	Bergfeld et al., 2016	RCT	25	52 weeks	53.2	6.98	40 (after optimization of DBS settings (T_2)) #	20 (T_2)	Suicide attempt: 4 Suicidal ideation: 3 Automutilation: 1
LHb	**Sartorius et al., 2009**	Case-report	1	60 weeks	64.0	9.0	-	-	-
MFB	**Schlaepfer et al., 2013**	Open-label	7	12–33 weeks	42.6	7.6	86, ¥	57	Cranial bleeding: 1
	Fenoy et al., 2016 (interim analysis)	Open-label	4	52 weeks	-	-	75 (7 days) ¥ 66 (26 weeks, OC) ¥	-	-

"-": has not been mentioned in this article, RCT; response criteria; #; 50% or greater reduction in Hamilton Depression Rating Scale (HDRS) $_{(17 \text{ or } 28)}$ scores, ¥; 50% or greater reduction in MADRS scores, ‡; 40% or greater reduction in MADRS scores, RCT: randomized controlled trial, ITT; intention to treat, OC; observed case.

Table 2. Targets for DBS in treatment resistant depression (TRD), functions, pathophysiology and the effect of DBS.

Brain Region	Function	Pathological Activity in MDD	HF-DBS Effect
SCG	Contains three white matter bundles; forceps minor + uncinate fasciculus connecting to the medial frontal cortex, cingulum connecting to the rostral and dorsal ACC and fronto-striatal fibers connecting to the NAcc, CN, Pt and anterior Th Connects higher 'top-down' cortical regions with subcortical modulatory regions Involvement in brain DMN [83]	Increased activity [84] Reduced volume in familial depression [85] Projections to: (1) NAcc may play a role in lack of interest, disruption in reward and underlie anhedonia (2) Hth and brainstem may play a role in circadian and sleep disturbances, problems with appetite and an abnormal stress responds and cortisol metabolism [84].	Disruption of pathological activity Modulation of multiple regions connected to the SCG [84]
NAcc	Receives projections from VTA, AG, OFC, mPFC, dCN, GP and Hip and projects to Cg25, mPFC, VP, Th, AG and Hth. Transmits information from emotion centers to motor control regions, causing motivational behavior to obtain rewards [31] Processes reward and pleasure information	In severe anhedonia; smaller size and less activation to reward [86]	Acute: Increase in exploratory motivation Chronic: reduction in anhedonia PET Imaging: ↑ **activity** in VS, bilateral dlPFC and dmPFC, cingulate cortex and bilateral AG. ↓ **activity** in vmPFC and vlPFC, dCN and Th [31]
VC/VS	Contains fibers connecting the dPFC, dACC, OFC and vmPFC with THAL, AG, Hth and brainstem (SN, VTA, RN and PTN) [87]	Increased activity [48] Activation of the connection from left vs. to left caudate has been associated with anhedonia Increased connectivity of vs. to DMN is positively correlated to higher depression scores in the CES-D score [88]	-
vALIC	Contains two fiber bundles: the anterior thalamic radiation and the supero-lateral branch of the MFB connecting the PFC to different subcortical structures such as the Th, NAcc, VTA and VS. Decreased integrity of the right vALIC in depressed patients [89]	-	Decreased metabolism in OFC, subgenual ACC and right DLPFC in patients with OCD [90]
LHb	Activity corresponds negatively to anticipation and reception of a reward [91]	Increased activity [92] Possible down regulation of serotonergic, noradrenergic and dopaminergic systems [93], volume reduction [94]	Localized metabolic increase in one patient with FDG-PET, presumably due to functional inhibition [57]
ITP	Interconnects the intralaminar nucleus and TRN with the OFC [60,83]	Hyperactivation in both TRN and OFC [95]	Cortical desynchronization Disruption of adrenergic and serotonergic malfunction [95]
MFB	Interconnects the Nacc, VTA, vmHth, lHth and AG ventromedial and lateral nuclei of the Hth and AG with convergence onto the PFC [62,96] Plays a crucial role in the reward pathway;	Dysfunctional reward system. Responders showed a strong connectivity between the active electrode contact and the mPFC pre-operatively using individual DTI [35]	Insignificant changes in metabolism in 3 patients with PET measurements pre-operatively, 6 and 12 months post-operatively [35]
BNST	Mayor output pathway of the AG Regulates stress response Integrates information from multiple brain areas to perform 'valence surveillance' [21,83]	Oscillatory activity with high a-power [97]	-

"-": not known.

4. TOWARDS A MORE PERSONALIZED DBS TREATMENT APPROACH FOR TREATMENT-RESISTANT DEPRESSION

Since open-label trials and RCT data on DBS in TRD show inconsistent results, this gives rise to discussion about the chosen study designs, the correct interpretation of results, and the best target(s) for neuromodulation. Depression entails different clinical subtypes and looking at homogenous subgroups of depressed patients may lead to a personalized DBS approach. This would be superior to looking at primary outcomes across all participants. Importantly, a prerequisite to this approach is the ability to determine pathoanatomical substrates of specific subtypes. How to implement such a more personalized approach to DBS treatment for TRD is discussed below.

4.1. Clinical and Neurophysiological Subtypes of Depression

Most response rates in depression treatments to date have been measured with changes in average levels among all patients treated. However, depressive symptomatology varies highly among individuals, making the standardization of positive outcomes challenging. Mood, sleep rhythm, concentration, psychomotor, and cognitive domains can all be disturbed in depression, while treating one selected brain structure within the mood circuit may not have an effect on all aforementioned symptoms nor have an effect on the main symptomatology of all depressed patients.

Subdividing TRD into different subtypes, involving distinct clinical symptoms as well as distinct patterns of dysfunctional connectivity in limbic and frontal striatal networks, may reveal different subtype-related outcomes for each investigated brain region, and if so, patient selection for a given brain target could enhance treatment effectiveness [65]. Analysis of resting-state connectivity biomarkers previously revealed four connectivity-based biotypes of depression characterized by either anxiety, increased anhedonia, psychomotor retardation, and/or increased anergia and fatigue. Moreover, patients could not be differentiated into a particular subtype based on clinical features alone and clustering them based on functional connectivity was needed [66]. Therefore, imaging procedures as well as featured symptoms should be taken into account when treating TRD with DBS. It is conceivable that subdividing TRD patients according to connectivity-based biotypes will shed new light on the interpretation of previous DBS study results, and that the integration of functional connectivity in future DBS studies will reveal clinically relevant subgroups that might respond to DBS of a specific target within the mood circuit. Altogether, it can be suggested that better assessment of therapeutic outcomes at symptom level might be accomplished when TRD patients with dominant anergia/fatigue symptoms (biotype 2) are stimulated within the Cg25; and patients characterized by more anxiety (biotype 4) are stimulated within the thalamic region, as suggested by Drysdale and coworkers [66]. Likewise, SCG stimulation could alleviate sleep disturbances and NAcc stimulation could improve anhedonia (Table 2).

4.2. Individual Tractography

Another way in which DBS efficiency can be improved is to ameliorate the implantation of electrodes with the usage of individualized, patient-specific, deterministic tractography targeting. Riva-Posse et al. (2018) used individualized patient-specific tractography targeting for SCC-DBS surgeries in TRD patients, aiming at the convergence of the four white matter bundles: the forceps minor, uncinate fasciculus, cingulum, and fronto-striatal fibers. This resulted in a response rate of 81.8% and a remission rate of 54% after a one year trial period, which proved greater than the previous open-label trials [67]. In a recent study, diffusion tensor imaging (DTI) tractography was used to target SCC-DBS more optimally, and the authors examined the impact of tract activation on clinical response at 6 and 12 months. Stimulation of vmPFC pathways by SCC-DBS was associated with a positive response and stimulation of the cingulum was associated with a 6 month, but not a 12 month DBS response. Monopolar stimulation of 130 Hz was applied with either pulse width (90–450 μs) or amplitude (4–8 V) progressively increased every month, based on response status. Patients were changed to bipolar settings if monopolar stimulation caused adverse effects. It was speculated that targeting more ventral, rather than the dorsal mPFC projections, might improve the response [68].

4.3. Combining Deep Brain Stimulation with Cognitive-Behavioral Therapy

It is plausible that better therapeutic outcomes could be achieved if DBS is applied in combination with concurrent treatments, such as pharmacotherapy with antidepressants or cognitive-behavioral therapy (CBT) in TRD. Studies focusing on the added effect of concurrent treatments to DBS have not been conducted in patients with TRD. The results from studies in OCD patients treated with DBS show that adding CBT to DBS has added beneficial effects [69]. Studies targeted at revealing the added effects of concomitant treatments after DBS in TRD would also provide information that may facilitate establishing a treatment algorithm to determine the place of these treatments in TRD patients.

4.4. Biomarkers

Biomarkers are quantifiable characteristics of biological processes, which could prove helpful in improving diagnostic objectivity of MDD and TRD as well as help in personalizing its treatment. For MDD, no specific biomarkers have yet been found, though several markers have been shown to be potential candidates, such as brain-derived neurotrophic factor (BDNF), interleukins (IL) 1 and 6, tumor necrosis factor (TNF), malondialdehyde (MDA), hypothalamic-pituitary-adrenal (HPA) activity, and cortisol responses [70,71]. Every biomarker as a standalone shows a low sensitivity and specificity, partly explained by the heterogeneity of MDD. To overcome this shortcoming, either examining a biological panel of several markers [72] or phenotyping MDD and TRD into distinct subtypes could be considered. However, a recent meta-analysis showed that only cortisol has a predictive effect on onset/relapse and recurrence of MDD making

the integration of biomarkers for personalizing TRD treatment a futuristic milestone yet to be discovered [73].

4.5. Insights into Symptomatic Improvement after Deep Brain Stimulation

For TRD, different regions in the mood circuit can be stimulated with DBS (Table 2), although it is still unclear which depressive-symptoms respond to the stimulation of a specific target. More research into the mood circuit is needed to untangle which emotions arise from specific brain regions. This may vary from basic animal research, disentangling neuronal function per brain region, and ultra-high field MR studies in humans, all of which could shed light on the dysfunctional brain circuits in TRD. In contrast to the motor system that is studied thoroughly [74,75], emotional circuitry is far less understood. One reason for this is that animal research into mood circuitry remains complicated as there is considerable heterogeneity between species [76]. Modeling depression in animals is complex as there are several depressive-like behavior models, such as the chronic unpredictable stress paradigm (CUS), which give insight into depression pathology [77]. DBS is investigated within these models to unravel behavioral and cellular changes following DBS [78].

Alongside the standard clinical rating scales, the use of momentary assessment techniques, such as the experience sampling method (ESM), could enhance the documentation of the momentary mood states [79]. The ESM includes short repeated assessments of experiences and behaviors, as well as moment-to-moment changes in mental states in the context of daily life. Research has shown that depressed patients can improve their depressive symptomology while using weekly ESM for six weeks, and add-on ESM derived feedback resulted in a significant decrease in HDRS scores compared to controls ($p < 0.01$; −5.5 point reduction in HDRS at 6 months) [80]. In add-on-derived feedback, a psychologist or psychiatrist gives feedback on the association between the participants momentary affective states and specific daily life contexts [81]. ESM-derived feedback could further improve treatment by showing within-subject changes in a heterogeneous TRD population and contribute to clinical decision-making [81]. In the case of DBS, the use of ESM may reveal specific response patterns depending on the brain region that is stimulated, which can provide valuable information about emotional circuitry. This can be done using well-evaluated day-to-day scores, including questionnaires that go into detail on current mood and adaptive functioning.

5. CONCLUSIONS

More personalized treatment approaches hold the potential to increase the overall efficacy of DBS in TRD. Precise evaluations of symptoms, biomarkers, and resting-state connectivity patterns are essential when distinguishing clinical subtypes of TRD. Moreover, subtyping may provide more insight into the working mechanisms of DBS and help in selecting optimal targets in patients.

Monitoring of biomarkers at multiple time points during treatment along with evaluation of ESM data, in parallel with clinical assessments of mood using standardized depression-rating scales, will lead to a better understanding of symptom changes when stimulating specific brain regions. Such considerations could further lead to optimal adjustments of stimulation parameters as long-term effects of DBS on mood occur.

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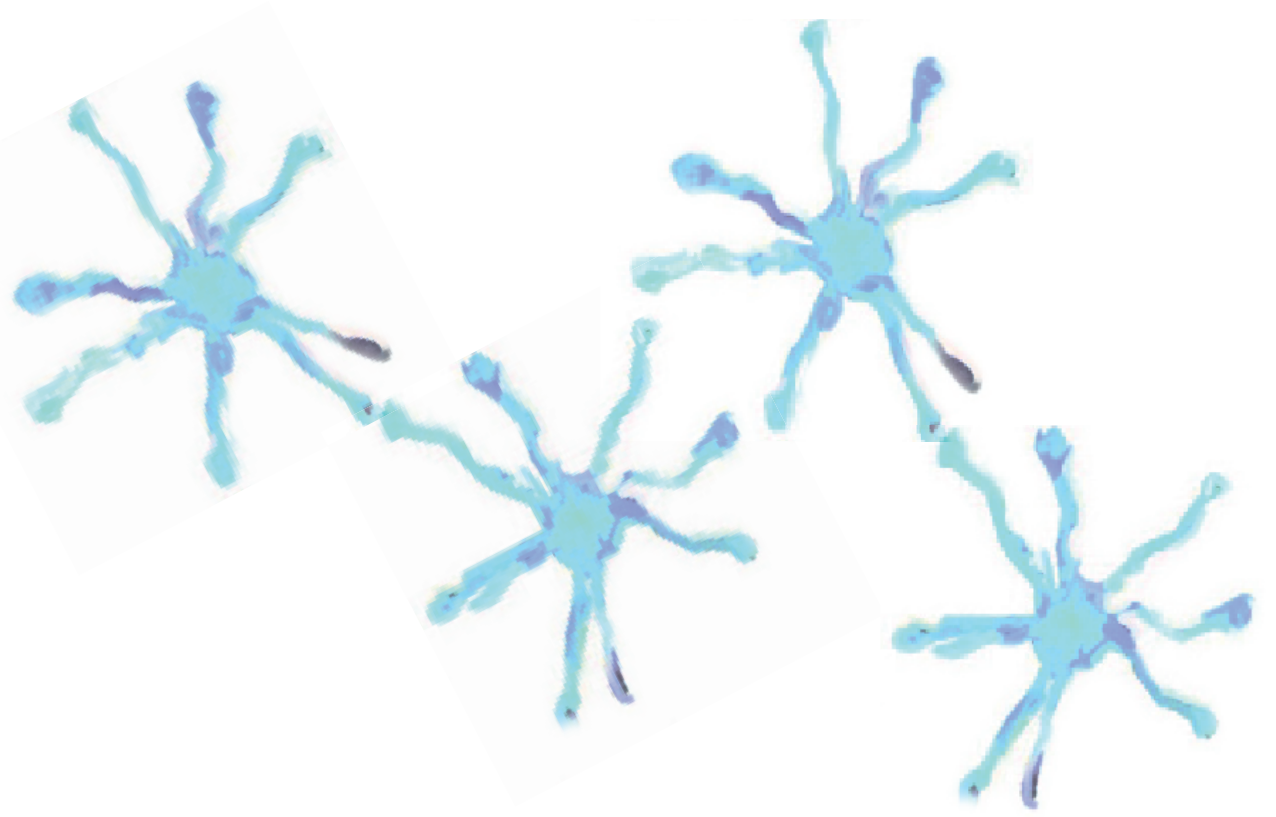

3 Deep brain stimulation of the prelimbic prefrontal cortex alleviates anhedonia-like symptoms in rats

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Submitted

ABSTRACT

Background: Major depressive disorder (MDD) is estimated to affect 4.4% of the population. Despite available therapies, approximately 30% of patients remain treatment resistant. Deep brain stimulation (DBS) has shown to be effective in treating depressive symptoms. Nevertheless, the outcomes have been contradictory. It has been postulated that depression might consist of different connectivity-based subtypes, which requires a tailored targeting. Herein, we addressed whether DBS in specific subregions of the prefrontal cortex could alleviate distinct symptom domains of experimental depression, aiming to improve the effectiveness of DBS for MDD.

Method: rats were implanted with DBS electrodes in either the prelimbic (PreL) or infralimbic (IL) subregion of the prefrontal cortex. They were assigned to chronic mild stress or non-stressed control groups. After four weeks, all animals underwent behavioral testing for different behavioral domains including anhedonia, anxiety and helplessness. Rats were stimulated using high frequency, monophasic and bipolar pulses for 15 minutes before and during the behavioral tasks.

Results: High frequency (HF) DBS in the PreL cortex but not the IL cortex alleviated anhedonia and behavioral despair revealed by the sucrose preference and forced swim tests, respectively. No differences were found for the home cage emergence test, food intake test and elevated zero maze.

Conclusion: these data suggest that modulation of specific sub-regions in the prefrontal cortex might be a potential approach towards providing tailored DBS therapy for different subtypes of depression.

Keywords: Deep brain stimulation; depression; anhedonia; symptom specific

1. INTRODUCTION

Major depressive disorder (MDD) is a psychiatric disorder characterize by a depressed mood and a loss of interest in otherwise enjoyable daily activities for more than two weeks. For the diagnosis of MDD, as stated in the Diagnostic and Statistical Manual of Mental Disorders number 5 (DSM-5), five of the following symptoms need to be present: a depressed mood, anhedonia, change in weight or appetite, insomnia or hypersomnia, psychomotor retardation or agitation, loss of energy or fatigue, worthlessness or guilt, impaired concentration or indecisiveness and thoughts of death or suicidal ideation or attempt (1). Worldwide over 300 million people suffer from the disease in 2015, equivalent to 4.4% of the world's population (2). The treatment of MDD consists of pharmacotherapy, psychological therapies such as cognitive behavioral therapy and interpersonal psychotherapy and electroconvulsive therapy (3-5). However, roughly 30% of treated patients do not respond adequately to these treatments. For treatment-resistant depression (TRD), non-pharmacological therapies such as transcranial magnetic stimulation (6) and deep brain stimulation (DBS) are proposed (7).

In depression, various cortical and subcortical brain areas have been indicated to play a role in its pathology (8, 9). These cortical areas involve the orbital frontal cortex (OFC) and the medial prefrontal cortices (MPFC) forming the orbital and medial networks (10, 11). In the orbital network, Brodmann area 13 and parts of area 12 form an associative network that processes visual, auditory, somatosensory, gustatory, and olfactory information. This information is projected to multiple nuclei within the amygdala, entorhinal, perirhinal and temporal cortices, the dorsal striatum and portions of the mediodorsal thalamic nuclei (MD). In these brain regions, sensory information is integrated, and reward and aversion values are provided to these experiences that guide behavior (10, 11). The medial prefrontal network consist of Brodmann areas 25, 32 and part of area 24, 14, 10 and 11. These areas, project to the amygdala, entorhinal, perirhinal and temporal cortices as well as the subiculum, hippocampal CA1, striatum, the medial part of the MD, the hypothalamus and periaqueductal grey. This network mainly plays a role in mood and emotion, and modulating visceral reactions to emotional stimuli. It is not directly related to a sensory modality, but resemble a 'default' system that is active in a resting state as determined by fMRI (10, 11).

Targets for DBS studies therefore, are mainly parts of the orbital or medial prefrontal networks. For example the subcallosal cingulate gyrus (SCG), which includes Brodmann area 25, parts of area 24, and 32 (12).

For TRD, DBS has shown promising results in open-label trials when stimulating the SCG, ventral capsule/ventral striatum, (VC/VS), nucleus accumbens, the inferior thalamic peduncle, lateral habenula and more, reviewed in detail elsewhere (13). However, randomized controlled trials stimulating the SCG, anterior limb of the internal capsule or VC/VS have revealed contradictory results (14-17). In this regard multiple questions have been raised; i) what is the optimal target for the DBS electrode? ii) what are the precise underlying brain circuits modulated by

stimulation? and iii) are certain targeted brain regions only effective for a subgroup of patients with specific symptoms?

There is growing agreement on subtypes of depression, reflecting the dysfunction of particular large scale neural circuits, rather than seeing the disorder as one unitary (18). In a resting state fMRI study, four different biotypes of depression has been reported (19). In this study all MDD patients shared a neuroanatomical core of pathology, consisting of the insula, orbitofrontal cortex, ventromedial prefrontal cortex (vmPFC) and multiple subcortical areas. These brain regions represent three symptoms of depression, namely; mood (feeling of sadness, hopelessness, and helplessness), anhedonia and fatigue. Beside this pathological core, distinct patterns of abnormal functional connectivity differentiated four biotypes associated with specific clinical-symptom profiles. A severe reduced connectivity was found in fronto-amygdala networks, which is responsible for fear-regulated behavior, in biotypes 1 and 4, characterized by increased anxiety. Hyperconnectivity in thalamic and fronto-striatal networks, which supports reward processing, adaptive motor control and action initiation, was found in biotypes 3 and 4, showing increased anhedonia and psychomotor retardation. Furthermore, a reduced connectivity in anterior cingulate and orbitofrontal areas, which supports motivation and incentive-salience evaluation, was most severe in biotype 1 and 2, characterized partly by anergia and fatigue. Seemingly, DBS in one specific brain region might not be an effective treatment for all depressed patients with heterogeneous symptoms.

To unravel if DBS in different cortical (sub)regions ameliorate particular depressive symptoms, animal research can be useful. In this research DBS was chosen and not TMS since DBS can be applied more regionally and within deep brain regions, additionally more stimulation settings can be explored. In rodents, the prefrontal cortex is homologous to the SCG in humans (20).

Our research group has previously showed that high frequency (HF) DBS of the vmPFC induced anti-depressive effects, however subdivisions of the vmPFC were not studied in detail (21). Likewise, other groups have found similar effects when stimulating the vmPFC (22). The vmPFC can be subdivided into the infralimbic (IL) prelimbic (PreL) and dorsal peduncular (DP) cortices. In rodents the IL cortex mainly plays a role in stress, autonomic responses and the extinction of conditioned responses, while the PreL cortex is involved in cognitive processes such as memory, delayed variable responses, the integration of behavioral sequences, planning of behavioral responses and the attention and behavioral flexibility (23). The IL cortex is most homologous to Brodmann area 25, where the PreL cortex is most homologous to Brodmann area 32 (24). Herein, we aimed to investigate if different microcircuits within the vmPFC are responsible for particular domains and symptoms of depression by applying HF DBS in the IL or PreL subregion of the vmPFC and test for different anti-depressive effects in a rat model of depression.

2. MATERIALS AND METHODS

2.1. Subjects

Fifty-one male rats (300–400 g; Sprague Dawley, Envigo) were used in this study. All animals were housed in standard individual ventilated cages (IVC) in a controlled environment (temperature 22 °C, humidity 59 (rH)) in a reversed 12:12 h light-dark cycle. After DBS surgery, all animals were housed individually. Water and rat chow was available *ad libitum*, except during the CUS period when the given stressor related to food or water intake. All animal procedures were carried out in accordance with the Netherlands Central Committee on Animal Testing (CCD).

2.2. Surgical procedure

A detailed description of the electrodes used for DBS and the surgical procedure for electrode implantation appeared in our earlier publications (25, 26). In summary, under isoflurane induction anesthesia, all animals were placed in a stereotactic frame. Bur wholes were made in the skull and bilateral electrodes were implanted in either the IL (anteroposterior (AP): + 3.00 mm. mediolateral (ML): ± 0.60 mm dorsoventral (DV): 5.00 mm), or PreL (anteroposterior (AP): + 3.00 mm. mediolateral (ML): ± 0.60 mm dorsoventral (DV): 3.50 mm), area of the vmPFC, according to the brain atlas of Paxinos and Watson 6th edition (27). All animals were given two weeks postoperative recovery before being introduced to the stress protocol.

2.3. Experimental groups

After the postoperative recovery period, all rats were randomly assigned to undergo either the chronic unpredictable stress (CUS) paradigm or no stress. The animals undergoing the CUS paradigm with DBS implants into the IL brain area were randomly assigned to the group receiving DBS during behavioral testing, i.e. IL-HF DBS (n=12) or the sham group not receiving stimulation. i.e. IL sham (n=5). The animals undergoing the CUS paradigm with DBS implants into the PreL brain area were randomly assigned to the group receiving DBS during behavioral testing, i.e. PreL-HF DBS (n=16) or the sham group not receiving stimulation. i.e. PreL sham (n=7). The stressed sham animals were pooled together as one group (n=12). The non-stressed sham animals implanted into the IL (n=4) or PreL (n=7), were also pooled together as one non-stressed sham group (n=11). Due to electrode loss, the number of IL and PreL implanted animals in stressed and non-stressed sham groups differ. Notably, for the sake of animal ethics we implanted fewer animals in sham groups to reduce the number of animals used in this study similar to our earlier studies. It has been shown that there were no significant differences in the behaviors with respect to the implantation site (21).

The DP cortex within the prefrontal cortex has not been researched within this paper, since our previous research showed severe seizure induction upon stimulation of this brain region (26).

2.4. The CUS model

To induce experimental depression, the CUS protocol was executed as described before (21, 26). The stressors consisted of; soiled-cage bedding with 300 ml of cold water (4 °C), intermittent illumination every 2 h during their dark cycle, stroboscopic light (2.5 Hz), food or water deprivation, housing in mouse cages, and paired-housing where the rat alternatingly was the intruder or resident. Also a condition with no stressors was given. Each stressor was given at a unpredictable time in a random order in both the morning and evening and lasted 10-14 h. Stressors were given for 4 consecutive weeks.

2.5. Deep brain stimulation

A digital stimulator (A-M systems 3800, USA) and stimulus isolator (A-M systems 3820, USA) were used to apply monophasic, bipolar DBS for 15 minutes before and during the behavioral tasks at a frequency of 100 Hz, an amplitude of 100 μA and a 100 μs pulse width. Sham rats were connected; but did not receive stimulation. An acute stimulation paradigm was chosen, since previous results done in our research group has shown antidepressant effects upon acute DBS in the PFC in this model of experimental depression (21).

2.6. Behavioral testing

Stimulated animals received HF DBS 15 minutes before and during the entire behavioral task. All animals, CUS-susceptible, CUS-resilient, sham and non-stressed animals underwent the behavioral paradigm including the sucrose preference test, home cage emergence test, food intake test, elevated zero maze and the forced swim test, each described below.

2.6.1. Sucrose preference test

One day before testing, all animals were exposed to a 1% sucrose solution instead of water for 1 h in a custom-made freely-moving stimulation setup without stimulation. The custom-made freely moving stimulation set-up consisted of a wooden square (50 by 50 cm) with high walls (100 cm) in which the home cage could be inserted and two drinking bottles in opposite direction could be placed. The sucrose 1% bottles were randomly placed in one of the two bottle holders. This was followed by a 14 h long period of food and water deprivation starting at the beginning of their dark cycle. After the deprivation, the actual sucrose preference test took 1 h. The sucrose 1% bottle was randomly placed in one of the two bottle holders and normal drinking water was placed in the other bottle holder. Both drinking bottles were weighted before and after the 1 hour testing. Sucrose preference index (SPI, %) was determined using the equitation SPI = (sucrose intake/ total liquid intake) x 100.

2.6.2. Home cage emergence test

In the Home cage emergence (HCE) test, the home-cage of the animal was opened and placed in the middle of an open field, which consisted of a Plexiglas square arena covered with cardboard

(100 x 100 cm with 40 cm high walls), and a dark floor. A custom-made iron grid was placed over the edge of the home-cage to ease leaving the home-cage. The latency to leave the home-cage onto the iron grid was recorded. The session lasted for 10 min. If the rat did not escape its home-cage within these 10 min, a score of 600 seconds was given.

2.6.3. Food Intake test

The food Intake test took place in the same custom made freely-moving stimulation setup as the STP. Prior to the test, the animals were food and water deprived for 24 h starting at the beginning of their dark cycle. During the test, all rats had access to a limited amount of food on a petri-dish. After 2h of testing, the total food intake was measured and corrected for their body weight. Food that was hidden by the animal in their home-cage, was taken into account.

2.6.4. Elevated zero maze

The elevated zero maze (EZM) was performed using a black circular arena (100 cm in diameter, 10 cm path width, 70 cm above floor level) with an integrated infrared light powered via an external adaptor. A long stimulation cable was attached next to the camera above the center of the circular arena, with a long enough reach so rats could move freely during stimulation. Each rat was placed in the middle of an open arm facing one of the closed arms, and was allowed to explore the maze for a period of 5 min. Rat's movement was recorded using an infrared video tracking system (EthoVision version 8.5, Noldus, The Netherlands). The arena was cleaned after every rat. Scoring of behavior was done manually by three blinded observers. As outcome measures, the percentage of time spend in closed arm was noted and corrected for the time the rat entered a closed arm for the first time. Also the latency to go into a closed arm for the first time was measured.

2.6.5. Forced swim test

The Forced Swim test (FST) was performed using a transparent Perspex cylinder (50 x 20 cm). The cylinder was filled with tap water (25 °C) to a depth of 30 cm. Testing was performed over two consecutive days. On day one, a pretest session was given in which the rat was placed in the water for 15 minutes. The following day, rats were tested in the water for 10 minutes. The test sessions were video-taped using a digital camera. The immobility time was measured using EthoVision tracking software (EthoVision version 8.5, Noldus, The Netherlands).

2.7. Electrode localization

The localization of electrodes was verified in sections containing the electrode trajectory from all rats. Sections were mounted on gelatin-coated glass and stained with hematoxylin and eosin.

2.8. Statistical analysis

All data were represented as mean ± standard error of the mean (SEM). Analysis were performed using IBM SPSS Statistics 25. Normality and homogeneity of variance of the data were checked using the Shapiro-Wilk test and levene's test. The data were analyzed using either a one-way analysis of variance (ANOVA) or a Kruskal–Wallis H test, as appropriate. Multiple comparison correction was done using the Tukey HSD post hoc test.

3. RESULTS

3.1.1. Sucrose preference test

The sucrose preference test significantly differed between the four animal groups, (one-way ANOVA, 614.871(6.595)=0.001, P<0.05). The Tukey HSD was used for multiple comparison and showed that HF DBS in the PreL cortex significantly enhanced the sucrose preference compared to sham animals (p=0.049). It showed a significant difference between the IL-HF DBS and PreL-HF DBS group (p=0.002), where the PreL-HF DBS animals consumed more sucrose water. Furthermore a significant difference was observed between the IL-HF DBS and non-stressed control animals (p=0.013), where the non-stressed animals consumed more sucrose water (Fig. 1).

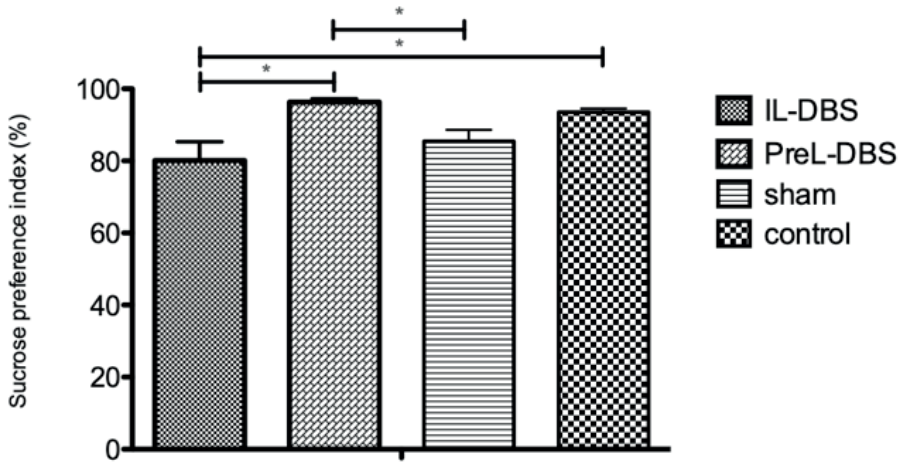

Figure 1. Sucrose preference test (SPT) following high frequency deep brain stimulation (HF-DBS); graph shows the quantitative sucrose preference index data obtained by SPT during HF-DBS in the prelimbic or infralimbic prefrontal cortices (PreL and IL, respectively) compared to sham and control groups. Data are represented as the mean sucrose preference index per group ± SEM; * p=<0.05.

3.1.2. Forced swim test

The immobility of the forced swim test significantly differed between the four animal groups, (one-way ANOVA, 198.399(6.223)=0.001, P<0.05). The Tukey HSD was used for multiple comparison and showed that HF DBS in the PreL cortex significantly decreased the immobility time in the FST compared to the sham group (P=0.034). Furthermore, a significant difference between PreL-HF DBS and IL-HF DBS (p=0.004) or non-stressed controls (p=0.005) was found (Fig. 2).

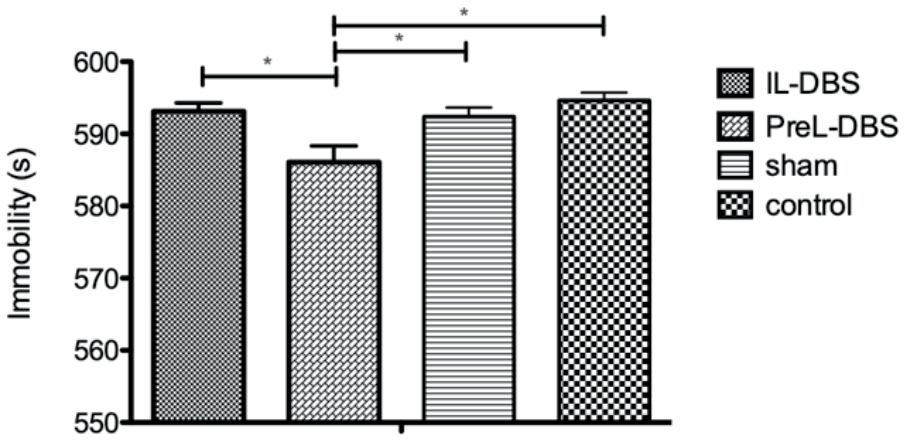

Figure 2. Forced swim test (FST) after 4 weeks of chronic unpredictable stress (CUS); graph shows the quantitative immobility time data obtained during 10 minutes of FST following HF-DBS in the prelimbic or infralimbic prefrontal cortices (PreL and IL, respectively) compared to sham and control groups. Data are represented as the mean immobility time per group ± SEM; * p=<0.05.

3.1.3 Home cage emergence test

HF DBS in the IL or PreL subregion did not have any significant effect on escape latencies in the HCE test compared to the sham animals (one-way ANOVA, 1473.615(0.399)=0.755) (Fig. 3).

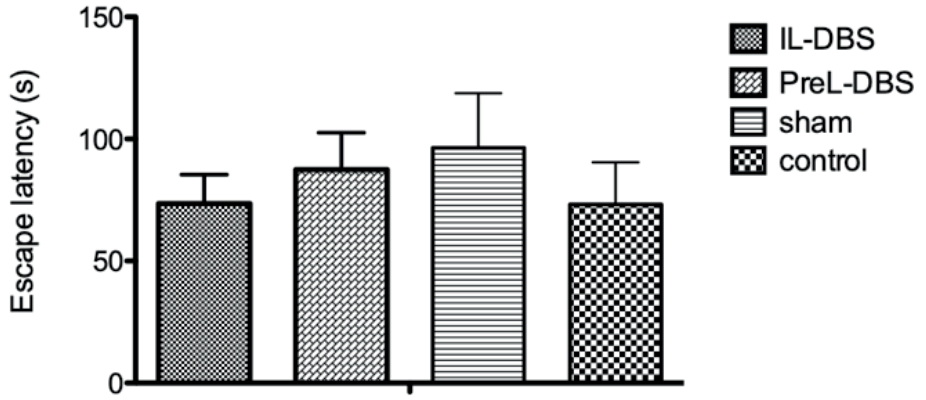

Figure 3. Home cage emergence test (HCE) following high frequency deep brain stimulation (HF-DBS); graph shows the quantitative escape latency data obtained by HCE test during HF-DBS in the prelimbic or infralimbic prefrontal cortices (PreL and IL, respectively) compared to sham and control groups. No significant effect was observed for DBS. Data are represented as the mean escape latency per group ± SEM.

3.1.4 Food Intake test

HF DBS in the IL or PreL subregion did not have any significant effect on food consumption in stimulated groups compared to the sham animals (one-way ANOVA, 0.000(2.077)=0.117) (Fig. 4).

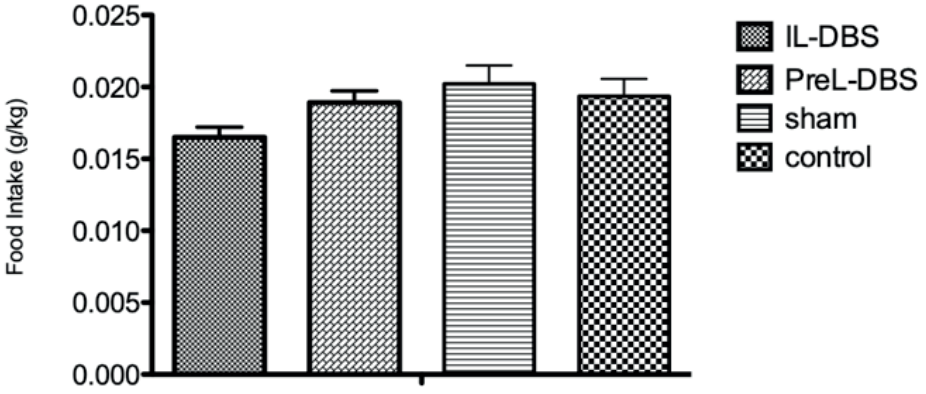

Figure 4. Food intake test following high frequency deep brain stimulation (HF-DBS); graph shows the quantitative food intake data obtained by food intake test during HF-DBS in the prelimbic or infralimbic prefrontal cortices (PreL and IL, respectively) compared to sham and control groups. No significant effect was observed for DBS. Data are represented as the mean food intake per group ± SEM.

3.1.5 Elevated Zero Maze

HF DBS in the IL or PreL subregion did not change the total time spend in open arms or escape latency to the first closed arm in the EZM compared to the sham group (one-way ANOVA, 3711.108(2.599)=0.065, Kruskal-Wallis 6.865(3)=0.76) (Figs. 5-6).

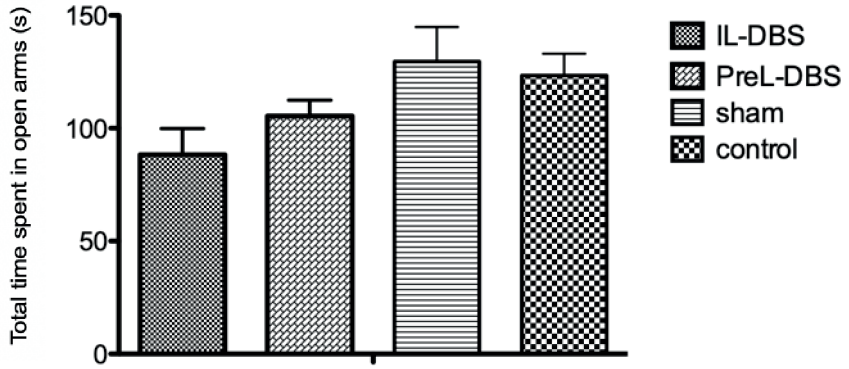

Figure 5. Elevated Zero Maze (EMZ) test; total time spent in open arms following high frequency deep brain stimulation (HF-DBS); graph shows the quantitative time data obtained by EZM test during HF-DBS in the prelimbic or infralimbic prefrontal cortices (PreL and IL, respectively) compared to sham and control groups. No significant effect was observed for DBS. Data are represented as the mean time spend in open arms per group ± SEM.

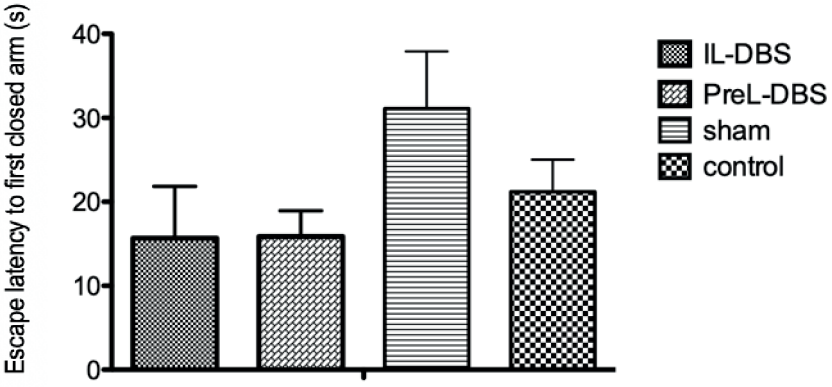

Figure 6. Elevated Zero Maze (EMZ) test; escape latency to the first closed arm following high frequency deep brain stimulation (HF-DBS); graph shows the quantitative escape latency obtained by EZM test during HF-DBS in the prelimbic or infralimbic prefrontal cortices (PreL and IL, respectively) compared to sham and control groups. No significant effect was observed for DBS. Data are represented as the average escape latency to the first closed arm per group ± SEM.

3.2. Verification of electrode placement

The DBS electrodes in stimulated, sham and non-stressed animals were placed correctly in 49 out of 51 animals in either the IL or PreL cortex as it was indicated in postmortem histology (Fig. 7). The two animals with incorrect electrode placement were excluded from analysis.

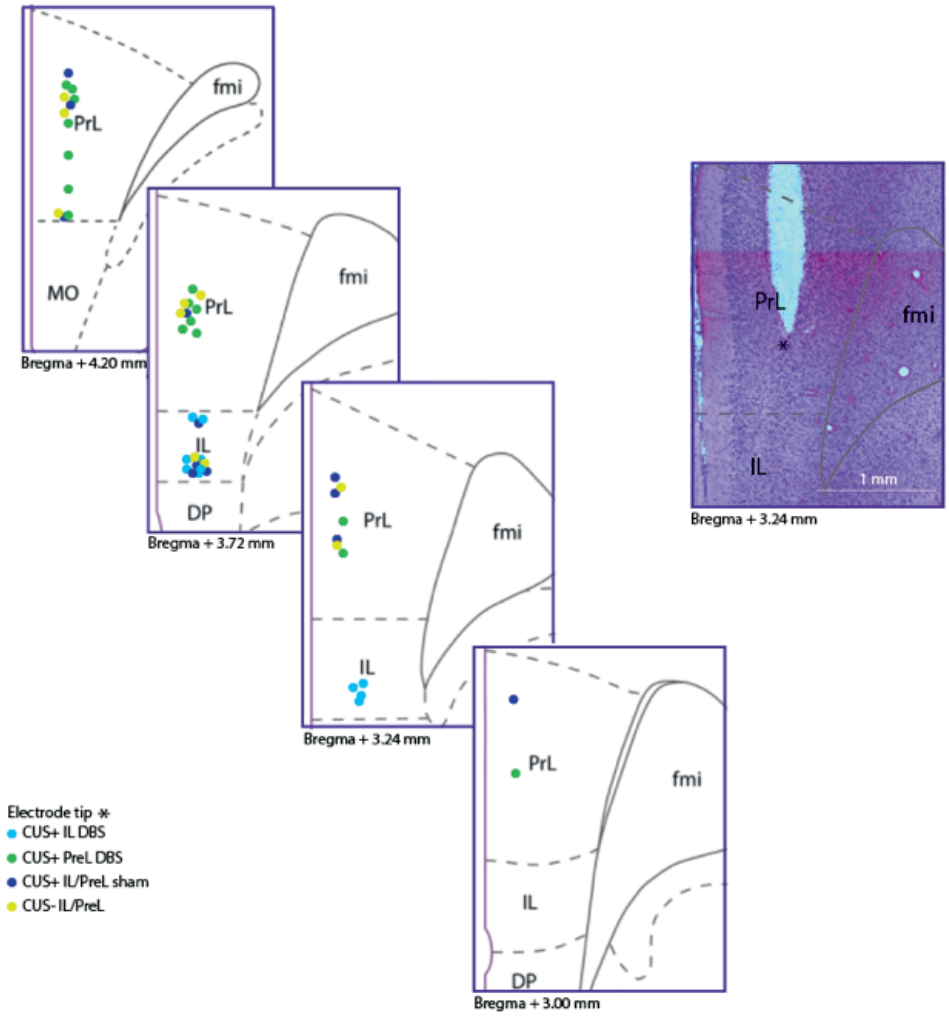

Figure 7. Electrode localization; figures show electrode tip localizations in the prelimbic and infralimbic prefrontal cortices (PreL and IL, respectively) in four experimental groups.

4. DISCUSSION

Our results showed that PreL-HF DBS alleviates anhedonia, indicated by an increase in sucrose preference and reduced behavioral despair in forced swim test. This implicates that two major symptom domains seen in depression, namely the anhedonia and helplessness, are regulated within a circuitry involving the PreL cortex and not the IL cortex in rats.

The IL cortex has been studied earlier in a rodent DBS study, where the authors showed no effect of IL-HF DBS on immobility in forced swim test in rats (28). However, as this study was conducted on naïve rats, the outcomes cannot be compared to our study. Nevertheless, these contradicting outcomes implies the importance of using relevant disease model when the therapeutics outcomes of DBS are desired.

In rats, the IL and PreL cortices receive projections from the orbitofrontal cortex, agranular insular, perirhinal and entorhinal cortices, hippocampus, subiculum, claustrum, basal forebrain structures, amygdala, midline thalamus and monoaminergic brainstem nuclei (29). However, projection patterns from the IL and PreL cortices are somewhat different. The IL cortex projects mainly to forebrain structures, amygdala, hypothalamus and parabrachial and solitary nuclei of the brainstem, while the PreL cortex projects to the agranular insular cortex, claustrum, nucleus accumbens, olfactory tubercle, thalamus, amygdala, and the dorsal and medial raphe nucleus of the brainstem (23). Although, both structures innervate parts of the orbitofrontal cortex, olfactory forebrain and midline thalamus (23). The IL cortex is mainly involved in stress regulation and autonomic responses functioning as a visceromotor region, while the PreL cortex not only has visceromotor activities but is also involved in 'higher-order' functions like memory and the integration of behavioral sequences and planning of behavioral responses (20, 23). These thereby explain why DBS of the PreL- and not the IL cortex alters states of anhedonia and helplessness.

Research from McKlveen et al. 2016 suggests that chronic stress increases synaptic inhibition onto prefrontal glutamatergic output neurons. In their research they used patch clamping of pyramidal neurons from layer V in the IL cortex of SD rats. To induce chronic stress they have used a two weeks CVS protocol using stressors such as cold room exposure (1 hour, 4 °C) and hypoxia (30 min of 8% oxygen), which slightly differs from our CUS protocol. Their results showed that chronic stressed rats had i) an increase of iPSCs frequency with no effect on the amplitude suggesting an increased GABA release in the ILPFC , ii) increased inhibitory appositions and terminals onto glutamatergic cells in all layers of the ilPFC and iii) GR downregulation in the ILPFC especially in PV+ interneurons(30) compared to controls.

Czéh et al. 2018, however, have shown opposite results of GABAergic disturbances in neurotransmission and a reduced number of GABAergic neurons in the medial PFC in anhedonic whistar rats. Their rats underwent a nine weeks CUS paradigm, after which a sucrose preference test was performed, subdividing the stressed animals into a CUS susceptible, hence the anhedonic animals, and a CUS-resilient group. Their results showed that anhedonic rats had i) a 37% reduction of spontaneous inhibitory postsynaptic currents (sIPSC's) frequency in the mPFC

(infralimbic and prelimbic region) and a reduced amplitude, ii) a decrease in release probability and thus GABA release in the mPFC and iii) a down regulation of $GABA_bR$-GIRK currents, indicating a reduced $GABA_b$ receptor mediated inhibition in the mPFC and iiii) less GABAergic cells in the MPFC compared to controls. No subdivision between the PreL- and IL cortex was made except in there histopathological analysis of GABAergic neurons in which both the IL- and PreL-cortex of anhedonic rats showed less CKK+ neurons, but only the IL cortex also showed less PV+ and CR+ neurons (31).

Their differences in outcome can be due to the different amount of stress given, two versus nine weeks, different forms of stress, CVS versus CUS paradigm, but can also be strain related, SD versus whistar rats. Furthermore, McKlveen et al. 2016 only measured the IL cortex, while Czéh et al 2018 measured in the prelimbic-infralimbic region.

In our research we stressed our SD rats for four consecutive weeks. Therefore, our research is mostly comparable with the work done by McKlveen et al. 2016, keeping in mind we investigated both the IL- and PreL cortex. We believe that when chronic stress increases synaptic inhibition onto prefrontal glutamatergic output neurons (30), DBS restores this imbalance between excitatory and inhibitory neurotransmission possibly by diminishing the elevated GABA release. Furthermore, it is known that gamma-oscillations in the mPFC, tightly controlled by PV neurons, are disrupted in MDD (32, 33). McKlveen et al. 2016 has shown that after two weeks of chronic stress, PV+ neurons are less expressed in the IL cortex not the PreL cortex, possibly disabling information outflow out of the mPFC. DBS could thereby restore these impaired gamma-oscillations, enhancing the information flow out of the mPFC. Research by Parthoens et al 2014 has shown that 60HZ DBS in the PreL cortex causes hypermetabolism in glucose using [(18)F]FDG microPET indicating cellular changes upon DBS (34).

We, however, found that HF DBS of the PreL cortex and not the IL cortex alleviates particular depressive-like symptoms, a region which according to McKleev et al. 2016 does not show less PV+ neurons. Nonetheless, we must highlight our differences in chronic stress time, therefore more abundant changes in the PreL cortex after four weeks of CUS could underlie our findings. Why HF DBS in the PreL- and not the IL cortex alleviates anhedonia and learned helplessness still remains unclear. Since these depressive domains are not directly anxiety related, a different pathway not including the basolateral amygdala (BLA) but higher order regions could be altered with HF DBS. Including electrophysiology and microdialysis in both the IL- and PreL cortex together with regions such as the DRN, amygdala and lateral habenula in future experiments might elucidate their different outcome.

Interestingly, we did not find any significant effects of HF DBS on other symptom domains of depression, such as anxiety, neophobia or motivation for food consumption. In the HCE test, we would have expected that non-stressed control rats tend to explore more and escape the home cage faster compared to stressed animals (21). However, the non-stressed control group was not handled as much as the stressed animals, as the various stressors require a lot of handling, which might have influenced behavioral outcomes. It has been shown that stressed rats emerge quicker

from their home cage because of increased behavioral agitation (35). Nonetheless, it should be noted that CUS is a challenging model to reproduce and that stress outcome vary greatly among trials and laboratories (36).

As mentioned above, the vmPFC is homologous to the human SCG. The SCG has shown to be hyperactive in MDD patients and DBS in this region elicited a significant reduction in depression rating scales in open-label studies while a RCT could not replicate this finding (12, 16, 37). In our rat model, HF DBS of the PreL (Brodmann are 32) but not the IL (Brodmann area 25) cortices within the vmPFC alleviated anhedonia and helplessness, suggesting that also in the human SCG subdivisions and microcircuits are responsible for different domains of depression showing contradictory results when SCG subdivisions are not taken into consideration in DBS trials.

5. CONCLUSION

Taken together, our findings suggest that circuits emerging from distinct subregions in the prefrontal cortex of rats are responsible for different depressive symptoms such as anhedonia and behavioral despair. This means that our study support the concept of targeting selective brain areas for specific subtypes of depression. These data might partially explain the contradictory outcomes of SCG DBS in depression, emphasizing that a DBS approach for TRD needs to be more individualized with a focus on microcircuits.

6. CONFLICT OF INTEREST

All authors declare to have no conflict of interest.

7. ACKNOWLEDGEMENTS

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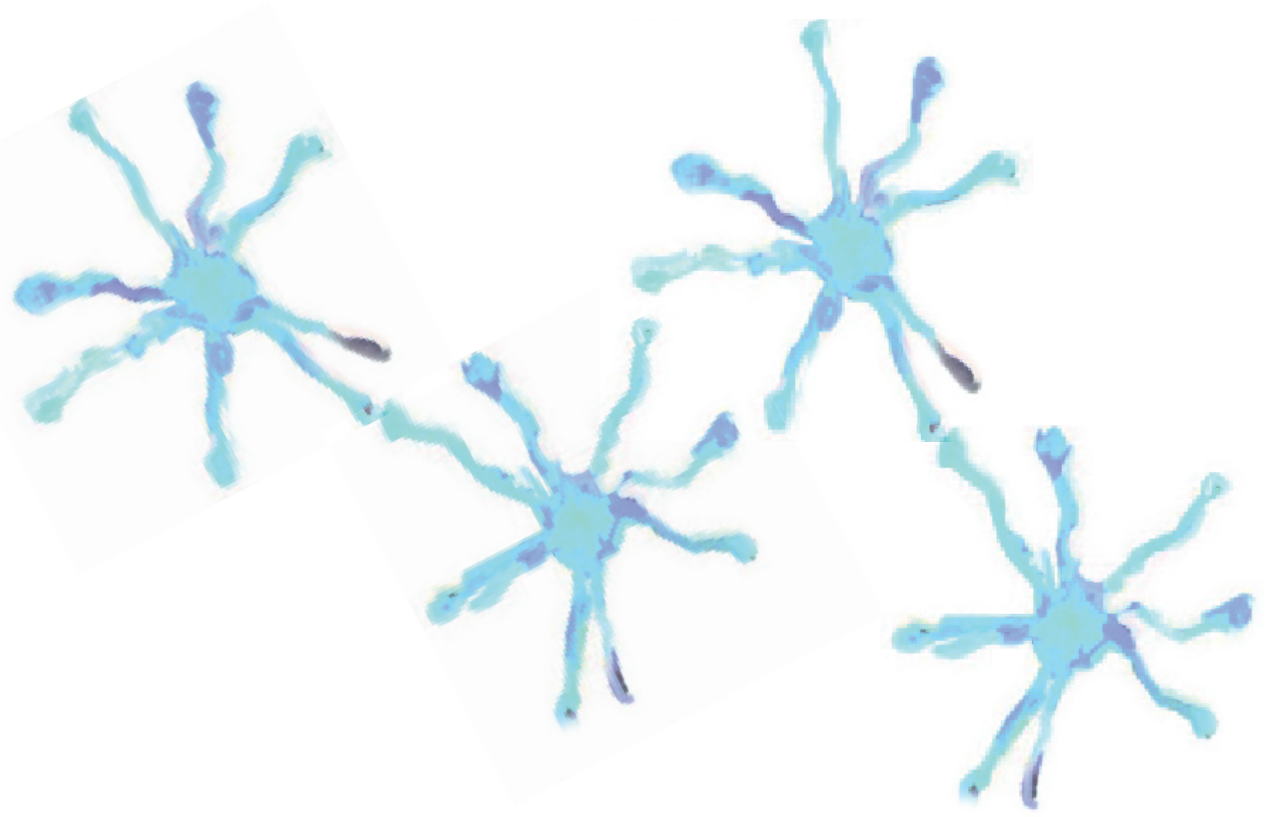

4 Severe seizures as a side effect of deep brain stimulation in the dorsal peduncular region of the prefrontal cortex in a rat model of depression

Milaine Roet, Sylvana Pol, Ali Jahanshahi, Fred Schaper and Yasin Temel

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ABSTRACT

Deep brain stimulation (DBS) has shown to have antidepressant effects in both human trials and animal studies. However, it remains to be determined what the best target is, and which mechanisms underlie therapeutic effects. In this study, we investigated if DBS in the dorsal peduncular (DP) subregion of the ventromedial prefrontal cortex (vmPFC) could alleviate depressive-like behavior in an experimental model of depression. Surprisingly, DBS in the DP cortex caused acute induction of seizures in ~40% of stimulated animals. Clinically relevant stimulation parameters were applied, and a bipolar stimulation approach was chosen to keep the current spread to a minimum. We therefore conclude that the DP subregion of the vmPFC is not a suitable target to conduct DBS in mood disorders but could be a potential model for seizure induction.

1. INTRODUCTION

Major depression is a common mental disorder affecting more than 300 million people worldwide [1]. Despite advances in antidepressant treatment, 10-30% of the patients show an inadequate respond to therapy. This treatment-resistant depression (TRD) is diagnosed when a patient shows a poor or unsatisfactory response to two to four medication and psychotherapy treatment sessions [2]. For these patients, alternative treatments are widely investigated. Deep brain stimulation (DBS) is one of these treatment options initially showing promising results in different open labelled trials. Nevertheless, more recent randomized controlled trials could not replicate these positive findings [3]. Factors that contribute to these varying results include differences in study design, stimulation settings, patient selection and targets for DBS. Regarding the latter, there is no consensus about the best target for TRD. To get a better insight into the neurobiology of depression and to study potentially better targets for DBS, we need to further investigate the different neuronal circuits of mood. Previous animal research done in our laboratory showed that DBS in the ventromedial prefrontal cortex (vmPFC) caused anti-depressant effects in behavior in a rat model of depression [4]. The vmPFC is part of the prefrontal cortex (PFC) and consist of multiple interconnected regions fulfilling numerous functions such as the regulation of emotion, decision making, the process of extinction and fear conditioning, and self-directed cognition [5]. In rodents, the mPFC can be subdivided into different subregions including the prelimbic cortex (PreL), the infralimbic cortex (IL) and the dorsal peduncular (DP) cortex, each fulfilling different functions [6, 7]. Further dissection of the pathways causing the anti-depressive effects may give important insights into the neurobiology of this behavior. In this study, we used an experimental model of depression induced by 'chronic unpredictable stress' (CUS). This model mimics the pathway to depression by chronic exposure to various unpredictable stressors inducing a range of behavioral and physiological changes parallel to symptoms of depression. Our aim was to electrically stimulate the DP cortex with DBS which has not been studied much. We expected to see different anti-depressive behavioral effects when stimulating the DP subregion then what is seen with stimulating the IL and PreL subregions. We believe that multiple small microcircuits are responsible for different traits seen in depression and that stimulating the DP cortex would alleviate particular traits. Our hypothesis was that the different subregions of the vmPFC are responsible for different modalities of mood related behavior such as anhedonia, anxiety, behavioral despair and motivation. This could be critically important for the treatment of various mood related disorders such as depression and will give the opportunity to integrate a symptom-based treatment.

Abbreviations; AcbC; midrostrocaudal level of the nucleus accumbens core, AcbS; dorsal caudomedial shell of the nucleus accumbens, BDA; biotinylated dextranamine, BLA; basolateral nucleus of the amygdala, BST; bed nucleus of stria terminalis, CeA; central nucleus of the amygdala, DBS; deep brain stimulation, CUS; chronic unpredictable stress, dlPAG; dorsolateral periaqueductal

gray, DP; dorsal peduncular, EEG; electroencephalographic, IL; infralimbic, mPFC; medial prefrontal cortex, NAcc; nucleus accumbens, PFC; prefrontal cortex, PreL; prelimbic, SIT; sucrose intake test, SPC; sulcal prefrontal cortex, STN; subthalamic nucleus, TRD; treatment-resistant depression, VMH; ventromedial hypothalamus, vmPFC; ventromedial prefrontal cortex, VS; ventral striatum

2. MATERIAL AND METHODS

2.1. Subjects

We used male rats (300-400 g; Sprague Dawley, n=29, Envigo), housed in standard individual ventilated cages (IVC) in a controlled environment (temperature 22°C, humidity 59 (rH)) using a 12/12-h reversed dark/light cycle (light on 07AM-07PM). After DBS surgery, all animals were housed individually. Food and water were given *ad libitum*, except during the CUS model when the given stressor related to food or water intake. All the experiments were carried out in accordance with the Animal Experiments and Ethics Committee of Maastricht University.

2.2. Electrode construct

All stimulations electrodes were custom made by the engineering department (IDEE) of Maastricht University [8]. A DBS electrode consist of a bilateral construct of two concentric bipolar coaxial gold-coated stimulation electrodes containing a platinum-iridium inner wire; shaft diameter 0.3 mm, tip (core) diameter 0.08 mm, with an interelectrode distance of 1.2 mm.

2.3. Electrode implantation

Following an induction of isoflurane anesthesia, rats were placed into a stereotactic frame and their body temperature was kept a 37°C using a thermo-regulated heating pad. Throughout the whole surgical procedure, rats were sedated with 2,5% isoflurane inhalation anesthesia. Initially a bur whole in the skull was made and the DBS electrode construct was implanted into the DP subregion of the vmPFC (AP: + 3.00 mm. ML: +/- 0.60 mm DV: 5.00 mm), according to the brain atlas of Paxinos and Watson 6th edition [9]. After surgery, all animals were given a post-operative recovery period before introducing the stress protocol.

2.4. The chronic unpredictable stress (CUS) model

The CUS protocol was executed as described before [4]. The stressors given consisted of soiled-cage bedding with 300 ml of cold water (4°C), intermittent illumination every 2 hours during their dark cycle, stroboscopic light (2.5 Hz), food or water deprivation, housing in mouse cages, paired-housing where the rat alternatingly was the intruder or resident and a condition with no stressor. Each stressor lasted between 10-14 hours and was given in a random order at an unpredictable time during both the morning and evening. Stressors were given for 3 consecutive weeks.

2.5. DBS stimulation parameters

We used clinically relevant stimulation parameters with a biphasic and monophasic, bipolar high frequency stimulus (100 Hz) with a stimulation amplitude of 100 μA and a pulse width of 100 μs. For a precise delivery of the stimulus we used an A-M systems model 3800 8 channel stimulator connected to stimulus isolation units' model 3820.

2.6. Behavioral testing

During behavioral testing, animals received either stimulation or sham stimulation. For sham stimulation the animals were attached to a DBS cable without attachment to the stimulator. In all our experiments, animals were stimulated 15 minutes before behavioral testing und during the entire behavioral test.

Sucrose Intake Test (SIT): The day before testing, all animals were habituated to a 1% sucrose solution instead of water for 1h. This was followed by a period of 14h of food and water deprivation, starting at the beginning of their dark phase. After the 14h fasting period all animals were offered a 1% sucrose solution for 1h. The sucrose intake was calculated from the total amount of 1% sucrose solution consumed divided by the bodyweight of the animal (g/kg).

Home-cage emergence test (HCE): In this test, the home-cage of the animal was opened and placed in an open field. An iron grid was placed over the edge of the home-cage to ease leaving the home-cage. The total amount of time it takes for the animal to get out of their home-cage onto the iron grid was measured. The session lasted for 10 minutes. If the rat did not escape its home cage within these 10 minutes, the rat was given a score of 600 sec.

2.7. Statistical analysis

All the data are represented as mean ± standard error of the mean (SEM) and the analyses were performed with IBM SPSS Statistics 24. Normality and homogeneity of variance was performed using the Kolmogorov–Smirnov test and normality plots. The data of our behavioral tests were analyzed using either an independent sample t-test or a nonparametric Mann–Whitney U-test, as appropriate. All P-values <0.05 were considered significant.

3. RESULTS

3.1. CUS model

To test for CUS susceptibility, we performed a sucrose intake test (SIT) right before the onset of CUS and 3 weeks after the onset of CUS. Rats exposed to the CUS model, showed less increase in 1% sucrose solution consumption over time compared to the non-stressed control animals. This finding indicates a state of anhedonia in the rats undergoing the CUS model for 3 consecutive weeks (Fig. 1).

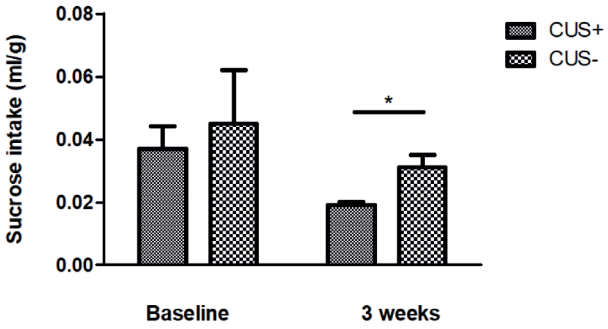

Figure 1. Sucrose Intake Test before and after 3 weeks of CUS. Results of the sucrose intake test at t=0 baseline and after 3 weeks of CUS. Data are represented as means ± SEM (CUS+ n=18, CUS+ sham n=6, CUS- n=6). *p<0.05. CUS+ Chronic unpredictable stress, CUS- non-stressed controls.
At baseline (t=0), no significant difference between the group of stressed (CUS+) and non-stressed (CUS-) animals was found (Mann-Whitney U, U=65.50, z=-.338, p=0.736). After 3 weeks of CUS, the 1% sucrose consumption levels showed a significant difference between the CUS+ and CUS- animals (Mann-Whitney U, U=14.500, z=-2.953, p=0.003).

3.2. Behavioral testing

After 3 weeks of CUS we started behavioral testing for all animals. Part of the stressed animals (n=18) underwent DBS and part of the stressed animals (n=6) served as sham controls (CUS+ sham) being coupled to a DBS cable but not to the stimulator. For DBS, biphasic bipolar stimulation with a frequency of a 100 Hz an amplitude of a 100 μA and a pulse width of a 100 μs was given. The animals not undergoing CUS (n=6) served as a non-stressed control group and therefore were also not stimulated.

The HCE test showed an effect between the CUS+ and CUS- group after the second day of testing, where the stressed group undergoing DBS remained in their cage for a longer period than the non-stressed controls. (HCE1: 1.844(2) = 0.398 and HCE2: 6.064(2) =0.048, P<0.05, post hoc Bonferroni correction showed that the significant difference was seen between the CUS+ and CUS- group, p=0.025 (Fig. 2). Since no differences between the CUS+ and CUS+ sham was found we cannot speak of a neophobia effect of DBS in the DP region of the vmPFC, but a certain trend might be seen when the number of animals in the CUS+ sham and CUS- groups are increased (p=0.122).

SIT with DBS in the DP was executed in which surprisingly, 5 out of the 18 stimulated animals showed involuntary movements and seizure-like behavior upon start of DBS in the 15 minutes of pre-stimulation. A significant difference between the different groups was found (One-way ANOVA, 4.934(3,25) = 0.008, p<0.05) in which the significant effect was seen between the CUS+ animals experiencing seizures and the CUS- group (p=0.005) (Fig. 3). A trend towards less 1% sucrose intake between the animals experiencing involuntary movements upon DBS and the CUS+ sham control group was seen (p=0.077).

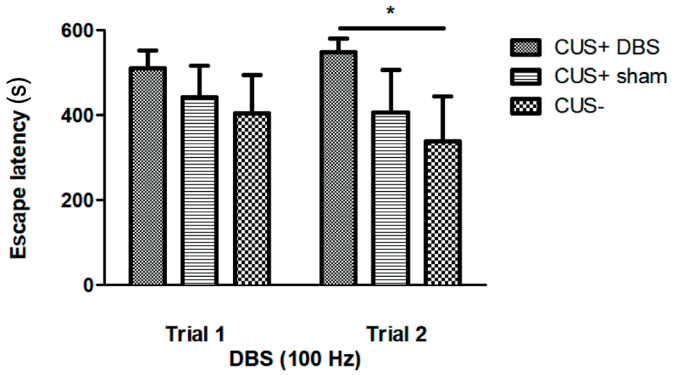

Figure 2. Home Cage Emergence Test with DBS. Results of the HCE test during DBS on two consecutive days. Data are represented as means ± SEM (CUS+ n=17, CUS+ sham n=6, CUS- n=6). *p<0.05. CUS+ Chronic unpredictable stress, CUS- non-stressed controls.

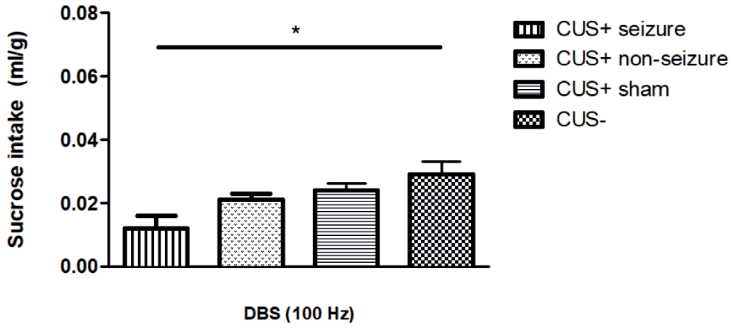

Figure 3. Sucrose Intake Test with DBS. Results of the SIT during DBS. Data are represented as means ± SEM (CUS+ seizure n=5, CUS+ non-seizure n=12, CUS+ sham n=6, CUS- n=6). *p<0.05. CUS+ Chronic unpredictable stress, CUS- non-stressed controls.

During further behavioral testing we observed that even more CUS+ animals receiving DBS began to show seizure like behavior upon stimulation (~40%, n=7 out of n=18). This seizure like behavior was not seen in the non-stimulated animals confirming that the observed involuntary movements were due to stimulation. Due to severe seizure like behavior, we stopped further behavioral testing and decided to acquire electroencephalographic (EEG) recordings upon DBS in the affected animals to investigate if indeed this seizure like behavior could be classified as seizures.

Seizure induction

To classify the observed seizure like behavior, EEG recordings were made during both biphasic and monophasic DBS. During baseline measurements, the rats were not stimulated but EEG recordings were made to record background EEG activity. Before DBS, the rats showed no signs

of discomfort with normal explorative and washing behavior. When starting DBS, the rats immediately froze and subsequently showed facials movements (Racine stage I) and head nodding (Racine stage II), quickly followed by a left forelimb clonus (Racine stage III) with rearing (Racine stage IV) and a full generalized clonus with falling (Racine stage IV-V). EEG recordings showed typical spike and wave discharges characteristic for seizures. Approximately 1 minute after the DBS is turned off, the rats still showed some automatism comprised of facial movements and post ictal behavior while the EEG signal normalized. Full recovery in which the rats were fully conscious and showing exploring behavior appeared roughly two to four minutes after cessation of DBS.

3.3. Electrode localization

Electrodes were traced in the DP of the vmPFC in 88% of the rats undergoing DBS, with a 100% accuracy in the rats experiencing seizures upon stimulation (n=7) (Fig. 5). Two animals were excluded from analysis due to misplacement or detachment of the electrode construct.

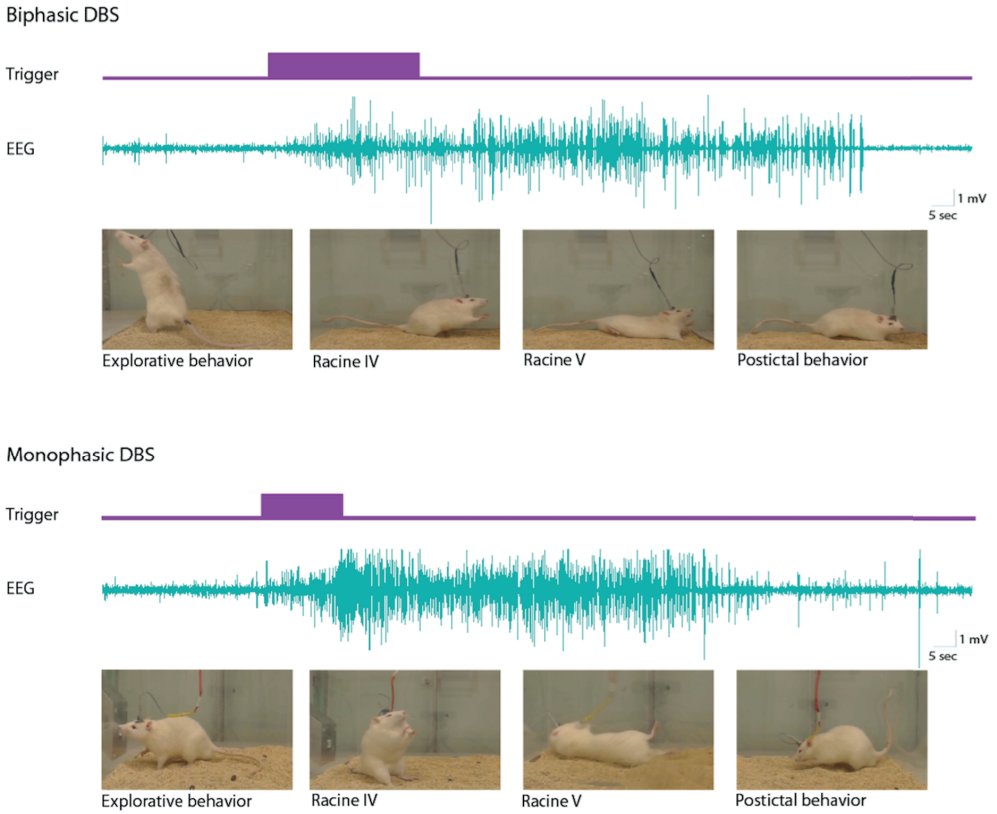

Figure 4. Seizure induction upon DBS. EEG recording during biphasic and monophasic bipolar DBS in de DP subregion of the vmPFC with a frequency of 100 Hz, an amplitude of 100 μA and a pulse width of 100 μs. Seizure induction (blue) can be seen right after the onset of the stimulus (purple) and continues for approximately one minute after the stimulation has stopped. Behavior is scored with the inclusion of Racine scores for epileptic seizures seen on the added pictures.
For supplementary video 1 and 2 see original article.

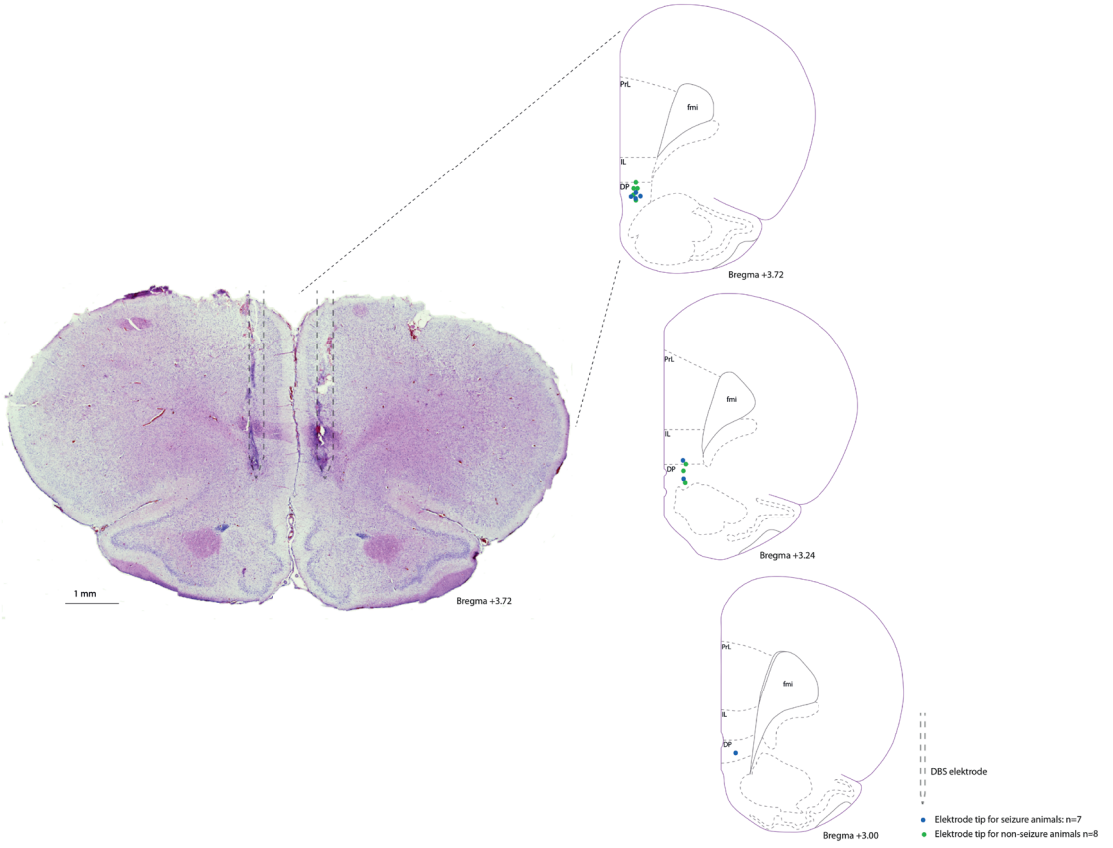

Figure 5. DBS electrode localization. Electrode localization in stimulated animals showing both non-seizure and seizure behavior upon DBS. A green circle indicates the electrode localization of an animal not experiencing seizures, while a blue circle indicates the electrode localization of an animal experiencing seizures upon DBS.

4. DISCUSSION

4.1. Dorsal peduncular region stimulation induced seizures

The present study showed that DBS in the DP region of the vmPFC can induce seizures while using clinically relevant stimulation parameters with both biphasic and monophasic stimulation paradigms. Due to this disabling side-effect, we can conclude that the DP cortex in the rat is not a feasible brain structure to conduct DBS experiments for mood disorders but can potentially be used as a model for seizure induction.

Seizure induction following cortical stimulation has been seen before in literature [10], however previous research stimulating the vmPFC in rats did not show this finding [4]. Experiencing seizure behavior upon cortical stimulation in our experimental model of depression was therefore unexpected and is extensively described in this paper to broaden our knowledge of this unforeseen finding and to prevent cases like this in the future.

The DP cortex has not been widely investigated so far, therefore we chose the stimulation parameters based on our previous experience with DBS experiments and stimulation in the PFC [4, 8]. The DP subregion seems to be far more sensitive to electrical current than the IL and PreL regions, therefore conventional DBS parameters do not seem to be appropriate for this region. However, the parameters used are clinically relevant and we have not experiences seizures so far in any of the regions used such as the subthalamic nucleus (STN), the nucleus accumbens (NAcc), the dorsolateral periaqueductal gray (dlPAG) and the ventromedial hypothalamus (VMH) [11-13]. In contrast, similar parameters used for DBS in the anterior nucleus of the thalamus have shown to be effective in animal models of epilepsy [14] and drug refractory epilepsy patients [15]. Nevertheless, our findings show that when given into the DP subregion of the vmPFC, these stimulation parameters are potent seizure inducers. These findings can be used as a new rat model of seizure induction in the PFC as opposed to stimulating temporal structures as performed in the amygdala kindling and post-status epilepticus models [16]. This model might have the advantage that classical structures involved in seizure induction remain undamaged by chemical or electrical lesioning and therefore its action during seizures can be accurately investigated using histological, imaging or electrophysiological measures. In addition, this model represents more a frontal seizure-model than the widely applied models of temporal seizures.

4.2. Other reports of stimulation induced seizures

The mechanism behind seizure induction when stimulating the DP cortex remains unclear. Previous research has shown that partial kindling of the PFC in rats with +/-11 after discharges of 5s stimulus train of 60 Hz frequency, a pulse duration of 0.5 ms and a 600-800 μA intensity, propagated into the hippocampus and Nacc, where postictal activity lasted for > 5 minutes [17]. However, the stimulation intensity used in their experiments was far greater than the intensity used in our experiments. Furthermore, current spread in this experiment was larger given that monopolar electrodes were used, while we stimulated with a bipolar electrode construct.

Nakamura-Palacois et al. have shown convulsion induction and behavioral responses such as head shaking upon bilaterally electrical activation (ten 30-sec trains, 60 Hz, 80-100μA) of the mPFC using monopolar electrodes, influenced by both diazepam and haloperidol [10]. Low frequency pulse stimulation in the cortex (2 ms monophasic, square wave pulses, frequency 9 Hz, intensity 400-800 μA) with monopolar constructs has been used as a procedure for inducing seizures by a different group [18]. Other researchers observed seizures during cortex self-stimulation acquisition in the sulcal prefrontal cortex (SPC) and medial prefrontal cortex (mPFC), but these seizures were not seen below currents of 100 μA [19].

Unlike other studies, we used a bipolar electrode constructs where the distance between the cathode and anode is approximately 50 micrometers. Therefore, the current spread should be significantly less in our experiments. Nevertheless, we do see overt seizures induced by direct stimulation in the PFC, possibly caused by its connections to the limbic system.

4.3. Anatomical connections of the dorsal peduncular region of the vmPFC

Since we lack a precise description of the DP cortex, tissue properties may differ substantially from those of the IL and PreL vmPFC subregions. Previous research has shown direct projections from the DP subregion of the vmPFC to the trigeminal brainstem sensory nuclear complex and other brain stem nuclei; where retrograde tracing by Fluorogold showed labeling of the rostrocaudal middle level of DP when injected into the rostro-dorsomedial part of the laminae I/II of the trigeminal subnucleus caudalis (rdm-I/II-Vc). Anterograde labeling with biotinylated dextranamine (BDA) into the mid-PD showed bilaterally labeling in the rdm-I/II-Vc, periaqueductal gray and solitary tract nucleus, and ipsilaterally in the parabrachial nucleus and trigeminal mesencephalic nucleus. Also, BDA-labeled axons and terminals were found reciprocally between the mid-PD and ipsilateral most caudal level of the granular and dysgranular insular cortex. These projections indicate a role for intraoral and perioral sensory processing, including nociceptive processing [20].

Retrograde labeling from the ventral striatum (VS), dorsal caudomedial shell of the nucleus accumbens (AcbS) the midrostrocaudal level of the nucleus accumbens core (AcbC), the parabrachial nuclei, the medial lateral septum, the bed nucleus of stria terminalis (BST), the lateral hypothalamus, the mediodorsal thalamus and the basolateral and central nucleus of the amygdala nucleus accumbens (BLA, CeA) to the dorsal peduncular cortex has been shown [21, 22].

The NAcc is part of the ventral striatum and a widely researched brain structure receiving dopaminergic input onto its GABAergic medium spiny neurons involved in functions such as motivation, reward and positive behavioral reinforcement. The parabrachial nuclei as discussed earlier mediate both ascending and descending nociceptive signaling. The medial lateral septum covers multiple nuclei receiving reciprocal information from regions such as the olfactory bulb, hippocampus, amygdala, hypothalamus, cingulate gyrus, habenula, thalamus and midbrain. This region mainly plays a role in reward and reinforcement [23]. The BST consist of multiple nuclei mainly containing GABAergic neurons that can express a variety of peptides and to some extent

glutamatergic cells [24]. It functions as a relay center where descending cortical information meets ascending interoceptive and exteroceptive information regarding homeostatic states or potential changes in homeostasis [25]. The lateral hypothalamus is involved in homeostasis, feeding behavior, arousal and reward and even receives input of the BST [26, 27]. The mediodorsal thalamus has a function in memory and cognition and mainly uses glutamate to communicate with the cortex [28, 29]. The amygdala consists of heterogenous nuclei where the BLA receives sensory input directly from the temporal lobes. The CeA processes this information serving as the major output nucleus of the amygdala. Mostly, GABA receptors are found in the BLA and CeA [30, 31].

Knowing that there are retrograde connections to the limbic system and in particular the amygdala, a region also known for kindling, the assumption that the current density upon stimulation in the PFC spreads into and activates the limbic system causing overt seizures becomes more plausible.

4.4. Functional connections of the dorsal peduncular region of the vmPFC

Despite the IL and PreL subregions of the vmPFC, the DP subregion is relatively unexplored [32]. Up to now, no actual functional connectivity studies of the DP subregion have been executed. A functional connection between the vmPFC and the basolateral nucleus of the amygdala (BLA) was shown in rats using optogenetics and electrophysiological recordings [33]. However, no clear distinction of the DP subregion has been made. Functional differences for dorsal and ventral subregions of the mPFC in controlling attention has been shown [34]. In their research, lesioning either the ventral (dorsal peduncular cortex and tenia tecta) or dorsal (prelimbic and infralimbic cortices) subregion resulted in differences in five-choice serial reaction time performance.

4.5. The synchronized parallel forebrain hypothesis

Another possible explanation for seizure induction following DBS in the DP region can be found in Penfield's prediction which describes the synchronized parallel forebrain hypothesis. In this hypothesis, the forebrain provides the neural apparatus necessary for temporal synchronization of multiple independent streams of processed information necessary for parallel processing [35]. Due to this property, stimulation in the cortex might be risky and cause uncontrolled propagation of the signal leading to seizures. It might be speculated that the cortex is more sensitive to stimulation than is thought before and therefore caution has to be taken when suggesting DBS for depression in cortical areas.

5. CONCLUSIONS

In summary, current results show that DBS in the DP subregion of the vmPFC in rats has a high incidence of inducing seizures up to Racine stage IV-V. The stimulation paradigms used are clinically relevant and do not seem to induce seizures in other brain regions.

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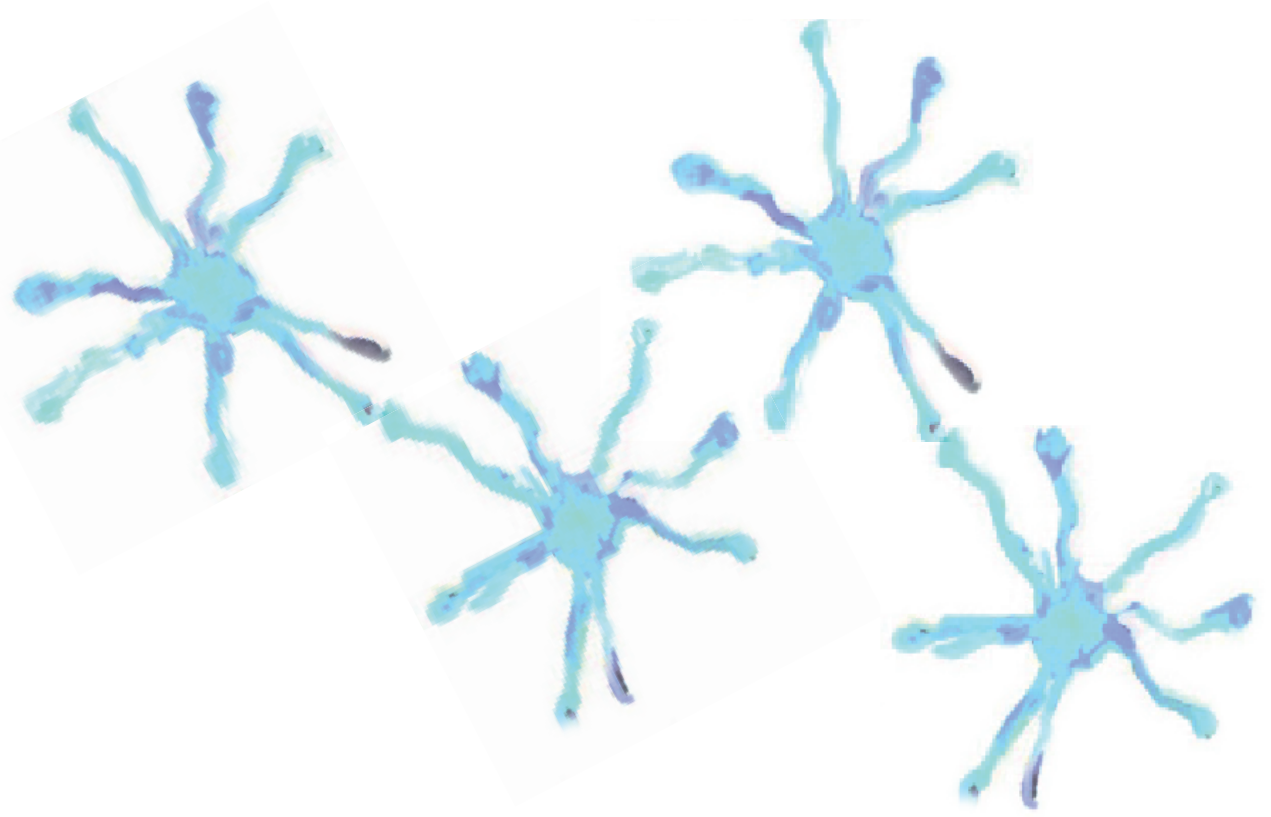

5 Progress in neuromodulation of the brain; a role for magnetic nanoparticles?

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ABSTRACT

The field of neuromodulation is developing rapidly. Current techniques, however, are still limited as they i) either depend on permanent implants, ii) require invasive procedures, iii) are not cell-type specific, iv) involve slow pharmacokinetics or v) have a restricted penetration depth making it difficult to stimulate regions deep within the brain. Refinements into the different fields of neuromodulation are thus needed. In this review, we will provide background information on the different techniques of neuromodulation discussing their latest refinements and future potentials including the implementation of nanoparticles (NPs). In particular we will highlight the usage of magnetic nanoparticles (MNPs) as transducers in advanced neuromodulation. When exposed to an alternating magnetic field (AMF), certain MNPs can generate heat through hysteresis. This MNP heating has been promising in the field of cancer therapy and has recently been introduced as a method for remote and wireless neuromodulation. This indicates that MNPs may aid in the exploration of brain functions via neuromodulation and may eventually be applied for treatment of neuropsychiatric disorders. We will address the materials chemistry of MNPs, their biomedical applications, their delivery into the brain, their mechanisms of stimulation with emphasis on MNP heating and their remote control in living tissue. The final section compares and discusses the parameters used for MNP heating in brain cancer treatment and neuromodulation. Concluding, using MNPs for nanomaterial-mediated neuromodulation seem promising in a variety of techniques and could be applied for different neuropsychiatric disorders when more extensively investigated.

1. INTRODUCTION

Neurological disorders are of huge impact in society. More than 90.000 disability adjusted life years (DALYs) are estimated for neurological disorders in the year 2015 increasing to a number of 100.000 DALYs in 2030 (WHO 2017). A larger part of these disorders includes both mental as well as neurodegenerative disorders. One example is Parkinson's disease (PD), increasing in incidence mainly due to the increase in human life expectancy (Schrag, Ben-Shlomo et al. 2000, van de Vijver, Stricker et al. 2001, Totaro, Marini et al. 2005, Havulinna, Tienari et al. 2008, Hirsch, Jette et al. 2016). The prevalence and thus disability due to PD has more than doubled from 1990 to 2015, with an estimation of 6.2 million people currently having PD worldwide. This number is expected to grow exponentially in the next decades (Dorsey and Bloem 2018) causing a substantial socio-economic burden for our society. For management of these disorders, we are in need of adequate treatment options. Unfortunately, up to now, preventive and drug-based therapies have shown limited progress and are not delivering the breakthroughs that the medical field needs to confront the challenges associated with population ageing (Temel and Jahanshahi 2015). Mainly improving the blood-brain barrier (BBB) permeability remains a challenge for drug-based therapies.

Contrary to this, the field of neuromodulation is progressing rapidly to continuously improve existing treatment strategies and to deliver new ones. In recent years, the application of transcranial magnetic stimulation (TMS), electric acoustic stimulation (EAS) and deep brain stimulation (DBS) have increased substantially in the clinics with focused ultrasound (FUS) as a newly emerging approach.

The clinical efficacy of DBS has been demonstrated in a number of disorders involving the basal ganglia and several neuropsychiatric disorders. The therapeutic concept of DBS is based on electrical stimulation through chronically implanted unilateral or bilateral electrodes into a specific subcortical structure in the brain. For PD, dystonia, Tourette's Syndrome and partial and generalized seizures, DBS has proven to be effective (Ackermans, Duits et al. 2011, Odekerken, van Laar et al. 2013, Schuepbach, Rau et al. 2013, Janssen, Duits et al. 2014, Dowd, Pourfar et al. 2017). Recently, new indications for DBS have emerged such as Alzheimer disease (AD) and intractable obesity (Whiting, Tomycz et al. 2013), needing greater follow up to show their effects.

Despite the proven clinical efficacy of DBS in the aforementioned indications, we lack a comprehensive understanding of the underlying mechanisms mediating these effects nor have we identified the exact distinct neural circuits underlying mental and behavioral sign and symptoms expressed in people diagnosed with the most prevalent mental and neurodegenerative disorders including depression, OCD, psychosis, dementia etc.

Current hypotheses about the key mechanisms involved in the effect of DBS are diverse. The 'inhibition hypothesis' suggests that local neuronal elements are inhibited upon stimulation showing similar effects as lesion therapy. This hypothesis fits well into the 'firing rate model' of movements disorders in which stimulating an overactive brain region inhibits the firing rate

(Lafreniere-Roula, Kim et al. 2010). The 'excitation hypothesis' suggest that DBS can also excite local neuronal elements, mainly axons, antidromically. This causes the activation of regions along efferent pathways (Deniau JM 2010, Reese, Leblois et al. 2011). Another hypothesis is 'the disruption hypothesis', proposing that the information flowing through the stimulated brain region is blocked upon DBS and thereby pathological activity is interrupted (Chiken and Nambu 2013). This can both be inhibitory or excitatory depending on the stimulated neural elements.

Although the underlying mechanisms of electrical DBS remain to be elucidated it is known to operate on a macroscale, lacking cell-type specificity. For this reason, its therapeutic effect will depend on the composition of neural elements in the targeted region causing interference with both pathological and physiological neural activities. This occasionally gives rise to side effects in a number of patients receiving DBS. For example, PD patients treated with DBS have reported speech deterioration as well as changes in mood, sleep and behavior which in turn range from new onset to worsening of pre-existing syndromes (Tan, Hartung et al. 2011, Kurtis, Rajah et al. 2017, Mucke, Hermes et al. 2018).

Another drawback of the current technique of DBS is that it requires the implantation of a relatively large, wired system which entails the risk of bleeding and infection peri- and postoperative. As a result, many patients are reluctant to undergo DBS when surgery is warranted (Kim, Yun et al. 2016). The first challenge thus addresses clinician and patient demands to develop new, wireless avenues for DBS technology. A second challenge addresses the continuous stimulation paradigm of current DBS which need improvements. New advancements are made introducing intermittent or adaptive DBS (aDBS) working with a closed-loop system (Herron, Thompson et al. 2017). This closed-loop system is created to measure and analyze biomarkers reflecting the patient's condition and to adapt its stimulation parameters accordingly improving treatment efficacy. Furthermore, this closed-loop systems benefit from less power consumption and therefore have a longer battery life. For PD, recent research has shown positive results when using aDBS of the subthalamic nucleus (STN) with LFPs in PD patients (Arlotti, Marceglia et al. 2018). A commercially available closed-loop system called responsive neuromodulation (RNS) has shown good results in patients suffering from refractory epilepsy (Sun and Morrell 2014). RNS includes an implanted neurostimulator that continuously records the electrocardiogram at the seizure focus and delivers brief pulses when abnormal electrographic activity is detected.

Another refinement for continuous stimulation is called coordinated reset (CR) DBS. In this method, brief high-frequency pulse trains are given through the different contacts of the stimulation electrode in treatment blocks for a few consecutive days resulting in desynchronizing effects lasting beyond cessation of the stimulus. In a non-human primate model of parkinsonism, CR DBS of the STN for 5 consecutive days resulted in acute motor improvements and, in contrast to traditional DBS, showed benefits persisting up to two weeks after stimulation (Wang, Nebeck et al. 2016). Moreover, the usage of rechargeable implantable pulse generators (rIPG) has made its entrance into the field and has been proven effective and applicable in OCD patients. These rlPGs have a longevity of nine years in contrast to the non-rechargeable IPGs showing a mean

longevity of 9 months (De Vloo, Raymaekers et al. 2017). Evaluation of the recharging process has been done with patients receiving CR DBS for PD, essential tremor (ET) and dystonia and was experienced as feasible with a low number of adverse events even in the elderly patients (Jakobs, Kloss et al. 2018).

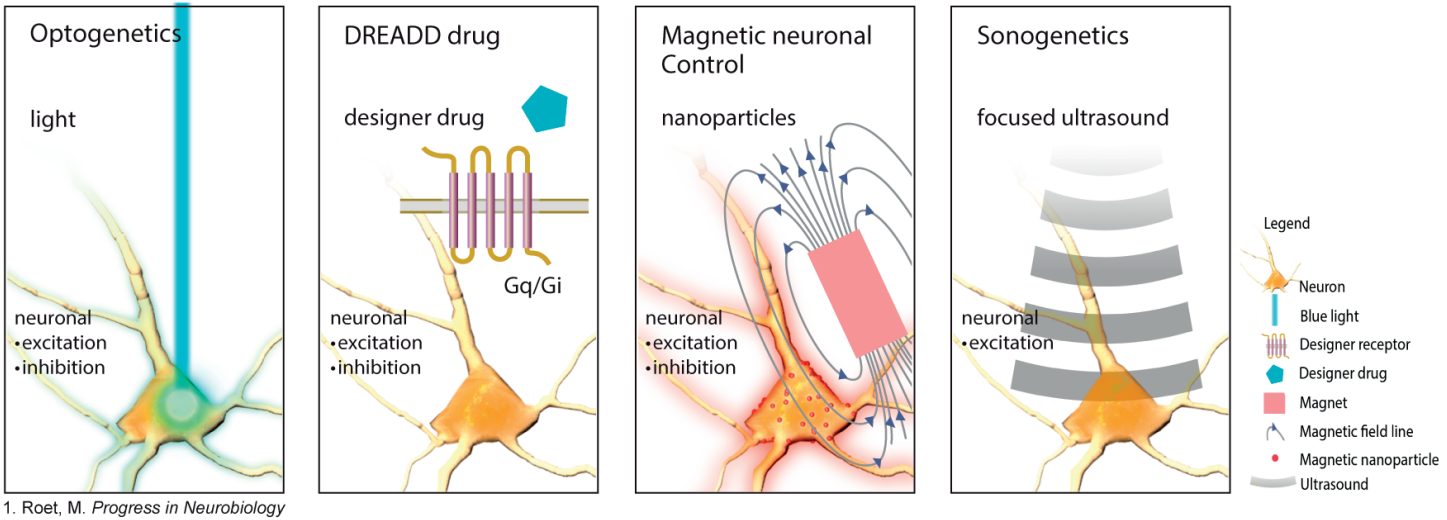
Despite these recent refinements, there remains a need for new advanced neuromodulation techniques in which the information transfer between the neuromodulation technique and the evoked neuronal signals can be performed more delicately. This ideally will only cause the modulation of pathological neural activity, leaving physiological neural activity in close vicinity unaltered, thereby minimalizing the possibility of side-effects. Here, we will discuss different advanced neuromodulation techniques of the brain and analyse their clinical potential. In particular, we will review relevant preclinical and clinical literature and evaluate the progress of our current understanding.

2. SEARCH STRATEGY

To describe and evaluate advanced techniques of neuromodulation of the brain and their latest refinements, we performed an extensive literature search. The literature for this review was identified by a PubMed search where the following keywords were queried either individually or combined: 'deep brain stimulation', 'adaptive deep brain stimulation (aDBS), closed-loop deep brain stimulation (closed-loop DBS), coordinated reset (CR) DBS, 'neuromodulation', 'optogenetics', 'DREADD', 'focused ultrasound' 'neuropsychiatric disorders', 'neurodegenerative disorders' and 'neurosensory disorders' with an additional search on ' nanoparticles (NP)', 'magnetic nanoparticles (MNP), 'iron oxide nanoparticles', 'alternating magnetic field', 'magnetic hyperthermia', 'hysteresis', 'MNP heating' and 'magnetothermal deep brain stimulation'. Relevant articles were chosen from review papers, original research articles and book chapters. Articles of interest within the reference lists of selected articles were also considered. The search was limited to studies published in English.

3. NEUROMODULATION OF THE BRAIN, NEW INSIGHTS

There are several advanced neuromodulation techniques besides electrical stimulation, such as optogenetics, Designer Receptors Exclusively Activated by Designer Drugs (DREADD), ultrasonic neuromodulation and magnetic neuronal control (Fig. 1). Each of these techniques has its own unique method of modulation and has broaden our insight into general neuronal function, numerous neural circuits underlying specific behavioral and pathological firing patterns responsible for various diseases. These advanced forms of neuromodulation might bring us closer to a



1. Roet, M. *Progress in Neurobiology*

Figure 1. Advanced techniques of neuromodulation, its modulation source and neuronal effect. This figure illustrates advanced techniques of neuromodulation. In optogenetics, genetically modified neurons expressing specific opsins, can be either excited or inhibited by visible light. With the DREADD technique, designer drugs can activate specific neurons expressing DREADDs-genetically engineered GPCRs. Ultrasonic neuromodulation offers a non-invasive way to stimulate or suppress neuronal activity by using focused ultrasound. Magnetic neuronal control, allows for remote and wireless activation of neural cells with or without transducers such as MNPs to convert magnetic fields into different stimuli.

more refined, clinically applicable technique of neuromodulation. Each technique, and its latest insights, is described in more detail below.

3.1. Optogenetics

In optogenetics, neurons are genetically modified to express microbial light-sensitive proteins termed 'opsins', which can be activated by visible light causing neuronal excitation or inhibition depending on the specific opsin. There are three classes of opsins, namely: the bacteriorhodopsins, the halorhodopsins and the channel-rhodopsins. Bacteriorhodopsins pump protons out of the cell causing hyperpolarization when inserted into a neuron and subsequently lead to neuronal inhibition. Inserted halorhodopsins cause hyperpolarization of neurons and neuronal inhibition by pumping negatively charged chloride ions into the cell. Channel-rhodopsins can either excite or inhibit neural systems when inserted into a neuron by allowing positively charged ions to flow into the cell or by chloride conduction, respectively (Deisseroth 2015). Early in its development, this groundbreaking method of neuromodulation has already demonstrated its ability to control the activity of specific mammalian neuronal populations with these engineered light switches (Boyden, Zhang et al. 2005). Shortly after, the modulation of a defined group of neurons in the hypothalamus, a structure deep in the brain, has been shown in freely moving mice (Adamantidis, Zhang et al. 2007). This research succeeded to show a causal relationship between frequent-dependent activity of these defined neurons and changes in the sleep-wake cycle as a behavioral outcome. Neuromodulation through optogenetics has continued to make remarkable progress over the past decade in animal models, illuminating the role of defined neural cell populations and their connectivity in healthy and disease-related states (Deisseroth 2015). Recently, implantable wireless optogenetic devices have been developed, allowing for untethered complex behavioral research in rodents. For instance, wireless optogenetic activation experiments in mice demonstrated both central and peripheral neural activation. One research group was able to design fully internal miniature light-emitting implants with a minimum size of 10 mm^3. These implants are wirelessly powered through a resonant cavity, activating a micro-LED embedded in the construct with electromagnetic energy coupling (Montgomery, Yeh et al. 2015). Other research demonstrated fully implantable soft optoelectronic devices. For peripheral nerves, a soft stretchable film with an incorporated LED was used and for stimulation of the spinal cord stretchable filaments which are able to be inserted into the epidural space were designed. Their research states that in order to minimize the constructs, a dynamically moving antenna coupling radio-frequency (RF) radiation is needed (Park, Brenner et al. 2015).

However, scaling up wireless optogenetic stimulation for the use in larger rodents or potentially even humans will remain a challenge since the amount of power required for RF-powered wireless optogenetic interfaces is considerably large. The need for an implanted light delivery device and the viral introduction of invertebrate genes, however, remain as the major challenges for clinical application of optogenetics. Furthermore, visible light needed to drive the inserted opsins is scattered and absorbed by neural tissue, thereby impeding its penetration into deep

brain regions in the absence of an invasive probe. These features hinder clinical application of optogenetics for neuromodulation in movement and neuropsychiatric disorders although recent translational efforts are underway. A recent study investigated a different approach of wireless optogenetics using NPs to serve as optogenetic actuators of transcranial near-infrared (NIR) light to stimulate neurons. In their experiments, upconversion NPs (UCNPs) were able to convert NIR light into blue or green light with enough intensity to activate corresponding opsins in the surroundings of these UCNPs. Their results show that in transgenic mice expressing ChR2 in the ventral tegmental area (VTA), in vivo neuronal activation is possible after the injection of UNCPs into the VTA and placing a NIR light probe 2 mm above the skull 4 weeks later. Furthermore, they demonstrated that neuronal silencing is also possible when using UCNPs that emit green light upon NIR light emission in transgenic mice expressing Arch in the hippocampus (Chen, Weitemier et al. 2018). These findings might be another different step towards wireless optogenetics, keeping in mind that the emission intensity of these particles decreased with an increase of the distance between the NIR light and UNCPs. Long distances might therefore pose a challenge. In non-human primates (NHP), the first optogenetics study showed the activation of neurons in the primary motor cortex upon optical stimulation (Han, Qian et al. 2009). Successive studies showed that optogenetics in NHP can also serve as a tool to modulate specific behavior, such as choice behavior when modulating dopamine activity and inducing saccadic dysmetria when stimulating cerebellar Purkinje cells (Stauffer, Lak et al. 2016, El-Shamayleh, Kojima et al. 2017). In recent years, different disease models in transgenic primates have been added, which create opportunities to explore new optogenetic therapies (Liu, Li et al. 2016). In the field of ophthalmology, optogenetics is taking its first step into the clinics with a clinical trial in which researchers try to restore vision in completely blind patients by placing channelrhodopsin-2 into retinal ganglion cells (Schmidt 2017).

3.2. Chemogenetics with DREADD

In chemogenetics, specific neurons are virally transduced to express DREADDs-genetically engineered G-protein coupled receptors (GPCRs) with high affinity to designer drugs, allowing for modulation of cellular functions through systemic administration of the drug. Clozapine N-oxide (CNO), is the most commonly used designer drug and is a pharmacologically inert metabolite of the antipsychotic drug clozapine (Armbruster, Li et al. 2007, Roth 2016). Following initial discovery in yeast, both excitatory and inhibitory DREADDs have been demonstrated. For enhancing neuronal firing, the Gq-DREADDs hM1Dq, hM3Dq and hM5Dq have been developed of which hM1Dq has been used the most. These DREADDs enhance neuronal activity by increasing intracellular calcium concentrations. The first study investigating the modulation of neurons via hM1Dq demonstrated the activation of hippocampal neurons after CNO administration in hM1Dq-expressing mice (Alexander, Rogan et al. 2009). To inhibited neuronal activity, Gi-DREADDs hM2Di, hM4Di, and KORD are being used. Both hM4Di and KORD inhibited neuronal activity via hyperpolarization of the cell and synaptic silencing. HM4Di

is the mostly used inhibitory DREADD and the first study investigating its property showed neuronal silencing when incorporated into hippocampal neurons (Armbruster, Li et al. 2007). Furthermore, the DREADD GsD has been used to modulate plasticity via an increase in cAMP and the DREADD Rq(R165L) is used to enhance β-arrestin specific signaling (Roth 2016). Chemogenetics enables genetically-precise control of cellular activity in both superficial and deep brain regions and is less invasive compared to optogenetics. Its temporal precision, however, is limited by the pharmacokinetics of the designer drugs (Guettier, Gautam et al. 2009, Gomez, Bonaventura et al. 2017). Nevertheless, this promising approach has led to the discovery of several behavioral circuits in rodents, including associative learning, memory and reward guided behavior. Moreover, it has been applied to various animal models of human disease, thereby enhancing its translational application (Urban and Roth 2015, Roth 2016, Whissell, Tohyama et al. 2016). In a chronic model of focal neocortical epilepsy in rats, it has been shown that virally introduced hM4Di into the seizure focus attenuates seizure frequency upon intraperitoneal CNO application. These results are promising as a possible intervention for intractable focal epilepsy (Katzel, Nicholson et al. 2014). Akin to opsins, DREADDs have recently been expressed in NHPs. Research done in rhesus monkeys demonstrated a repeatable disruption in relative reward value when the functional connection between two different brain regions, namely the orbitofrontal and rhinal cortices, was temporarily disrupted by inhibitory DREADD modulation (Eldridge, Lerchner et al. 2016).

Just recently, however, researchers have shown that not CNO but clozapine binds to DREADD. This finding might have implications for the interpretation of observed effects in previous research that used this technique. Based on the previous findings, it has been suggested that DREADD is an inaccurate name since the receptors are not activated by a designer drug nor are they exclusive. For future research, it has been proposed that scientists may simply apply clozapine as the actuator of this technique, keeping in mind to use proper controls. Clozapine is already an approved drug; nonetheless, due to its high affinity to various other receptors, scientists should use it at a low dose and carefully evaluate possible off-target effects (Gomez, Bonaventura et al. 2017).

Altogether, this technique seems promising, but still requires either the use of viral DREADD introduction or genetically engineered animals and its temporal precision is limited by slow pharmacokinetics. One advancement for DREADD could be the implementation of NPs as a safer alternative for gene delivery than viruses. Previous research has already shown cellular siRNA delivery with gold NPs and nanocarriers (Kakizawa, Furukawa et al. 2006, Elbakry, Zaky et al. 2009). This approach could simplify DREADD for clinical applications since now lentiviral delivery is one of its drawbacks.

3.3. Ultrasonic neuromodulation

Ultrasound (US) is acoustic energy in the form of sound pressure waves at very high frequencies not audible to the human ear. It has been shown that these sound pressure waves can interact

with biological tissue, making US a well-known biomedical imaging modality. US can penetrate through the skull and be focused at specific regions deep within the brain without losing its signal. Focused ultrasound (FUS) can produce thermal and non-thermal effects depending on various parameters such as its frequency, intensity and exposure time. High-intensity FUS (HIFU) produces thermal effects on targeted tissue resulting in tissue ablation. A recently conducted randomized controlled clinical trial amongst patients suffering from ET showed that MRI-guided FUS lesioning of the thalamus resulted in an improvement in hand-tremor scores (Elias, Lipsman et al. 2016). HIFU has also been widely investigated as a form of cancer therapy including prostate, breast, liver, kidney, pancreatic cancer and bone malignancies showing mixed results (Hsiao, Kuo et al. 2016). Recently a study investigated whether adding MNPs to HIFU could enhance the thermal effects showing promising results (Devarakonda, Myers et al. 2017). In contrary to HIFU, low-intensity FUS has shown to be able to stimulate neuronal circuits by non-thermal (mechanical) effects without causing any neuronal damage. The first *in vivo* experiments demonstrated that transcranial pulsed US to the motor cortex in anaesthetized mice could evoke motor behavior (Tufail, Matyushov et al. 2010). The following years, different brain circuits in various species have been modulated using transcranial FUS (tFUS), as reviewed in more detail elsewhere (Fini and Tyler 2017). In NHP, low-intensity FUS was able to modulate visuomotor behavior due to the disruption of information processing across the frontal eye fields (Deffieux, Younan et al. 2013). Also human applications of ultrasonic neuromodulation have already been investigated. In healthy volunteers it is shown that tFUS is able to produce changes in sensory-evoked brain activity. In these experiments, healthy volunteers were given median nerve stimulation while recording these sensory evoked brain oscillations. Subsequently tFUS was given, which caused suppression of the evoked somatosensory potentials (Legon, Sato et al. 2014). Other research showed that when stimulating the primary somatosensory cortex (S1) in healthy volunteers, a transient tactile sensation on the contralateral side of the stimulated hemisphere could be observed. Simultaneously EEG recordings showed sonication specific potentials in the S1 (Lee, Kim et al. 2015). When stimulating the visual cortex with tFUS in healthy volunteers, a visual sensation could be evoked. Furthermore their results show that not only the sonicated brain area, but also other regions involved in visual processing were activated, demonstrated by simultaneously acquisition of blood-oxygenation-level-dependent functional MRI (Lee, Kim et al. 2016).

Ultrasonic neuromodulation is a promising technique since its application does not require the use of exogenous agents. However, the underlying mechanisms of FUS induced neuromodulation are still unclear. One hypothesis is that the mechanical force of FUS activates mechanosensitive ion channels embedded within cell membranes (Tyler 2012). The applied pressure waves may stretch or deform the cellular membrane altering the state of mechanosensitive ion channels embedded within these membranes leading to transmembrane currents and consecutive neural activity. Recent research elucidated that FUS is indeed capable of modulating sodium and potassium mechanosensitive ion channels expressed in Xenopus oocytes resulting in transmembrane

currents (Kubanek 2016). Another research group introducing a technique called 'sonogenetics' showed that low pressure US is capable of inducing specific behavior in the *Caenorhabditis elegans* (c. elegans) by misexpressing a pore-forming subunit of the mechanotransduction channel TRP-4 (Ibsen, Tong et al. 2015). All these observations together make ultrasonic neuromodulation an interesting non-invasive technique for future clinical application and we believe this field of neuromodulation will grow rapidly within the upcoming years. Future advancements in spatial resolution is expected to further improve this technique. One interesting finding is the combination of FUS with drug carrying NPs as is recently investigated in rats. In this research, ultrasound-gated NPs that encapsulated propofol were given intravascular and released their drug due to a conformation change of the NPs by FUS (Airan, Meyer et al. 2017). This enables a more targeting drug release. Additionally, FUS seems to be a promising method of delivering NPs to brain targets due to the possibility of BBB disruption on its own (Liu, Chen et al. 2011, Chu, Chai et al. 2016). Combining these two modalities could make the delivery of MNPs into the brain less invasive.

3.4. Magnetic neuronal control

Magnetic neuronal control is a recently discovered technique, which may hold promise as a clinical neuronal activation approach since it does not require implantation of invasive electrodes or optical devices, it can penetrate into the brain and has a lower response latency than that achieved with drug delivery. Magnetic fields with magnitudes in millitesla range are able to penetrate into the brain without attenuation of the signal or given side effects because of the negligible magnetic susceptibility and low conductivity of biological tissue (Young 1980). Several research groups are investigating magnetic neuronal control by activating ion channels on membranes using purely the magnetic field itself or by the usage of transducers responding to this magnetic field such as MNPs. The latter can be subdivided into either magnetothermal activation, magnetomechanical activation and magnetoelectric activation. All will be discussed in detail.

3.5. Transcranial magnetic stimulation (TMS)

TMS is a technique used for neuromodulation in which an electric current generated in a copper wire coil induces a non-invasive magnetic field able to penetrate thought the skull into brain regions directly below the coil. This magnetic field subsequently induces another electric current in the underlying brain capable of inducing neuromodulation. Since the magnetic field strongly decays with distance, TMS is mainly limited to cortical stimulation. Depending on the given stimulation protocol, TMS can induce immediate effects through stimulation and disruption and after-effects through neuroplastic changes when multiple consecutive magnetic pulses are given called 'repetitive TMS' (rTMS). A general belief is that low frequency rTMS causes long-term depression (LTD) in the underlying brain region while high-frequency rTMS causes long-term potentiation (LTP) (Klomjai, Katz et al. 2015). Nowadays, rTMS for major depression disorder if

FDA approved and other disorder such as stroke and OCD are investigated in research context (Demitrack and Thase 2009, Avenanti, Coccia et al. 2012, Elbeh, Elserogy et al. 2016).

One problem with rTMS is its variability between individuals generating a bimodal pattern of response with responders and non-responders to a certain given TMS protocol (Fitzgerald, Hoy et al. 2016). In some patients low-frequency rTMS has an inhibitory effect while in other patients it has an excitatory effect and vice versa. As a consequence, the responds to rTMS therapy is very patient-specific and applying multiple rTMS protocols might be necessary (Eldaief, Halko et al. 2011).

To be less stressful and time consuming for the patient, shortening the time of a TMS protocol is desired. For this reason, theta-burst (iTBS) and accelerated rTMS protocols have been established. rTMS protocols last 30 to 45 minutes, while iTBS paradigms require 1 to 3 minutes. In iTBS, short trains of stimuli at a high frequency are repeated in intervals of 200 ms. In the THREE-D study, 3 minutes iTBS has shown to have equal effects to 37,5 minutes of high frequency rTMS (Han, Chen et al. 2018). In accelerated rTMs protocols, multiple rTMS session are given within one day. Research has shown that accelerated rTMS given for depression can shorten the days of treatment in 'fast responding' patients, however other patients still need the extra days of treatment to show the same decline in BDI-II Scores (Holtzheimer, McDonald et al. 2010).

One recent advantage in TMS is the introduction of deep TMS (dTMS). DTMS uses so called 'H' coils providing a magnetic field which penetrates deeper into the brain but automatically also generates a bigger field spread making the signal less specific. For this reason different 'H' coils are designed for different disorders. For Alzheimer disease, findings suggest some improvements in the Alzheimer's disease assessment scale-cognitive subscale when treated with dTMS (Coppi 2016). A possible refinement of this technique could be the combination of dTMS with MNPs to enhance or transduce the signal. Combining these two approaches might lead to the stimulation of more defined brain regions deeper in the brain.

3.6. Magnetic stimulation using transducers

Combining magnetic neuromodulation with transducers converting or enhancing the magnetic signal has indeed been done before. Magnetothermal activation uses AMFs to induce MNP heating through hysteresis and triggers heat-sensitive cation channels from the Transient Receptor Potential Vanilloid (TRPV) family causing depolarization and action potential firing (Huang, Delikanli et al. 2010, Stanley, Gagner et al. 2012, Chen, Romero et al. 2015, Munshi, Qadri et al. 2017). Magnetomechanical activation uses the force exerted by iron-containing particles or proteins tethered to the cell membrane in the presence of magnetic fields to trigger pressure sensitive receptors that convert this signal into neural modulation (Stanley, Sauer et al. 2015). Magnetoelectric activation uses magnetoelectric NPs composed of magnetostrictive core and a piezoelectric shell to generate local electric fields when exposed to an external magnetic field (Guduru, Liang et al. 2015, X. Chen and E. Siringil 2016). In contrast to standard DBS and TMS,

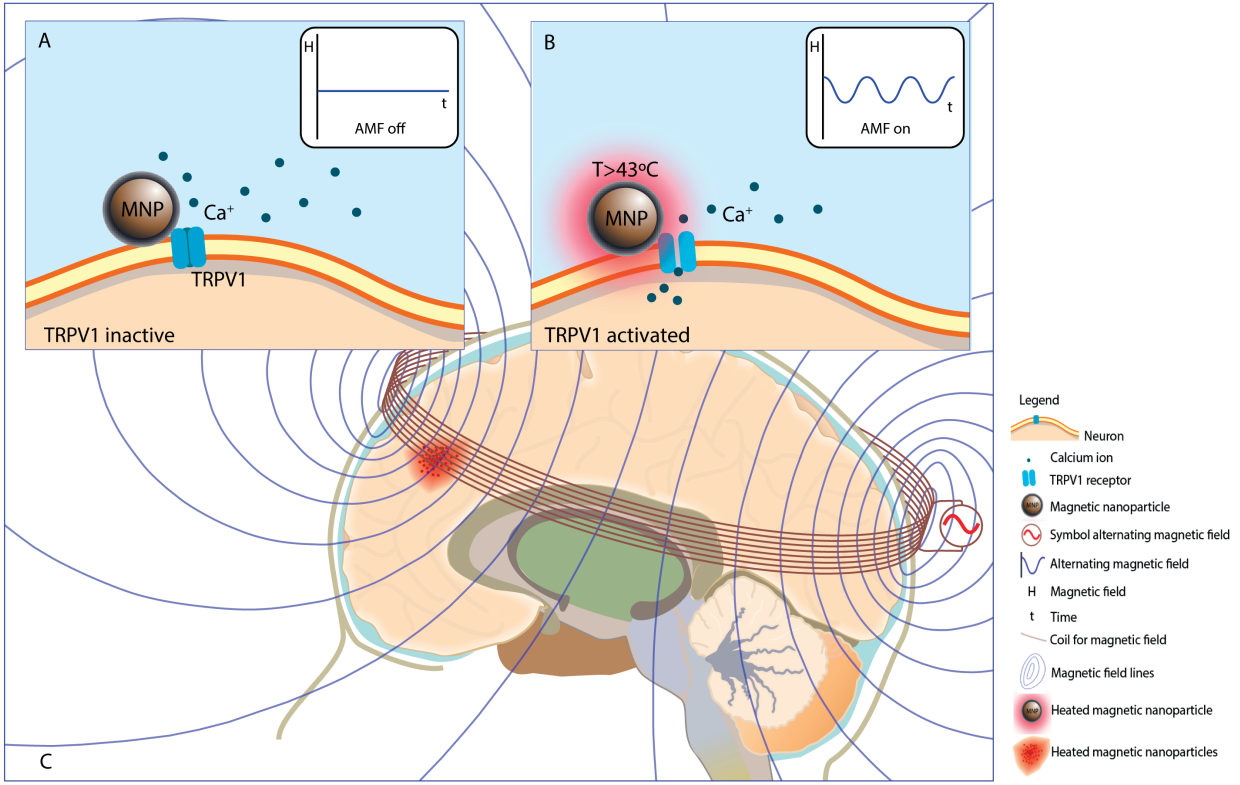
adding these transducers allows to operate on a nanoscale increasing its precision and region or cellular specific targeting.

The first study to establish remote neuronal control of cell function leveraged RF AMFs and MNP heating to activate the capsaicin receptor TRPV1, which resulted in the calcium influx into human embryonic kidney (HEK) 293 cells. The authors also showed that the MNP heating triggered behavioral responses in C. elegans (Huang, Delikanli et al. 2010). Other researchers investigating magnetic cellular activation demonstrated that modified TRPV1 receptors with extracellular antibody-coated iron oxide NPs could regulate protein production *in vivo* when exposed to a magnetic field. This work also indicated that a fusion of an iron-binding protein ferritin to TRPV enables control of calcium influx *in vitro* when exposed to a magnetic field (Stanley, Gagner et al. 2012). Based on these findings, several groups went on to investigate TRPV-ferritin fusion constructs in the context of magnetic manipulation of cellular function in behaving rodents (Stanley, Sauer et al. 2015, Stanley, Kelly et al. 2016, Wheeler, Smith et al. 2016). The mechanisms of neural modulation in these studies, however, remain poorly understood since ferritin is weakly paramagnetic and thereby the physical ability of this protein to activate TRPV by either thermal or mechanical stimuli appears unlikely (Meister 2016). Although a number of biophysical studies are currently underway (Duret 2017), several questions remain, including which mechanism is causing this neuronal activation, what is the extent of excitation, and which cell types are affected.

Magnetothermal activation in the mammalian brain was recently demonstrated by AMF induced bulk heating of MNP solution injected into the VTA of anaesthetized mice expressing TRPV1 following viral delivery (Chen, Romero et al. 2015). Hysteretic heating of MNPs in the presence of AMF activates TRPV1, which causes calcium ion influx into heat-sensitized cells and yields membrane depolarization and neural excitation. The latter suggests that such an approach could be a possible candidate for neuromodulation. As such, the first application of magnetothermal activation to control behavior of awake freely moving mice has been recently demonstrated (Munshi, Qadri et al. 2017). This work showed that magnetothermal stimulation of motor cortex evoked locomotor activity and stimulation of different parts of the striatum induced rotation or freezing-of-gate. The same research group recently showed MNP induced neuronal silencing. Hippocampal neurons were transfected with the chloride channel Anoctamin 1 (TMEM16A) and spontaneous firing was suppressed using MNP induced heating opening this inhibitory channel (Munshi, Qadri et al. 2018).

The studies discussed above employed genetic tools to achieve TRPV1 expression in the given brain areas in mice. This ion channel is endogenously expressed in neurons in the mammalian central nervous system (CNS), which suggests that it can be a promising target for future investigations for delivery of magnetic neuronal control (Marinelli, Pascucci et al. 2005, Starowicz, Cristino et al. 2008, Sun, Guo et al. 2013, Terzian, dos Reis et al. 2014, Nam, Park et al. 2015).

To develop magnetic neuronal control for biomedical applications, leveraging endogenously expressed receptors of physical stimuli may provide a convenient approach. The use of MNP



2. Roet, M. *Progress in Neurobiology*

Figure 2. A schematic view of magnetothermal DBS in the human brain. In part A, a MNP surrounds a neuronal cell close to its cell membrane. No external AMF is applied, therefore the TRPV1 receptor remains closed. In part B, the external AMF is turned on leading to MNP heating adjacent to the neuronal cell and TRPV1 receptor. This heat signal opens the TRPV1 receptor causing a calcium influx and subsequent activation of the neuronal cell. Part C illustrates a magnetic coil surrounding the human head, which is needed to generate an AMF to activate the MNPs in a particular brain region. MNP heating is shown by the red dots.

heating and TRPV1 may offer a route to clinical applications with a clear mechanism of stimulation and without genetically engineered TRPV-ferritin fusion constructs (Fig. 2: Schematic view of magnetothermal DBS in human). Similarly, the use of magnetoelectric particles has the potential to improve upon resolution and cell-type specificity of traditional electrical DBS.

MNPs have already shown promising results in a variety of biomedical applications, ranging from cancer hyperthermia to magnetic resonance imaging, and wireless neuromodulation may be another intriguing possibility.

4. MAGNETIC NANOPARTICLES

4.1. Biomedical applications

MNPs can be used in a wide range of biomedical application since they have several beneficial characteristics. Firstly, MNPs contain paramagnetic properties making them good candidates as contrast agents in imaging. For MRI, the MNPs can alter the relaxation mechanism of protons resulting in sharper images (Lee, Yoo et al. 2015). For fluorescence imaging quantum dot nanocrystals are used as fluorophores emitting light when excited by long wavelengths as thoroughly reviewed elsewhere (Utkin 2018). Secondly, MNPs can be targeted toward specific tissue by the appliance of an external magnetic field or by coating the NPs with targeting moieties (Steichen, Caldorera-Moore et al. 2013). Thirdly, MNPs can be coated with therapeutic agents enhancing drug bioavailability while keeping the drug dose low (Latorre, Couleaud et al. 2014). Fourthly, MNPs can serve as transducers producing and or converting incoming stimuli. Hyperthermia is one of these examples being used in cancer therapy and to transiently increase BBB permeability (Wankhede, Bouras et al. 2012, Wegscheid, Morshed et al. 2014, Tabatabaei, Girouard et al. 2015, Wang and Guo 2016). Furthermore, magnetomechanical destruction and heat transfer beneficial in cryosurgery can be accomplished by MNPs (Yu, Yi et al. 2014, Wang and Guo 2016). Combining multiple characteristics within the same particle to enable both imaging, diagnostic and therapy purposes is desired, and this concept is reviewed in more detail elsewhere (Gobbo, Sjaastad et al. 2015).

Several decades of research in nanomaterials have delivered a diversity of particles with different composition, structure, properties, and functions (Kateb, Chiu et al. 2011, Chen, Roy et al. 2016). MNPs constitute a class of NPs composed of magnetic materials. Some MNPs can undergo hysteretic power loss in externally applied AMF resulting in heat dissipation. Depending on the thermal dosage released by the MNPs, this remote-controlled heating can be used for tumor therapy that induces apoptosis of malignant cells or for neural activation as discussed above (Maier-Hauff, Ulrich et al. 2011, Chen, Romero et al. 2015). Combined with their utility for imaging and drug delivery, MNPs constitute a promising platform for nanotheranostics. With the new concept of magnetic neuronal control using MNPs, MNP heating for the purpose of neuromodulation might become a next step in nanotheranostics.

4.2. Materials chemistry

MNPs are crystal structures with their linear dimensions in the order of 100th of nm or less (Mody, Siwale et al. 2010). For the purpose of MNP-heating in AMFs, their inorganic core needs to be composed of a magnetic material and their chemistry needs to be optimized to maximize heat dissipation under specific AMF conditions (Chen, Christiansen et al. 2016). The commonly used MNPs consist of magnetite phase (Fe3O4) of iron-oxide (Silva, Oliveira et al. 2011). The common methods of MNP synthesis are chemical coprecipitation, thermal decomposition, and microemulsion. In chemical coprecipitation, a mixture of Fe^{2+} and Fe^{3+} ions are submitted to hydrolysis forming magnetite precipitates. The particle-size depends on the ratio of different ions and the temperature during hydrolysis (Petcharoena K. 2012, Verma, Lal et al. 2014). Thermal decomposition is a process in which organometallic iron precursors are decomposed under high temperatures of up to 473 K. The advantages of this method compared to coprecipitation include superior control over chemical composition, size, and shape of the MNPs. The solvent in which the MNPs are synthesized greatly influences the consistency of magnetic properties of the particles, and optimizing the solvent's redox activity is important for appropriate magnetic phases with desirable magnetic properties (Chen, Christiansen et al. 2016). Another approach of producing uniform size-controlled NPs is microemulsion. In this method Fe^{2+} salts are oxidized in a microemulsion in which the controlled temperature and added surfactant concentration determines the particle size. However, large amounts of solvent are necessary to synthesize substantial amounts of MNPs, which makes this process challenging to scale (Lee Y. 2005, Lu, Salabas et al. 2007, Laurent, Forge et al. 2008, Verma, Lal et al. 2014, Wegscheid, Morshed et al. 2014).

4.3. Particle coating

Besides an iron-oxide core, MNPs need a surface coating of a biocompatible material to ensure their solubility in aqueous physiological solutions, to minimize potential cytotoxicity, and to enhance their biocompatibility. Iron oxide can give iron-mediated radical formation and oxidative stress in the brain if not coated, so adding coating is a must (Petters, Irrsack et al. 2014). However, it must be noted that coating can also limit characteristics of the particles such as heating, therefore choosing the right surface coating is critical. Various *in vitro* and *in vivo* studies have applied a variety of coatings including dextran, carboxydextran, glycosaminoglycan, N-(a-trimethyl ammonioacetyl)-didodecyl-D-glutamate chloride, and polyethylene glycol (PEG) (Laurent, Forge et al. 2008, Silva, Oliveira et al. 2011). For human studies, however, only aminosilane-coated MNPs have been used to date (Maier-Hauff, Rothe et al. 2007, Maier-Hauff, Ulrich et al. 2011). It is also important to consider possible coating effects on the particle's pharmacokinetics and biodistribution. Table 1 summarizes different types of MNP coating material for studies using magnetic hyperthermia. To induce target delivery of MNPs to specific cells, MNPs can be decorated with targeting moieties such as short peptides, binding proteins, and antibodies (Wankhede, Bouras et al. 2012, Shah, Pasquale et al. 2014, Wegscheid, Morshed et

al. 2014, Yin, Shah et al. 2014, Munshi, Qadri et al. 2017). These target moieties will recognize the cells of interest and only stimulate the neuronal cells in close vicinity due to a small span of signal transduction. While the majority of targeting strategies have been explored in the context of tumor therapies, similar approaches may permit targeting of specific neurons deep in the brain for neuromodulation (Gobbo, Sjaastad et al. 2015).

5. MNPS IN THE CENTRAL NERVOUS SYSTEM

5.1. Delivery

In order to reach specific neurons in the brain, MNPs either need to cross the BBB or should be delivered directly into the brain via invasive means. Crossing the BBB remains a challenge for systemic delivery of MNPs and other substances due to its selective nature. For this reason, either direct intratumoral delivery or convection-enhanced delivery (CED) has been used in clinical applications of MNPs (Hadjipanayis, Machaidze et al. 2010, Maier-Hauff, Ulrich et al. 2011). Although these methods allow for the delivery of high concentrations of MNPs or other therapeutic substances into the brain, they are invasive and carry a potential risk of hemorrhage and infection. Many ways to cross the BBB has been researched including intra-carotid arterial infusion of hyperosmotic solutions, CNS vaccines, the lipidization of small molecules and receptor mediated transport all being inappropriate for nanoparticle transport (Pardridge 2007). Photodynamic therapy has also been investigated to increase BBB resulting into several studies showing a high accumulation of a photosensitizer into glial tumors (Stummer, Novotny et al. 2000), but only scarcely detectable amounts in intact BBB of the rat (Madsen, Angell-Petersen et al. 2006). Notable, it was recently shown that MNP heating in brain capillaries can transiently increase BBB permeability without causing inflammation or neurovascular damage (Tabatabaei, Girouard et al. 2015). It seems that this technique offers promise as an approach to deliver MNPs or other substances directly into the brain with minimal invasiveness. Focused ultrasound in the presence of microbubbles provides another way to transiently increase BBB permeability (Chu, Chai et al. 2016). However, in the context of magnetic hyperthermia tumor therapy or magneto-thermal neuromodulation, increasing the BBB permeability may not be sufficient and magnetic field gradients may be necessary to aid transport of MNPs across the disrupted BBB (Liu, Hua et al. 2010). Another way to transiently open the BBB has been demonstrated in patients suffering from malignant glia tumors. In this study, dTMS was able to increase the BBB permeability for contrast agents in 10 out of 15 patients. Increased BBB permeability was found not only directly in the tumor region but also peritumoral, in the ipsilateral and contralateral hemisphere (Vazana, Veksler et al. 2016). This method could be combined with the administration of NPs and might be a promising application for the future.

5.2. Biodistribution, uptake and clearing

What happens with MNPs once they are in the CNS? A rodent study showed that intratumoral instillation of aminosilane-coated MNPs led to the formation of stable deposits, which allowed for repeated magnetic field treatments (Jordan, Scholz et al. 2006). For the purpose of neuromodulation, it has been shown that in mice NPs are distributed in extracellular spaces close to cell membranes and synaptic clefts, with a small fraction taken up by microglia and neuronal axons (Chen, Weitemier et al. 2018). In multiple mice studies NPs seem to minimally disperse within one month after injection (Chen, Romero et al. 2015, Chen, Weitemier et al. 2018). Several other research groups have demonstrated that activated microglia *in vitro* can display macrophagic properties and internalize MNPs primarily into vesicles, albeit the fate of MNPs following macrophagic internalization remains to be investigated (Rogers and Basu 2005, Ribot, Bouzier-Sore et al. 2007). Furthermore, research showed that coating MNPs with PEG prevents non-specific cellular MNP uptake when incubated in human plasma (Schottler, Becker et al. 2016). A group investigating magnetoelectric NPs in human astrocyte cells and peripheral blood mononuclear cells showed that there is no significant toxicity of these particles when analyzed with a Cell Proliferation Assay Kit (XTT) (Guduru, Liang et al. 2015). Post mortem analysis of patients treated with magnetic hyperthermia for glioblastoma multiforme (GBM), the most aggressive malignant form of brain cancer, revealed that the majority of MNPs were aggregated in areas of necrosis within the tumor and largely distributed around the site of instillation. The survival of patients after MNP-injection ranged from two weeks to 7.9 months. The MNPs at the border of the aggregates were internalized mainly by macrophages (95%) and only a few by tumor cells (5%) (van Landeghem, Maier-Hauff et al. 2009). The larger clinical trials investigating MNP heating for GBM did not explicitly report the location of the MNPs after a certain exposure time (Maier-Hauff, Ulrich et al. 2011). Further research is warranted to investigate the long-term effects and clearance of MNPs.

6. MNP HEATING AND ITS APPLICATIONS IN THE BIOMEDICINE AND NEUROSCIENCE

6.1. Thermal dosage

MNPs dissipate heat when exposed to an AMF (Maier-Hauff, Rothe et al. 2007, Nair, Nagaoka et al. 2010, Maier-Hauff, Ulrich et al. 2011, Silva, Oliveira et al. 2011, Wankhede, Bouras et al. 2012, Lee Titsworth, Murad et al. 2014, Rivet, Yuan et al. 2014, Schaub, Rende et al. 2014, Verma, Lal et al. 2014, Wegscheid, Morshed et al. 2014, Dan, Bae et al. 2015, Tabatabaei, Girouard et al. 2015). Prolonged local rise in temperature above the normal body temperature is applied in cancer therapy to induce apoptosis in malignant cells. When applied in conjunction with standard treatments, MHT using MNPs enhances the overall survival rate of patients following diagnosis of first tumor recurrence of GBM (Maier-Hauff, Ulrich et al. 2011). Applied AMF parameters

can be adjusted in order to decrease the thermal dosage delivered by MNPs, thereby making the heating signal suitable for neuromodulation without inducing cell damage. Importantly, when a MNP solution was employed for magnetothermal neural excitation in the ventral tegmental area, only the neurons within a 200 μm border experienced local heating and no significant damage was observed following repeated cycles of AMF exposure (Chen, Romero et al. 2015).

MNP heating originates from hysteresis when the particles are exposed to an AMF with a given field frequency and amplitude. The amount of heat released by a MNP during one cycle of an AMF equals to the area of its hysteresis loop. The amount of heat produced by MNPs in a given AMF depends on their properties, such as the magnetic anisotropy, the saturation magnetization, its volume, and the magnetic interactions between the particles. The MNP heating efficiency can be quantified either by the specific loss power (SLP), which refers to the power achievable per gram of iron in the MNPs at a given AMF, or by the specific absorption rate (SAR), which refers to the amount of energy converted into heat per time and mass. Both metrics are expressed in watts per gram. Table 1 summarizes studies that used MNP heating for either GBM therapy, cellular or neuronal modulation, showing SLP and SAR for particular MNPs and AMF parameters used.

This table shows different articles investigating MNP heating in either GBM therapy, cellular or neuronal modulation. It states the composition of the MNPs, the AMF parameters used and the amount of energy produced by their MNPs in their experiments.

6.2. Treatment of brain cancer

In clinical trials for the treatment of recurrent GBM, an aqueous MNPs dispersion is instilled within the tumor using neuronavigation. Postoperatively, the patient is placed in an alternating magnetic field applicator MFH 300F causing an AMF and subsequent MNP heating within the tumor. Results from Maier-Hauff et al show that the intratumoral median temperature following AMF varied between 39-51.2°C with a maximum intratumoral temperature of 82°C (Maier-Hauff, Rothe et al. 2007, Maier-Hauff, Ulrich et al. 2011). The maximal duration of hyperthermia lasted for 60 minutes per session, which was enough to produce a cytotoxic effect in the tumor cells. Hyperthermia treatment consisted of six semi-weekly treatment sessions, combined with stereotactic radiotherapy. In a rat model of GBM, 40 minutes of AMF was already sufficient to cause this cytotoxic effect (Jordan, Scholz et al. 2006). Comparisons between treatment paradigms are summarized in table 1.

6.3. The path towards neuromodulation

The application of MNP heating for cellular activation and neuromodulation requires a lower thermal dosage and median increase in temperature, as compared to hyperthermia used for GBM treatment (Table 1). As a consequence, optimization strategies for MNP properties and AMF parameters differ between the two applications. In the study by Chen et al. 2015, the combination of high MNP SLPs and short 10 second AMF stimulation pulses enabled a rapid raise in median

Table 1. Materials chemistry of MNPs used for hyperthermia in either GBM therapy, cellular of neuronal modulation and the AMF parameters used.

Study		MNP core	$Ø_{core}$ (nm)	Coating	MNP conc. (mg/ml)	Iron weight administrated	Genetic Construct
Jordan Maier-Hauff et al 2006	*In vivo*	Iron oxide	3 15	Carboxydextran Aminosilane	NI NI	1.80 mol/L 2.00 mol/L	-
Maier-Hauff et al 2007	*In vivo*	Iron oxide	15	Aminosilane	NI	112 mg/ml	-
Maier-Hauff et al 2011	*In vivo*	Magnetite	12	Aminosilane	NI	112 mg/ml	-
Silva et al 2011 review	*In vivo*	Magnetite, Maghemite	1-35	TMAG/DLPC/ DOPE/CMC/ Carboxydextran/ Dextran/ Aminosilane	NI	$2.00E^{-7}$-20 mg/ml	-
		Magnetite	12-15	Aminosilane	NI	1.12-112 mg/ml	-
Shah et al 2014	*In vitro*	Zn-doped iron oxide	15.40	Au-coated + ATAP	$5.00E^{-3}$-0.02	NI	-
Yin et al 2014	*In vitro*	Zn-doped iron oxide	22.92 ± 3.70	MNP-PEI/miRNA/ PEI complexes	0.01	NI	-
Pralle et al 2010	*In vitro*	Magnetite	6	-	**	NI	-
	In vivo	Magnetite	6	Polyethylene glycol (PEG)-phospholipid	**		
Stanley et al 2012	*In vivo*	Iron oxide	20-25	Anti-His	8	NI	TRPV1His
Stanley et al 2015	*In vivo*	Ferritin	-	-	-	100 mg/ml iron dextran intraperitoneal	GFP-TRPV1/ GFP-ferritin
Chen et al 2015	*In vivo*	Iron oxide	22	(Polyethylene glycol) PEG	100	NI	-

Subject	Function	Injected volume (ml)	Magnetic field frequency (kHz)	Magnetic field amplitude (kA/m)	Stimulation paradigm	SLP/ SAR (W/g)	Temperature (°C)
GBM cells injected in rats	HT for GBM	$2.00E^{-2}$	100	0-18	kA/m gradually increased to desired level for 10 min and sustained for 30 min	0-35*	Max: 39 Max: 43-47
Human	HT for GBM	0.25/ml tumor	100	2.5-18	60 min	2-35*	Med: 44.60 Max: 49.50
Human	HT for GBM	0.28/ml tumor	100	2-15	60 min	2-30*	Med: 51.20 Max: 82
Gliomas in mice or rat	HT for GBM	0.1-0.4	88.90-150	11-30.60	20-60 min	96-286	39-47
Human	HT for GBM	0.25-0.28ml/ml tumor	15-18	100	60 min	NI	49.60-65.60
GBM cells, metastatic breast cancer cells	Peptide therapeutics and HT	NI	300	5	45 min	NI	NI
GBM cells	HT for GBM	NI	225	5	0-60 min	341	44.10
Hippocampal neurons	Remote control neurons	NI	40000	0.67-1	45 sec	2.51	Max: 43
C. elegans	Worm retraction	NI	40000	0.67	17 sec	2.51	Bulk: 34
PC-12 cells injected in mice	Remote regulation of gene expression	$5.00E^{-2}$	465	3.99	30 min	0.63	NI
MSC cells injected in mice	Remote regulation of gene expression	0.05 iron dextran intraperitoneal	465	23.13 or 25.53	60 min	NI	NI
Mice injected with TRPV1 lentivirus	Control of cellular signaling in non-excitable and electro-active cells	$2.50E^{-3}$	500	15	10 seconds field pulses with 50 seconds rest interval 20 min	660 +/- 50	Med: 43 Max: 45

Table 1. Materials chemistry of MNPs used for hyperthermia in either GBM therapy, cellular of neuronal modulation and the AMF parameters used. (*continued*)

Study		MNP core	$Ø_{core}$ (nm)	Coating	MNP conc. (mg/ml)	Iron weight administrated	Genetic Construct
Stanley et al 2016	*In vivo*	-	-	-	-	-	GFP–TRPV1/ GFP–ferritin, GFP–TRPV-1mutant/ GFP–ferritin
Pralle et al 2017	*In vivo*	Co-ferrite core, (MN-ferrite shell)	10.25 ± 1	PMA (poly -isobutylene -maleic anhydride), NeutrAvidin coupled to antibodies	1	NI	-
Pralle et al 2018	*In vitro*	Co-Mn-ferrite core	10.25 ± 1	PMA (poly -isobutylene -maleic anhydride), NeutrAvidin coupled to antibodies	10	NI	-

NI: Not indicated in paper. * Not indicated in paper, values deducted from 'Description and characterization of the novel hyperthermia- and thermoablation-system MFH®300F for clinical magnetic fluid hyperthermia. Uwe Gneveckow,a) Andreas Jordan,b) and Regina Scholz c). ** Hippocampal cells incubated in 10nM nanoparticle solution for 1 minute. C. Elegans incubated in 1nM nanoparticle solution for 1 minute. *** Measured at 500 kHz and 15 kA/m.

temperature up to 43°C with a maximum increase to 45°C. During the 50 seconds rest epochs, the tissue cooled back down to 37°C. This short intermittent exposure to AMF induced neural activation and prevented the harmful heating of cells by prolonged AMF exposure, thereby avoiding cytotoxicity (Table 1). In the study of Munshi et al. 2017, the combination of high MNP SLPs and three to four one-minute stimulation epochs during a 10-15 minutes experiment enabled control of motor behavior in mice while avoiding brain tissue damage. Their results show that magnetothermal stimulation of the motor cortex elicited running. Magnetothermal stimulation of the striatum caused rotation around the body axis, while stimulation of the ridge between dorsal and ventral striatum caused freezing of gait. Furthermore, their findings demonstrate short latencies between starting or terminating the AMF stimulation and the observed behavior (Munshi, Qadri et al. 2017). These reports show that a short intermittent AMF stimulation induces well dosed and temporarily precise MNP heating, which is the key to safe and effective magnetic neuromodulation.

Subject	Function	Injected volume (ml)	Magnetic field frequency (kHz)	Magnetic field amplitude (kA/m)	Stimulation paradigm	SLP/ SAR (W/g)	Temperature (°C)
Cre mice	Activation or inhibition of glucose-sensing neurons	4.00^{E-3} iron dextran into lateral ventricle	465	18.35, 21.54 or 24.73	30 min	NI	NI
Mice injected with TRPV1 AAV virus	Remote control neurons, behavioral output	6.00^{E-4}	570	7.5	Four one-min field applications within a 15-min trial	450 ***	0.1 to 0.5°C/s, membrane bound: 0.1 to 1.0°C/s
Hippo-campal neurons	Remote control neurons, Ca^{2+} imaging and AP firing pattern	0.2	412.5	28.87 +/- 1.03	5 seconds intervals	553 +/- 10	3°C in 5 seconds

7. ADVERSE EFFECTS OF MNP HEATING

Adverse effects of MNP heating are subject of vigorous investigation. Clinical research for MHT in GBM treatment indicated that adverse effects, such as swelling of the brain and rise of intracranial pressure, could be avoided by very slow injection of the magnetic fluid (Maier-Hauff, Rothe et al. 2007). In these studies, moderate adverse effects included sweating (50%), a general sensation of warmth in the treated area (47.0%), headaches during hyperthermia (13,8%), focal convulsions (22.7%), motor disturbances (21.2%), and perifocal edema (9%). Focal convulsions stemmed primarily from a pre-existing hemiparesis. Only 2% of the patients who experienced motor disturbances or focal convulsions had developed these side effects during MHT. Despite worsening of pre-existing hemiparesis, none of the side effects persisted in the long term, and their physiological origins remain unclear (Maier-Hauff, Ulrich et al. 2011).

Another adverse effect of increased temperature could be the aggregation of the MNPs. A study investigating citrate-coated-iron- oxide MNPs, observed accelerated aggregation of the particles following hyperthermia *in vitro*. This clustering of MNPs can change their magnetic properties and cause occlusion when administered into a blood vessel (Wegscheid, Morshed et al. 2014). This could have great disadvantageous clinical consequences, therefore preventing this is utterly important. Surface chemistry plays a significant role in avoiding MNP aggregation so a carefully designed surface passivation is essential for clinical efficacy of MNPs.

The effects of magnetic hyperthermia on the viability of healthy neurons greatly differ among different MNP studies. For MHT on healthy rat astrocytes, it has been found that stimulating for 2 consecutive hours led to decreased astrocyte viability, even at physiological temperatures (Schaub, Rende et al. 2014). Another study found that 2 hours exposure of healthy chick embryonic cortical neurons to hyperthermia did not yield any negative effects (Rivet, Yuan et al. 2014). Intermittent magnetothermal stimulation of healthy neurons in the VTA of mice showed no difference in neuronal or glial density between stimulated and non-stimulated groups (Chen, Romero et al. 2015). Therefore, the effects of magnetic hyperthermia on neuronal viability depend for a large part on MNPs composition, magnetic field stimulation paradigms, the interval of increased temperature, the maximum increased temperature reached, and the type of tissue stimulated. On top of that, such results highlight the importance of conducting studies that directly compare neuronal viability outcomes using the same magnetic stimulation paradigms.

Another area lacking experimental investigation is the long-term effect of MNP-heating on microvasculature. It is plausible that the nearby microvasculature adapts to repetitive exposure of heat and, therefore, should be taken into consideration.

Due to the dearth of studies and lack of consistency between MNP chemistries, AMF parameters, as well as exposure paradigms, toxicological data concerning hyperthermia with MNPs remain inconclusive (Table 1) (Nano, Lascialfari et al. 2012, Wankhede, Bouras et al. 2012). Some evidence point in the direction of astrocytic mitochondrial stress and attachment defects after nanoparticle administration *in vitro* (Au, Mutkus et al. 2007). In patients treated with MNP heating for GBM, key parameters for iron metabolism were determined before and after the administration of MNPs, showing no indication of iron release from intratumoral deposits or iron being metabolized (Maier-Hauff, Ulrich et al. 2011). Nonetheless, the long-term toxicological effects of NPs located in the CNS and their clearance require further investigation, in which different magnetic stimulation parameters should be taken into account as well.

8. FUTURE CHALLENGES FOR MNP INDUCED NEUROMODULATION

Magnetic coils suitable for cancer hyperthermia and magnetothermal neuromodulation in rodents can be engineered to efficiently generate appropriate AMF conditions over small experimental volumes (Attaluri, Kandala et al. 2015, Kossatz, Grandke et al. 2015, Christiansen, Howe et al. 2017, Munshi, Qadri et al. 2017). Scaling AMF coils to volumes necessary for neuromodulation or tumor therapy in deep brain regions of human patients present a formidable challenge, as the power requirements to achieve comparable AMF conditions increase substantially. Despite these challenges, recent engineering efforts build upon techniques in the field of power electronics to pave the way toward development of scaling approaches for AMF coils (Lacroix, Carrey et al. 2008, Christiansen, Howe et al. 2017). The next step for this neuromodulation approach could

be the implementation of magnetic neuronal control into different animal models mimicking human diseases such as PD and upscaling the size to non-human primates. For instance, MNPs injected into the STN expressing TRPV1 of 6-OHDA rats, a rat model of PD, could possible revert Parkinson's-like behavior such as circling motor abnormalities upon stimulation. Another robust experiment could be the injection of MNPs into the VTA expressing TRPV1 to modulate both rewarding and aversive drug-dependent behavior.

A different approach for MNP induced neuromodulation could be by the usage of dTMS. dTMS is able to penetrate slightly deeper in the brain than TMS, however deep brain regions such as the STN can still not be reached. One possibility could be to combine dTMS with the usage of MNPs. The magnetic field might not be strong enough to modulate the tissue on its own but added MNPs might be able to detect the magnetic signal, transducing it into a signal for neuromodulation. The challenge here remains making MNPs that respond to low frequencies or a TMS device working at frequencies in the kHz range. So far, the MNPs discussed above are activated by frequencies in the kHz range while dTMS for MDD works at a frequency of 18 Hz (Tendler, Barnea Ygael et al. 2016).

Other forms of neuromodulation such as optogenetics and DREADD can incorporate the usage of MNPs for a more wireless approach and to incorporate genes virus free making it more clinically applicable.

To get MNPs and or drugs into a desired brain region, FUS or dTMS seem to be a promising candidates. Both are capable of transiently increasing BBB permeability and future research combining these techniques with targeting neuromodulation using moieties need to prove its feasibility.

9. CONCLUSION

Current techniques of neuromodulation are limited as they require permanent implants, are invasive, lack cell-type specificity, have limited penetration depth into different brain regions, or rely on slow pharmacokinetics. Refinements are needed and are slowly making their entrance into the field. DBS is a neuromodulation technique already widely used in the clinics but interferes with both pathological and physiological neural activity due to its lack in cell-specificity causing unwanted side-effects in some patients. At the moment mostly continuous stimulation is given, but promising improvements like aDBS and CR DBS are now being investigated. For optogenetics, the limitation is the need of visible light through an invasive probe to drive neurons. Nonetheless, refinements are on the way using other actuators like NPs to convert the light signal and overcome invasive light probes. Also, small, fully implantable, optoelectronic devices converting RF radiation into visible light are now being researched. Chemogenetics, still requires DREADD introduction via viral components or genetic engineering and is mostly limited in temporal precision due to slow pharmacokinetics of the administered drug. Since the actuator

of DREADD is now assumed to be Clozapine instead of CNO, previous study results need to be interpreted with caution. Nanoparticle-based gene delivery could circumvent the need of viruses in DREADD, making it more convenient for clinical applications. Ultrasonic neuromodulation seems a promising technique not needing permanent implants and being less invasive than the aforementioned techniques. These sound pressure waves can penetrate the skull and interact with deep brain structures without losing its signal, making it an interesting candidate for clinical neuromodulation purposes. Furthermore, it can disrupt the BBB, making it an interesting candidate to deliver NPs and or NP encapsulated drugs into the brain. Magnetic neuronal control is another promising technique since it also does not require the implantation of invasive electrodes or optical devices. With this method, stimulation of deep brain regions is possible because of the negligible magnetic susceptibility and low conductivity of biological tissue. In addition, this technique has a faster response rate than that achieved with drug delivery.

Subsequently, we discussed the application of MNPs for nanomaterial-mediated neuromodulation in more detail and compared this application method to the current use of these particles in treating recurrent GBM. The application of MNPs as transducers of magnetic field into thermal, electrical, mechanical or chemical stimuli offers a possibility to remotely and wirelessly modulate specific groups of cells in arbitrarily deep regions of the brain. Magnetothermal stimulation application of AMF pulses only causes a short and modest temperature increase, which modulates cells whilst avoiding cytotoxicity due to prolonged exposure. Further research should implement this new technique in various animal models of signs and symptoms as expressed in mental, neuropsychiatric, neurosensory and neurodegenerative disorders in order to restore physiological brain functions and to define the therapeutic value of magnetothermal DBS in these disorders.

10. DISCLOSURE

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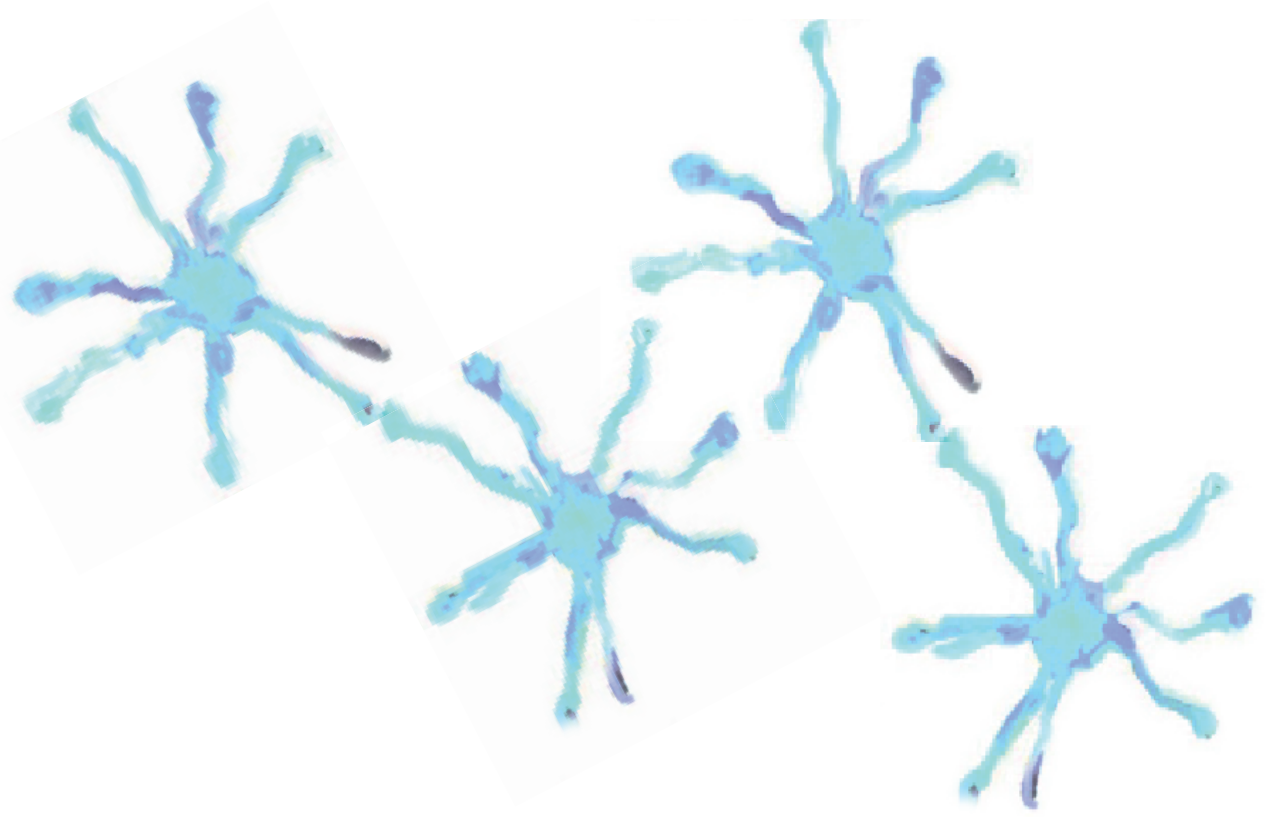

6 Magnetothermal deep brain stimulation of the subthalamic nucleus causes rotational behavior in freely moving mice

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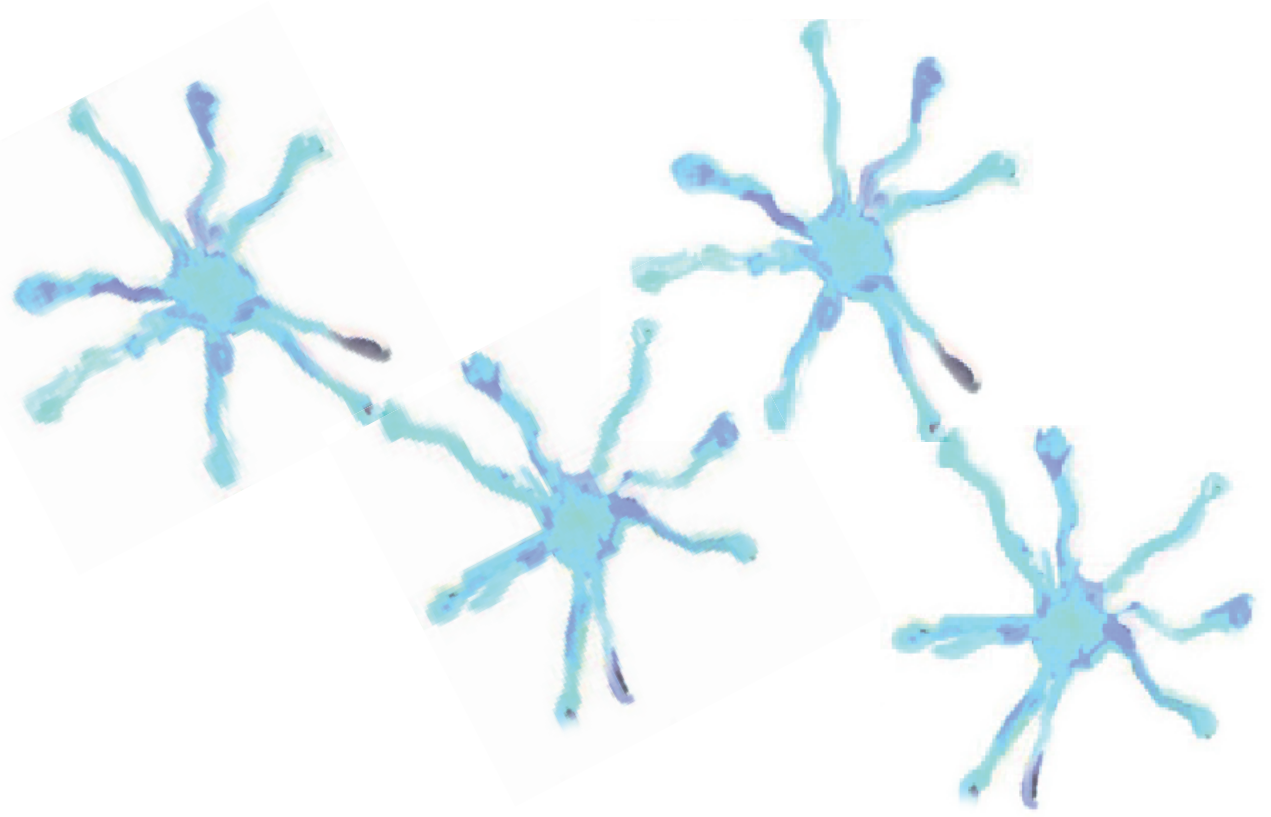
Submitted



7 Endogenous TRPV1 expression in the human cingulate- and medial frontal gyrus

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ABSTRACT

Background: The transient receptor potential vanilloid subtype-1 (TRPV1) channel is a calcium selective ion channel that responds to various stimuli such as heat, low pH, and capsaicin. Recently this channel was studied as an actuator for wireless neuromodulation in rodents, e.g., heat-induced activation of TRPV1 resulted in neuronal excitation. From a translational perspective, we addressed if TRPV1 is endogenously expressed in the human medial frontal gyrus (MFG) and cingulate gyrus (CG) in depressed and control subjects and if it can be used as a means for neuromodulation in mood and also other neuropsychiatric disorders.

Methods: We assessed TRPV1 expression levels by Western blotting and evaluated its tissue and cellular distribution by means of immunohistochemistry.

Results: TRPV1 was observed in all tissue samples, i.e., depressed and control, MFG and CG, yet the expression level as assessed by Western blotting varied between individuals. No intra-individual differences were seen between the MFG and CG. Immunohistochemistry showed that TRPV1 was expressed by glial-like cells but also in neurites, endothelial cells, and to a lesser extent in neuronal cell bodies. Fluorescent co-labeling of TRPV1 and glial fibrillary acidic protein (GFAP) identified most glial cells expressing TRPV1 to be astrocytes.

Conclusion: These findings indicate that TRPV1 is endogenously expressed in the human CG and MFG. As TRPV1 is predominantly expressed by glial cells, this may suggest an opportunity for non-neuronal network modulation.

1. INTRODUCTION

The transient receptor potential vanilloid subtype-1 (TRPV1) is a subfamily of the transient receptor potential cation channels and functions as a molecular integrator for multiple types of sensory input. It is selective for calcium ions and responds to capsaicin, noxious heat, low extracellular pH, divalent cations, and particular toxins [1]. TRPV1 has been described in the peripheral pain pathway where the receptor can initiate nociceptive signaling by generating a receptor potential [2]. In addition to peripheral expression, various reports state that TRPV1 can be found in the brain [3]. Recently TRPV1 was used as an actuator for neuromodulation in mouse brain [4]. However, due to low endogenous expression of TRPV1 in the central nervous system of rodents, the TRPV1 channel was introduced with lentiviral delivery. This method of neuromodulation seems to be a promising approach for future clinical application, although lentiviral delivery of TRPV1 might be undesirable.

To explore the possible clinical use of TRPV1 for neuromodulation we investigated if TRPV1 is endogenously expressed in the human brain. A sufficient expression of TRPV1 in neurons is necessary for this technique to work properly. We focused on subjects with depression since TRPV1 channels have been implicated in depression and anxiety [5-9].

We investigated the medial frontal gyrus (MFG) and cingulate gyrus (CG) of subjects whom experienced depression in their medical histories and compared them to non-demented control subjects. The MFG, which is part of the dorsolateral prefrontal cortex, is hypoactive in MDD while the CG is hyperactive in MDD [10, 11]. Therefore, these brain regions are potential targets for neuromodulation. In this paper we aim to examine whether TRPV1 is sufficiently expressed in these brain regions to be considered as a target for neuromodulation.

Abbreviations: DBS; deep brain stimulation, CG; cingulate gyrus, GFAP; glial fibrillary acidic protein, MDD; major depressive disorder, MFG; medial frontal gyrus, PD; Parkinson's disease, SN; substantia nigra, TRPV1; transient receptor potential vanilloid subtype 1.

2. MATERIALS AND METHODS

2.1. Subjects

Fresh-frozen and paraffin embedded brain tissue of depressed and control subjects containing the CG and MFG were provided by the Netherlands Brain Bank (NBB) (Table 1). As a positive control, we used temporal neocortical tissue of an epileptic patient provided by Maastricht UMC+ (MUMC+) for an abundant expression of TRPV1 in patients with mesial temporal lobe epilepsy has been reported earlier [12]. All experiments have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Table 1. Clinical characteristics of the subjects in the NBB samples.

Subject No.	Diagnosis	Gender	Age (years)	Resected tissue	Amyloid	Braak	Post-mortem delay
P1	Depression	F	84	MFG	O	2	08:45
P2	Depression	M	89	CG	O	1	04:35
				MFG			
P3	Depression	F	66	CG	O	1	07:55
				MFG			
P4	Non-demented control	F	84	MFG	A	2	05:36
P5	Non-demented control	M	89	CG	O	2	06:50
				MFG			
P6	Non-demented control	F	82	CG	A	1	07:45

2.2. Western blot

Western blot analysis was performed to investigate and compare TRPV1 expression in the CG and MFG of depressed and control subjects. Fresh frozen brain tissue was cut on a cryostat and lysed in homogenization buffer (1 g/ 9 mL) containing 10% protease inhibitor (cat. no. 11697498001; Roche). Total tissue lysates were centrifuged at 12,000 rpm for 10 min at 4°C. Protein concentrations in the supernatants were estimated using the Lowry protein assay (Bio-Rad). Odyssey protein molecular weight marker and samples (100 µg/lane) were loaded onto a 4% stacking gel (acrylamide/Bis 29:1, 1M Tris-HCl pH 6.8, 20% SDS, 10% APS, TEMED) / 8% running gel (acrylamide/Bis 29:1, 3M Tris-HCl pH 8.8, 20% SDS, 10% APS, TEMED). The resolved proteins were transferred onto a PVDF membrane using a mini-protein transfer system (Bio-Rad) at 100 V for 2 h in transfer buffer (1.4% glycine, 0.3% trizma base, 20% methanol). Next, membranes were blocked for 1 h at room temperature (RT) with Odyssey blocking buffer (cat. no. 927-40003; LI-COR) and then incubated overnight at 4°C with primary antibodies (mouse anti-glycerylaldehyde-3-phosphate-dehydrogenase (GAPDH) (1:1,000,000, cat. no. 10R-G109a; Fitzgerald) and rabbit anti-TRPV1 (1:1,000; cat. no. PA1-748; Thermo Fisher Scientific) diluted in Odyssey blocking buffer. The following day, the membranes were washed and subsequently incubated with secondary antibody (goat anti-rabbit IR Dye 800 CW, Alexa 488 (1:10,000, 925-32211; LI-COR) and donkey anti-mouse IR Dye 680, Alexa 700 (1:10,000, 925-68072; LI-COR) for 1 h at RT in the dark. After washing, the membranes were dried between filter paper and analyzed using an Odyssey scanner.

To validate TRPV1 specificity and to visualize TRPV1 degradation products, one membrane was stained with anti-TRPV1 (1:1,000; cat. no. PEP-202; Thermo Fisher Scientific) that was preabsorbed with TRPV1 synthetic peptide (1:1).

Optical densities (OD) of TRPV1 for each sample were calculated relative to the GAPDH protein band in that same sample with the use of the program ImageJ. To plot TRPV1 degradation against post-mortem delay, we calculated the ratio of the relative OD of TRPV1 degradation products and TRPV1 degradation products together with intact TRPV1.

2.3. Immunohistochemistry

Paraffin-embedded tissue was cut into 5 µm thick sections using a microtome.

All sections were deparaffinized followed by antigen retrieval in boiled citrate buffer (10 mmol) for 10 min and allowed to cool down a consecutive 20 min. Sections were blocked for endogenous peroxidase activity using a 0.3% H_2O_2 in TBS solution for 30 minutes. After washing, primary antibody was added overnight at 4°C (TRPV1; 1:100 or 1:200; cat. no. PA1-748; Thermo Fisher Scientific). The following day, all sections were incubated with secondary antibody for 1 h at RT (biotinylated donkey anti-rabbit; 1:200; cat. no. 711-065-152; Jackson ImmunoResearch). All sections were then washed and stained with an ABC kit (1:400; cat. no: PK-6100; Vector Labs) followed by an incubation in DAB-NiCl (1 DAB : 1 Tris-HCl, 0.5 % NiCL); cat. no: d5637-10g; Sigma-Aldrich) for 10 min. After washing, the sections were dehydrated in ethanol and coverslipped with Pertex. Photomicrographs were taking using an AX-70 microscope (Olympus Provis) and Cell^P software.

2.4. Immunofluorescence double-labeling

For immunofluorescence all sections underwent deparaffinization and antigen retrieval as described above. The primary antibodies were added overnight at 4°C (TRPV1; 1:50; cat. no. PA1-748; Thermo Fisher Scientific and anti-GFAP, 1:50, G3893; Sigma-Aldrich). The next day secondary antibodies were added for 1 h at RT (for TRPV1; donkey anti-rabbit Alexa 647 (1:100, cat. no. A-31573, Invitrogen), for GFAP; donkey anti-mouse Alexa 488 (1:100, cat. no. A-21202, Invitrogen)). All sections were washed and stained with Hoechst (1:500). A final step of 10 min incubation with Sudan black (0.5% in 100% ethanol) at RT in the dark was done to prevent autofluorescence from lipofuscin. All sections were coverslipped with glycerol. Microscopy was performed using an upright fluorescence microscope (Olympus BX51WI) and Stereo Investigator software.

3. RESULTS

3.1. Western blotting

Results showed an immunoreactive band of 95 kDa in control tissue and the MFG and CG in both depressed and control subjects (Fig. 1 and supplementary Fig. 1).

Pre-absorption of the anti-TRPV1 antibody with synthetic peptide resulted in a loss of the 95 kDa immunoreactive band. In addition, this condition also resulted in the loss of several lower immunoreactive bands (supplementary Figs. 2 and 3). Analysis of the OD showed a high inter-individual variability of the 95 kDa immunoreactive band and thus TRPV1 expression (Fig. 2).

Relative OD values were compared between the CG and MFG regions of all subjects. For the CG, both samples from the depression group showed a higher relative OD as compared to the controls. However, a univariate ANOVA showed no significant difference in the interaction effect

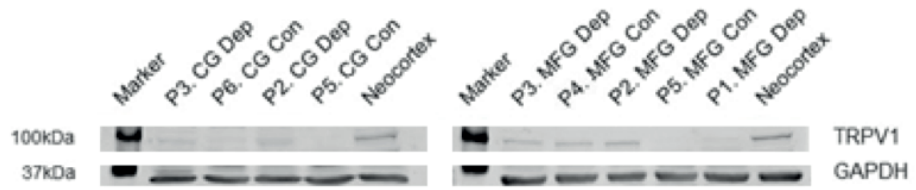

Figure 1. Western blot TRPV1 expression. Western blot of TRPV1 expression per subject and brain region. CG: cingulate gyrus, MFG: medial frontal gyrus, Con: Control, Dep: depressed, P#: subject number.

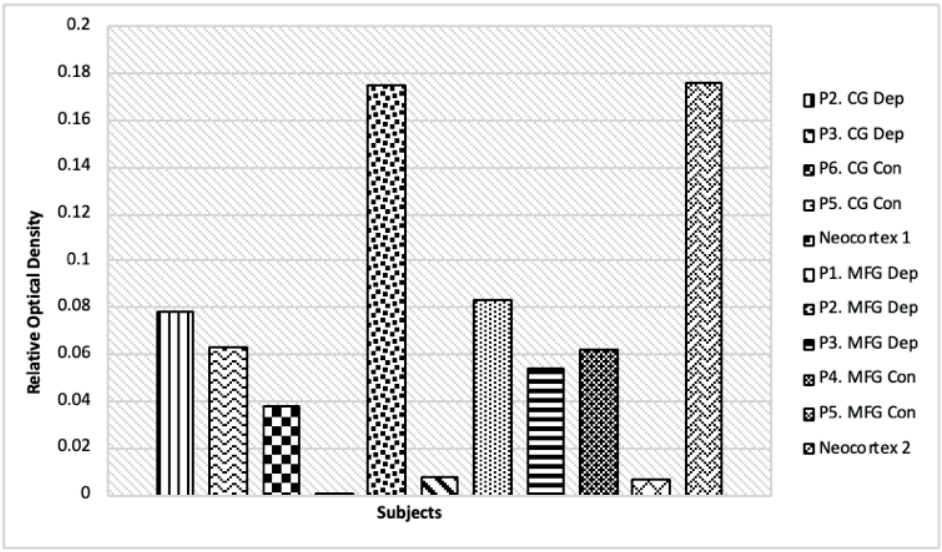

Figure 2. Individual TRPV1 expression levels. The individual TRPV1 relative OD values per subject and brain region represented by bar charts. CG: cingulate gyrus, MFG: medial frontal gyrus, Con: Control, Dep: depressed, P#: subject number.

between regions and the subjects state (depression vs control; p=0.442). No differences were found for the MFG region (Fig. 3).

In subjects of whom we were able to investigate both the CG and MFG, no significant difference in relative OD values of TRPV1 expression was found (n=3; Student's paired t-test p=0.083; Fig. 4). For the other subjects either the CG or MFG was not available for research.

We found a moderate negative correlation between the post-mortem time and the expression level of TRPV1 (Spearman correlation coefficient r_s=-.456). A short post-mortem time of 4.5 h resulted in 91.48% degradation of TRPV1 95 kDa into 50-75 kDa bands compared to 99.34% with a post-mortem time of 7 h (Fig. 5).

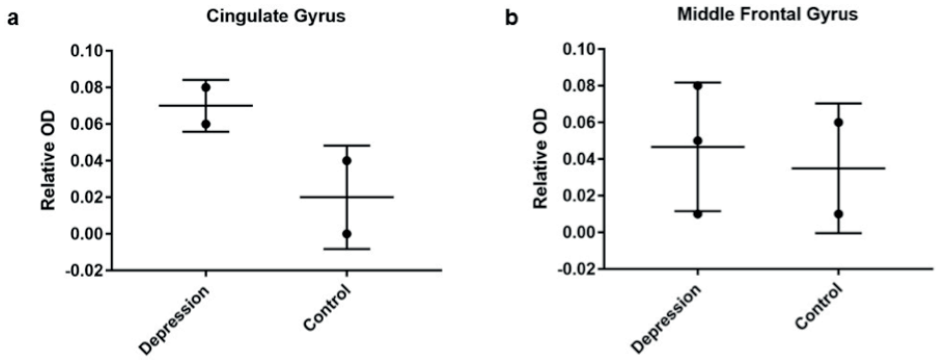

Figure 3. Differences in TRPV1 expression levels between depression and control within one brain region. Relative OD values measured in **a)** the depression (n=2) and control group (n=2) in the CG. **b)** the depression (n=3) and control group (n=2) in the MFG. Each dot represents one individual value and the average is represented with a horizontal line. CG: cingulate gyrus, MFG: medial frontal gyrus.

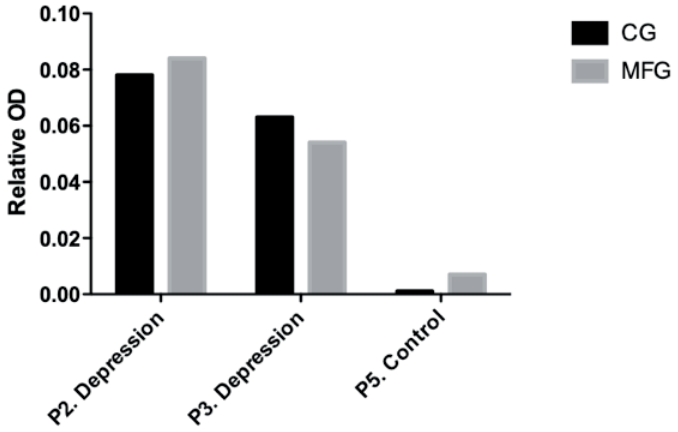

Figure 4. Relative OD values of the CG and MFG in the same subjects. The relative OD values of TRPV1 in the CG and MFG of the same subject (n=3) represented by bar charts. CG: cingulate gyrus, MFG: medial frontal gyrus.

3.2. Immunohistochemistry and double immunofluorescence labelling

To investigate in which cell types TRPV1 is expressed we executed immunohistochemistry and *double immunofluorescence labelling* on paraffin-embedded tissue of the CG and MFG of the subject that showed the highest TRPV1 expression in the western blots. We observed a high TRPV1 expression in glial-like cells in both the CG and MFG (Figs. 6 and 7), endothelial cells in the CG and MFG, neurite structures in the CG, and to a lesser extend in the MFG and neuronal cell bodies in the CG and MFG. Neuronal cell bodies were found scarcely and stained less intensely than the others mentioned structures (Figs. 6 and 7).

Fluorescent double labelling revealed TRPV1 co-expression with GFAP containing cells both in the CG and MFG (Fig. 8).

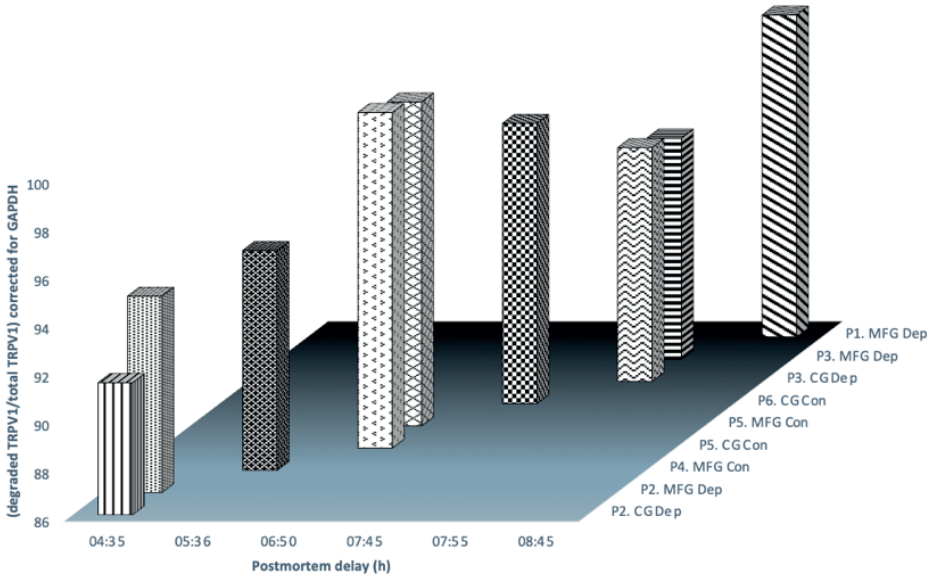

Figure 5. TRPV1 degradation and post-mortem delay. The amount of degraded TRPV1 and post-mortem delay per subject and brain region represented by bar charts. Degraded TRPV1 is represented as a percentage by calculating the ratio of the relative OD of 50-75 kDa : (the relative ODs of 50-75 kDa + 95 kDa).

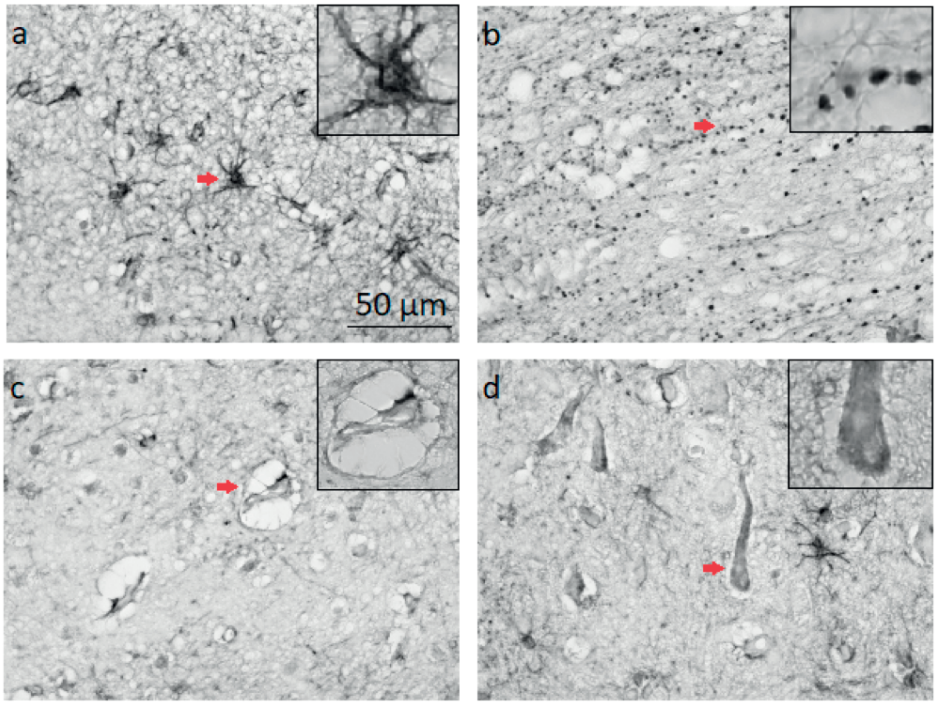

Figure 6. Cellular distribution of TRPV1 in the CG. Representative photomicrographs of anti-TRPV1 stained sections containing the CG from subject #2. Note the expression of TRPV1 (1:200) in cells with a morphological like appearance of a) glial cells, b) dendritic structures in white matter, c) endothelial cells, and d) neuronal cells. The images were acquired using a 40x objective. The scale bar of 50 µm is applicable to a, b, c, and d. Insets in the upper right corner: photomicrograph of cells indicated by the arrow at 100x magnification.

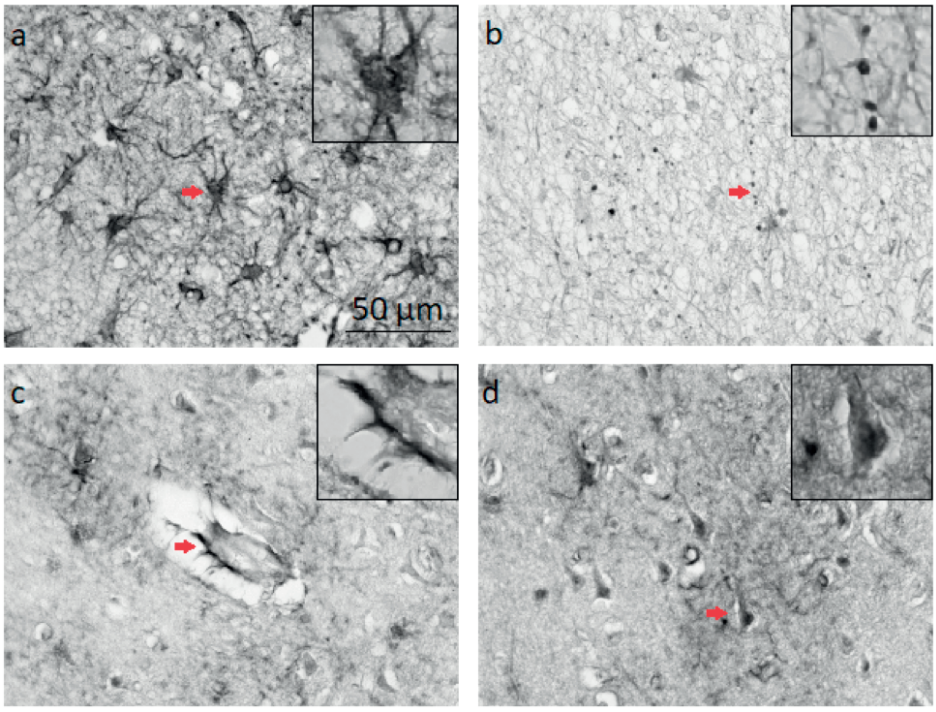

Figure 7. Cellular distribution of TRPV1 in the MFG. Representative photomicrographs of anti-TRPV1 stained sections containing the MFG from subject #2. Note the expression of TRPV1 (1:200) in cells with a morphological like appearance of a) glial cells, b) dendritic structures in white matter, c) endothelial cells, and d) neuronal cells. The images were acquired using a 40x objective. The scale bar of 50 µm is applicable to a, b, c, and d. Insets in the upper right corner: photomicrograph of cells indicated by the red arrow at 100x magnification.

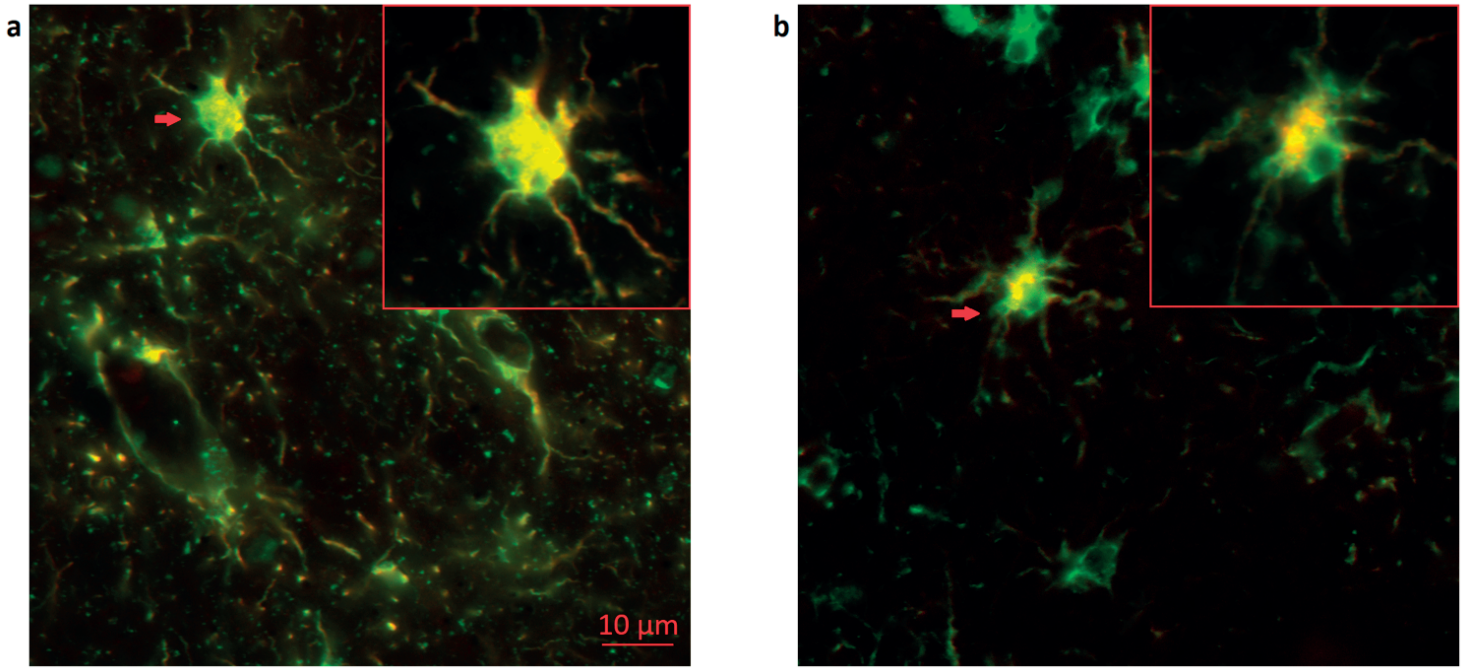

Figure 8. TRPV1 expression in astrocytes in the CG and MFG of subject no. 2. Representative photomicrographs of anti-TRPV1 (1:50, red), anti-GFAP (1:50, green) stained sections containing the a) CG and b) MFG from subject #2 showing double-labeling of TRPV1 and GFAP. The images were acquired using a 40x objective. The scale bar of 10 µm is applicable to a. and b. Insets in the upper right corner: photomicrograph of a co-labeling cell indicated by the red arrow at 100x magnification.

4. DISCUSSION

In the present study we showed that the TRPV1 channel was expressed in both the CG and MFG of subjects with a history of depression as well as controls. No differences in TRPV1 expression levels were found between the depression and control groups. However, strong inter-individual differences in TRPV1 expression level were detected. Immunohistochemistry revealed TRPV1 expression in morphologically glial-like cells, endothelial cells, neurites, and neurons. In addition, fluorescent double labeling showed abundant co-expression of TRPV1 with GFAP indicating its expression in astrocytes.

The TRPV1 channel has been implicated in depression and anxiety in rodents before [8]. However, TRPV1 expression in the human brain has only been reported scarcely [12]. In rodents, the function of TRPV1 in depression has shown some contradictory results. Reports have stated both antidepressant-like and depressant-like effects when activating TRPV1 channels [7-9]. In this study, TRPV1 did not seem to significantly differ between the depressed and control groups, indicating that TRPV1 expression is neither up- nor downregulated in depression. Nevertheless, it should be noted that as we studied post-mortem tissue a potential *in vivo* difference may have been missed. Furthermore, within subjects no differences were found in TRPV1 expression between the CG and MFG, although only three subjects were examined due to scarcity of available tissue.

In this study, in addition to observing the presence of TRPV1 in our western blots, preabsorption with anti-TRPV1 resulted in the absence of not only the 95 kDa but also several lower immunoreactive bands. This illustrates that the 95 kDa band is TRPV1 specific and that the few bands below the 95 kDa are most likely degradation products of TRPV1. Additionally, we found a moderate negative correlation between the post-mortem time and TRPV1 expression indicating that TRPV1 is an unstable protein sensitive to degradation. We therefore conclude that the interpretation of post-mortem TRPV1 expression levels needs to be done with precaution.

Immunohistochemistry showed that TRPV1 is expressed in presumably glial cells, neurites, endothelial cells, and neuronal cell bodies. Neuronal expression of TRPV1, however, was rather scares. More abundant TRPV1 expression was seen in astrocytes, shown by the co-labeling of TRPV1 and GFAP. Expression of TRPV1 in microglia was also considered, but given the fact that microglia cells are much more mobile than astrocytes, we did not consider them stable enough to fulfill the purpose of neuromodulation. For this reason TRPV1 expression in microglia cells was not studied. TRPV1 expression in astrocytes in rats and humans has been shown before [13]. Research stated that TRPV1 in astrocytes can mediate the production of ciliary neurotrophic factor inhibiting the degeneration of nigral dopaminergic neurons in rodent models of Parkinson's disease (PD). Furthermore, PD individuals express more TRPV1 and GFAP in their substantia nigra compared to healthy subjects [13]. Given these results, astrocytic TRPV1 might play a more crucial role than previously expected.

DBS outcomes have thus far mainly been attributed to a direct effect on neural elements. However, there is a growing insight into the role of astrocytes in neuronal communication and DBS [14, 15]. Astrocytes communicate with neurons via so called 'tripartite synapses' and potentially participate in synaptic transmission through Ca^{2+} and gliotransmitter signaling such as ATP [14, 16]. One human astrocyte interacts with approximately two million synapses making it a feasible candidate for the modulation of a neural network [17]. Astrocytes can be triggered directly by electrical stimulation, releasing the neuromodulators ATP and glutamate [18, 19]. This makes it plausible that DBS-induced modulation of network activity is partially due to astrocytic gliotransmission. Modulation of astrocytes, and thereby network activity, could potentially be done using the TRPV1 channel.

Given our results, using endogenous TRPV1 as a mean for neuromodulation can be a less effective approach. TRPV1 expression shows a great inter-individual variability, so using TRPV1 for this purpose requires the assessment of TRPV1 expression in each individual which does not seem to be feasible at this time. Furthermore, TRPV1 appears to be scarcely expressed in neurons, thereby restricting direct neuronal neuromodulation. Nevertheless, abundant expression of TRPV1 was shown in astrocytes making neuronal modulation through these cells a potential approach in future research.

5. CONCLUSION

This study showed that TRPV1 is present in the human CG and MFG in both depressed and control subjects. TRPV1 expression levels differ between subjects and do not seem to be dependent on depression in one's medical history. TRPV1 expression was extensively co-localized with GFAP indicating its abundant expression in astrocytes. Since endogenously neuronal TRPV1 expression is scarce, the potential use of endogenously TRPV1 for neuromodulation seems restricted. Interestingly, endogenous glial expression of TRPV1 may indicate an alternative approach for neuromodulation.

6. CONFLICT OF INTEREST

All authors declare to have no conflict of interest.

7. ACKNOWLEDGEMENTS

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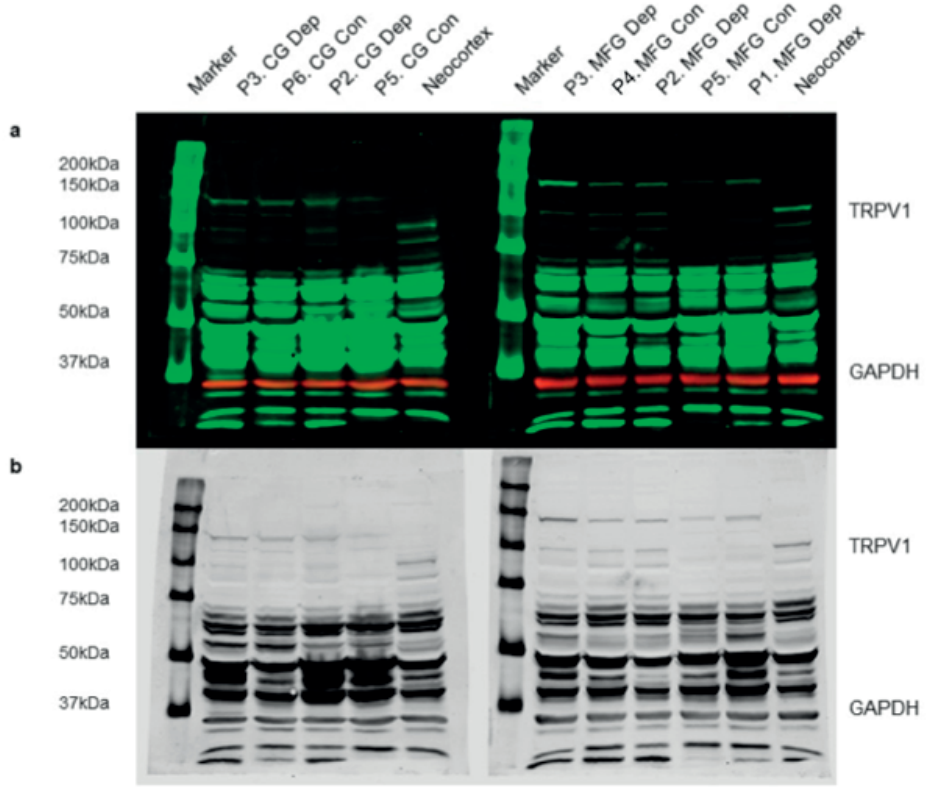
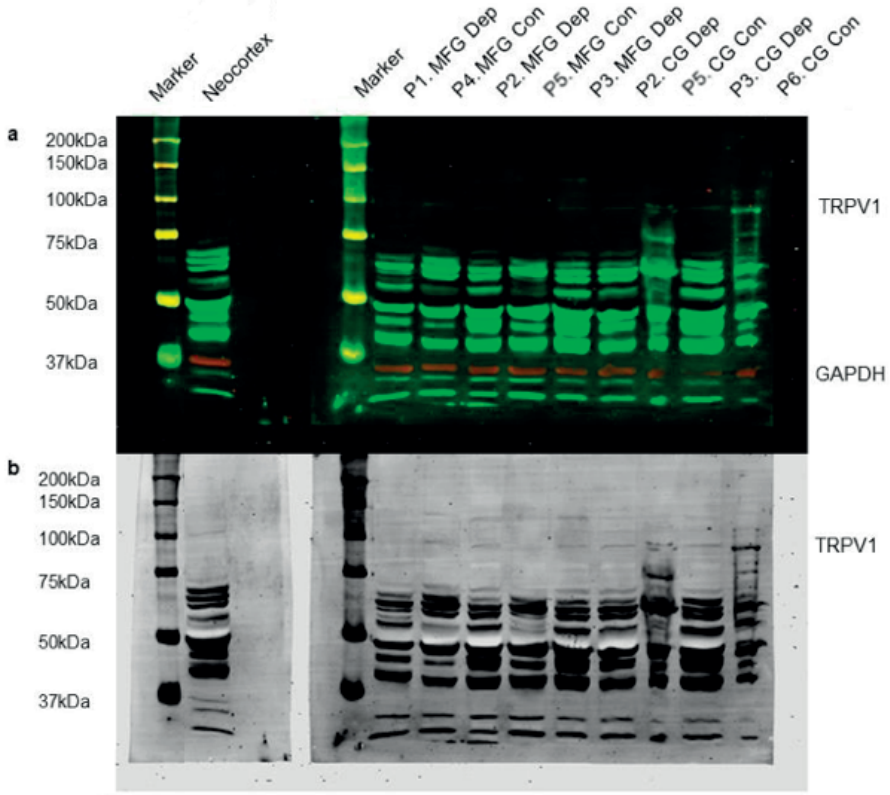
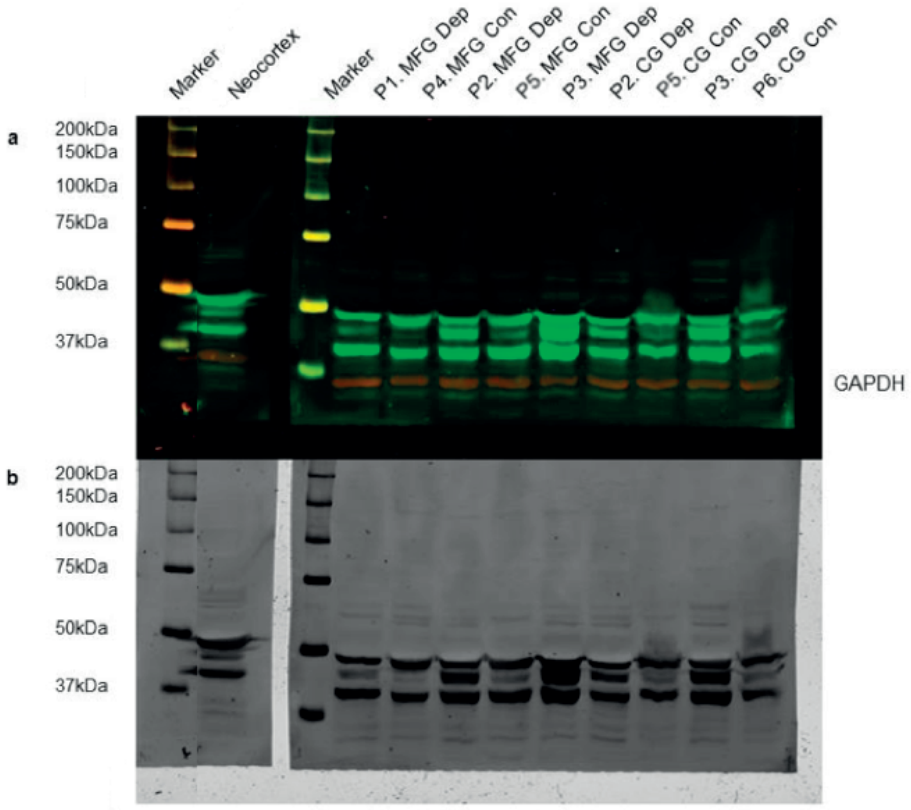
SUPPLEMENTARY FIGURES



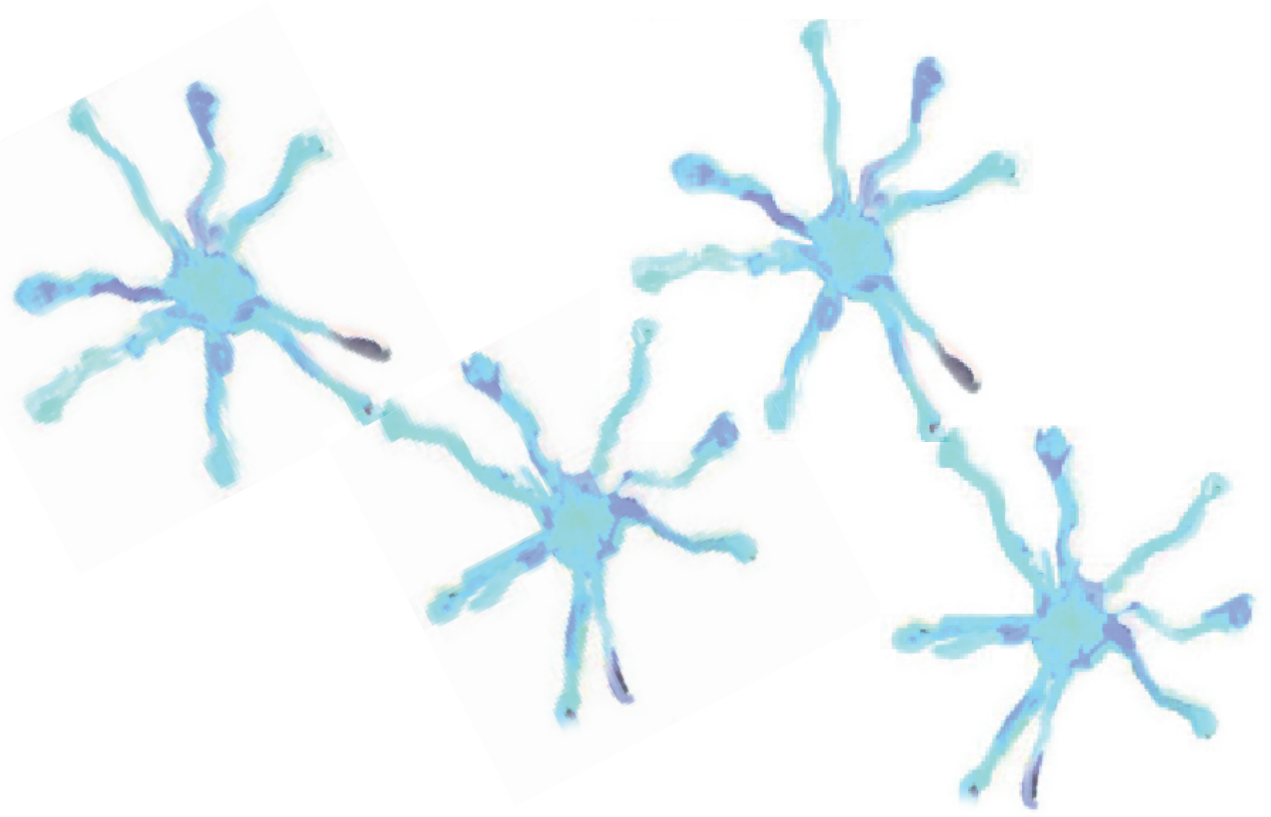
Supplementary figure 1. Western blot TRPV1 expression, raw data. Western blot of TRPV1 expression per subject and brain region. a) Colored channel green: TRPV1, red: GAPDH b) Black on white green channel: TRPV1. CG: cingulate gyrus, MFG: medial frontal gyrus, Con: Control, Dep: depression, P#: subject number.



Supplementary figure 2. Western blot TRPV1 expression, raw data, without peptide incubation. Western blot of TRPV1 expression per subject and brain region. a) Colored channel green: TRPV1, red: GAPDH b) Black on white green channel: TRPV1. CG: cingulate gyrus, MFG: medial frontal gyrus, Con: Control, Dep: depression, P#: subject number.



Supplementary figure 3. Western blot TRPV1 expression, raw data, with peptide incubation. Western blot of TRPV1 expression when incubated with anti-TRPV1 peptide per subject and brain region. a) Colored channel green: TRPV1, red: GAPDH b) Black on white green channel: TRPV1. CG: cingulate gyrus, MFG: medial frontal gyrus, Con: Control, Dep: depression, P#: subject number.



8 General discussion and conclusion

This general discussion and conclusion will start with the research questions formulated in the introduction. Following these research questions, I will address the limitations of my studies, future perspectives, and will end with a conclusion.

Research question 1

- How can we further improve deep brain stimulation outcomes for treatment-resistant depression?

Open-label trials and randomized controlled trials (RCTs) have shown inconsistent results for deep brain stimulation (DBS) in patients suffering from treatment-resistant depression (TRD). While open-label trials show promising effects, not all RCTs can replicate these findings [1-4]. Discussions arise concerning the criteria for patient selection, the choice of stimulation target, the optimal stimulation parameters, the correct interpretation of trial results and if subtypes of depression exist, which is in favor of a more personalized approach for DBS. Challenges remain to find the correct patient-specific target for stimulation and to discover the disrupted neural circuits in depression. In my animal research, I tried to enhance the knowledge of microcircuits in the prefrontal cortex responsible for different depressive domains in an animal model of depression. This enhanced my understanding of different microcircuits causing different depressive traits in animals, which might be extrapolated to the human state of depression, showing different inter-individual depressive traits.

From literature I have concluded that a personalized treatment approach holds the potential to increase the overall efficacy of DBS for TRD. A precise evaluation of patient specific symptoms, biomarkers, and resting-state connectivity patterns are essential to discriminate clinical subtypes of TRD [5-8]. Furthermore, this data might provide more insights into the working mechanism of DBS and help in selecting patient specific optimal DBS targets for TRD.

Research question 2

- Can we disentangle depression into multiple microcircuits responsible for different modalities seen is this disorder using an animal model of depression?

In my research, I have used the 'chronic unpredictable stress' (CUS) model, which is a well-validated and widely used animal model of depression [9]. In this model, rats are sequentially exposed to a variety of severe stressors in a random order over a period of four weeks. This model mimics the natural induction of depression, in which a chronic depressive-like state develops gradually over time in response to stress and unfortunate events. After the stress induction, I stimulated three different subregions of the ventromedial prefrontal cortex (vmPFC) with DBS and tested depressive-like behavior in different modalities of depression, such as anhedonia, anxiety and behavioral despair. In this thesis, I showed that prelimbic (PreL)-DBS decreased anhedonia, as is shown with an enhanced sucrose preference in the sucrose preference test (SPT),

and decreased helplessness, as is showed by a decrease in immobility time in the forced swim test (FST). These depressive symptoms were not alleviated when stimulating the infralimbic (IL)-cortex. These findings indicate the existence of different microcircuits in the vmPFC, responsible for different domains of 'depressive-like' behavior in rats. This might indicate that also in the human subgenual cingulate gyrus (SCG), the human analog of the rodents vmPFC [10], micro-circuits are present which might partially explain the inconsistent outcomes of SCG DBS trials in TRD. This emphasizes that DBS needs to be more individualized.

When stimulating the dorsal peduncular (DP)-cortex, I found a high incidence in seizure induction. The same stimulation paradigm did not induce seizures in the PreL- and IL-cortex. This finding showed that investigating mood-related behavior in the DP-cortex with DBS is not practicable and that serious side-effects of DBS can occur when stimulating particular brain regions. It emphasis that the region for stimulation should be chosen carefully and that accidentally activated nearby brain structures can give rise to adverse side-effects, making the correct placement of electrodes utterly important.

Research question 3

- Can the current method of deep brain stimulation be improved with the usage of nanoparticles, so that deep brain stimulation could potentially stimulate microcircuits and work wirelessly?

The field of neuromodulation is developing rapidly. However, current techniques of modulation are still limited as they depend on implants [11], require invasive procedures, are not cell-type specific [12], involve slow pharmacokinetics [13] or have a restricted penetration depth [14, 15] complicating stimulation of deep brain regions. Magnetic nanoparticles (MNP) can work as transducers in neuromodulation, which can improve existing methods of neuromodulation [16-18]. For DBS, replacing the stimulation electrodes for MNPs could advance its technique, making it potentially remote and wirelessly, and possibly more region specific by stimulating microcircuits [19].

Research question 4

- Does magnetothermal deep brain stimulation, which operates with nanoparticles, work in animal models?

The current technique of DBS requires the implantation of electrodes through invasive neurosurgery, powered by a chronically implanted battery. These electrodes work through a nonspecific electrical signal which is unable to selectively target specific neural subregions. To overcome some of these limitations we, in collaboration with the Massachusetts Institute of Technology (MIT) (Boston, USA) investigated a wireless alternative to DBS, called magnetothermal DBS (mDBS), which uses MNPs to induce neural excitation [19]. Our results showed that unilateral mDBS of

the subthalamic nucleus (STN) in wild-type mice resulted in more contralateral rotations around the body axis compared to sham animals. This finding indicates that unilateral mDBS of the STN can wirelessly control contralateral rotational behavior in wild-type mice through MNPs.

Research question 5

- Is it possible to apply magnetothermal deep brain stimulation in humans?

In this thesis, we have demonstrated that unilateral mDBS of the STN can induce contralateral rotational behavior in awake, freely moving and naïve mice. For mDBS in mice, lentiviral delivery of the transient receptor potential vanilloid subtype-1 (TRPV1) protein was needed, since endogenous expression of TRPV1 in mice is very low. I investigated if in the human medial frontal gyrus (MFG) and cingulate gyrus (CG), TRPV1 is expressed endogenously which might make mDBS without lentiviral introduction of TRPV1 possible. Results showed that TRPV1 is endogenously expressed in the human CG and MFG in both depressed and control subjects. The expression level of TRPV, however, did not seem to be dependent on depression, but rather showed strong inter-individual expression level differences. Furthermore, TRPV1 extensively co-localized with glial fibrillary acidic protein (GFAP), suggesting its profuse expression in astrocytes. Neuronal TRPV1 expression was limited, restricting its potential in neuromodulation. However, glial modulation through TRPV1 might be an interesting approach as the role of astrocytes in mediating neuromodulation is more and more investigated.

Limitations of our studies

The studies presented in this thesis show promising results, but like any research, are also bound to limitations. The biggest limitation is the exploration of depression in an animal model, in which a lot of the etiology of the disorder is lost. This makes behavioral and cellular outcomes of animal research hard to translate back to humans. However, fundamental research is needed to gain insights into the various disturbed microcircuits in TRD and the effect of DBS. In my thesis, I used the 'chronic unpredictable stress' (CUS) animal model, which to my knowledge, is one of the best animal models to simulate and investigate 'depression' with great validity and translational potential [9, 20], however its success can be user dependent [21]. With my research, I gained more insight into possible microcircuits in depressive-like behavior in the vmPFC in rats and possibly in the SCG of humans, partially explaining the inconsistent results in SCG DBS trials.

Another limitation of my studies is the sole use of male rats in my research. Since the prevalence of depression is higher in women, researching both male and female rats would have had the preference. However, I only used male rats since previous research has shown that the estrogen cycle of female rodents interfere with behavior and the release of neurotransmitters, causing a larger spread in behavioral outcomes [22, 23]. This would have interfered with my behavioral readouts and with the comparison to previously done research in our lab, in which also only used

male rats were used [24]. For these reasons, I only investigated male rats. However, following the results of my research in which DBS of the PreL-cortex and not IL-cortex resulted in alleviation of anhedonia and helplessness, it would be interesting to research this finding in female rats.

Another issue to address is the restricted amount of available human brain tissue for depression research. Due to the scarcity of brain tissue of depressed patients, I was only able to investigate a small number of subjects for my TRPV1 project. Due to this limitation, I was only able to make assumptions regarding TRPV1 expression in the CG and MFG of depressed subjects but no hard conclusion could be drawn.

FUTURE PERSPECTIVES

My research suggest that circuits emerging from distinct subregions in the prefrontal cortex are responsible for different depressive modalities in rats, such as anhedonia and behavioral despair. Future research is needed to disentangle other microcircuits in depression, investigating other brain regions possibly responsible for different modalities seen in depression. Furthermore, we need to discover the working mechanism of DBS within these microcircuits by analyzing neurotransmitter changes using microdialysis and cellular changes by the usage of electrophysiology.

Finally, in this thesis, we have shown that mDBS of the unilateral STN in wild-type mice caused contralateral rotational behavior. The next step would be to incorporate mDBS into different animal disease models to investigate if mDBS could alleviate or recover various disease states. Also for mDBS, it is needed to know what cellular changes it induces using microdialysis and electrophysiology and what side-effects could occur.

CONCLUSION

In conclusion, major depressive disorder (MDD) is a circuitopathy that involves a wide range of brain structures and exhibits diverse clinical manifestations. A precise evaluation of symptoms, biomarkers, and resting-state connectivity patterns are essential to distinguish clinical subtypes of TRD, might provide insight into the working mechanisms of DBS, and help in selecting optimal DBS targets in patients.

In 'depressed' rats, microcircuits in the vmPFC are responsible for different modalities of depression shown with different 'depressive-like behavior'. These data suggest that also in humans, microcircuits are present which might partially explain the inconsistent outcomes of SCG DBS trials in TRD. This also indicates that a more personalized approach in DBS holds the potential to increase the overall efficacy of DBS for TRD.

Furthermore, advancing the method of current DBS with the insertion of MNPs could overcome the limitation of electrode and device implantation, making its application wirelessly. We

have shown that unilateral mDBS of the STN is able to induce contralateral rotational behavior, which paves the way to its induction in various animal disease models and potentially might function as a treatment modality in the future.

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SUMMARY

Major depressive disorder (MDD) is globally the leading cause of disability with a worldwide prevalence of 4.4 %, affecting 322 million people in 2015. For the diagnosis of MDD, according to the Diagnostic and Statistical Manual of Mental Disorders number 5 (DMS-5), five of the following symptoms need to be present: a depressed mood, anhedonia, insomnia or hypersomnia, psychomotor retardation or agitation, loss of energy or fatigue, worthlessness or guilt, change in weight or appetite, impaired concentration or indecisiveness, and thoughts of death or suicidal ideation or an attempt. The treatment of MDD include antidepressant medication and psychological therapies. However, approximately one-third of treated patients do not respond adequately to these treatments. These patients suffer from treatment-resistant depression (TRD) which is associated with more hospitalizations and past suicide attempts. For TRD, different therapies modalities can be given such as electroconvulsive therapy (ECT), vagal nerve stimulation (VNS), transcranial magnetic stimulation (TMS) and deep brain stimulation (DBS). In this thesis we have focused on deep brain stimulation in TRD, if we can disentangle TRD into different microcircuits, how we can improve clinical DBS outcomes and if we can refine DBS with a non-invasive technique called magnetothermal DBS introducing nanomaterial-mediated neuromodulation.

Chapter 1 is a general introduction into the theme and aims of this thesis. It gives information about major depressive disorder, treatment-resistant depression, current treatment options such as DBS and our view of possible microcircuits responsible of different traits in TRD. It provides our research questions and outline of this thesis.

Chapter 2 provides a narrative review of recent studies investigating the effectiveness of DBS in TRD. We especially focus on the relationship between the targeted brain structures and clinical outcomes. It discusses the importance of clinical subtypes of TRD. We concluded that precise evaluations of symptoms, biomarkers, and resting-state connectivity patterns are essential when distinguishing clinical subtypes of TRD. Subtyping TRD may provide more insight into the working mechanisms of DBS and help in selecting optimal targets in patients allowing for more personalized symptom-based treatment approaches.

Chapter 3 aimed to investigate different microcircuits within the prefrontal cortex of rats in an model of depression. We hypothesized that different microcircuits cause different behavioral traits in depressive-like behavior, and therefore, the treatment of depression and depressive traits lies in the modulation of different neural microcircuits. In this study, we found that High frequency (HF) DBS in the prelimbic (PreL) cortex but not the infralimbic (IL) cortex alleviated anhedonia and behavioral despair revealed by the sucrose preference and forced swim tests, respectively. These results suggest that modulation of specific sub-regions with its own microcircuits in the prefrontal cortex might be a potential approach towards providing tailored DBS therapy for different subtypes of depression.

Chapter 4 presents the adverse side-effects when stimulating a different subregion in the prefrontal cortex named the dorsal peduncular (DP) cortex. Stimulation in this DP subregion caused acute induction of seizures in ~40% of stimulated animals. Clinically relevant stimulation parameters were applied. We therefore conclude that the DP subregion of the vmPFC is not a suitable target to conduct DBS in mood disorders. It emphasis that the region for stimulation should be chosen carefully and that nearby brain structures can give rise to adverse side-effects.

Chapter 5 provides a review in which we describe and evaluate advanced techniques of neuromodulation of the brain and their latest refinements incorporating the usage of nanoparticles. We emphasize on DBS and magnethothermal deep brain stimulation (mDBS) using magnetic nanoparticles (MNPs) for nanomaterial-mediated neuromodulation. We conclude the application of MNPs as transducers of magnetic field into thermal, electrical, mechanical or chemical stimuli offers a possibility to remotely and wirelessly modulate specific groups of cells in arbitrarily deep regions of the brain.

Chapter 6 presents a study of mDBS in awake, freely moving mice done in collaboration with the research group of prof. dr. P. Anikeeva at the research laboratory of electronics (rle) at the Massachusetts Institute of Technology (MIT) (Boston, USA). We found that unilateral subthalamic nucleus (STN) mDBS in mice injected with MNPs results in more contralateral rotations around the body axis when compared to mice injected with non-MNPs. This results showed that mDBS in mice works and offers opportunities to further explore this techniques in various animal models of neuropsychiatric, neurosensory and neurodegenerative disorders.

Chapter 7 describes endogenously transient receptor potential vanilloid subtype-1 (TRPV1) expression in the human cingulate gyrus (CG) and medial frontal gyrus (MFG). Thus far, TRPV1 is needed for mDBS and has shown to be present in the CG and MFG of humans, albeit more in glial cells than in neurons. The potential use of endogenously TRPV1 for neuromodulation seems restricted. However, endogenously glial expression of TRPV1 may indicate an alternative approach for neuromodulation.

Chapter 8 summarizes the main findings in this thesis and provides answers to our research questions formulated in chapter 1. It addresses the limitations of our studies and future perspectives.

VALORIZATION ADDENDUM

This Valorization Addendum describes how the knowledge obtained from our research in this thesis can be of value for both clinical and societal use.

Societal relevance

Major depressive disorder (MDD) is a medical condition affecting around 322 million people worldwide in 2015. This disorder includes a wide range of neuropsychiatric symptoms that vary greatly between patients. Approximately one third of treated patients do not responds to standard therapy including deep brain stimulation (DBS) and suffer from treatment resistant depression (TRD). For these people, treatment remains a challenge and research is needed to explore underlying neural mechanisms in depression.

Implementing clinical subtypes of TRD into future studies investigating DBS for TRD could increase its overall efficacy. Looking at homogenous subtypes of depressed patients and investigating which DBS target works the best for each particular subtype may lead to a more personalized DBS approach. This would be superior to looking at primary outcomes across all participants. With this approach we hopefully find correct patient-specific targets for stimulation.

We believe in the existence of microcircuits in depression and TRD and have showed that these exist in the prefrontal cortex in rats. This might be extrapolated to the human state of depression showing different inter-individual depressive traits. These findings are relevant for science and society so that further research into microcircuits in TRD can lead to more patient-specific targeting based on their depressive traits.

A lesson learned from our side-effect study is that adjacent brain regions can cause severe side-effects upon stimulation. This implicates the importance of correct electrode placement and indicates that caution is vital when exploring new brain regions for stimulation.

With our literature study into nanoparticle-mediated neuromodulation we have shown that magnetic nanoparticles (MNPs) can acts as transducers of a magnetic field into thermal, electrical, mechanical or chemical stimuli. This offers a possibility to remotely and wirelessly modulate specific groups of cells in arbitrarily deep regions of the brain. We hope to stimulate research groups to implement these advanced neuromodulation techniques, advancing the field of neuroscience and improving the specificity of these techniques.

We thoroughly described a new method called 'magnetothermal deep brain stimulation' (mDBS) in this thesis and in collaboration with the Massachusetts Institute of Technology (MIT) in Boston (MA, USA) are one of the first researcher groups to research mDBS in freely moving mice. Further research should implement this new technique in various animal models of signs and symptoms as expressed in mental, neuropsychiatric, neurosensory and neurodegenerative disorders in order to restore physiological brain functions and to define its therapeutic value. For clinicians such as neurosurgeons, mDBS could overcome invasive surgery for neuromodulation.

Our TRPV1 study (chapter 7) shows that the use of endogenously TRPV1 for neuromodulation seems restricted, but glial expression of TRPV1 may indicate an alternative approach for neuromodulation. This is mainly interesting for researchers trying to overcome the viral induction of TRPV1 for mDBS, or researchers interested in the function and modulation of glial cells in neuromodulation. Thus far, most studies only research the direct effect of modulation on neurons, various new insight could be obtained when investigating the role of glial cells in this process.

Target audience

Findings from our literature study (chapter 2) are relevant for patients, clinicians and researchers, since they can incorporate the precise evaluation of symptoms, biomarkers, and resting-state connectivity patterns to distinguishing clinical subtypes of TRD.

The findings from our DBS study (chapter 3) acknowledging the existence of microcircuits are relevant for both patients, clinicians as well as researchers since these insight pave a way to more patient-specific targeting based on depressive traits.

Our findings from the side-effect study (chapter 4) are relevant for clinicians and researchers showing that correct electrode placement is of the utmost importance and that simulating adjacent brain regions with the same clinically relevant parameters can cause severe side-effects which needs to be considered when researching new brain regions.

Implementing nanoparticles into neuromodulation (chapter 5 and 6) is relevant for both researchers and clinicians. For researchers, nanoparticles can refine current methods of neuromodulation. For clinicians such as neurosurgeons, these refinements could overcome invasive surgery for neuromodulation in the future.

Finally, results from our TRPV1 study (chapter 7) are mainly interesting for researchers since it showed that the use of endogenously TRPV1 for neuromodulation seems restricted, but glial expression of TRPV1 may indicate an alternative approach for neuromodulation.

Products/innovation

The studies of this thesis can be considered innovative in several ways. Firstly, this work included a novel concept of clinical subtypes of TRD comprising of different microcircuits. This theory was implemented into an animal model of depression showing the existence of different microcircuits responsible for different behavioral traits within the prefrontal cortex of rats. This is crucial for future research into patient-specific neuromodulation targeting in depression.

Another innovative approach in this thesis is the review of the implementation of nanoparticles into different forms of neuromodulation with an emphasis on DBS. We describe a the method mDBS and in collaboration with colleagues from MIT, are one of the first researchers to research magnetothermal DBS in freely moving mice.

Furthermore, we were the first to describe the existence of TRPV1 in the human cingulate- and medial frontal gyrus paving the way to magnetothermal DBS in humans.

Implementation

The knowledge and novel insights obtained from the studies in this thesis will be shared with health care professionals, patient organizations and scientific societies.

Future studies investigating TRD will hopefully implement clinical subtypes of TRD and explore the underlying microcircuits of different behavioral traits. We will encourage this by presenting our results at different neuroscientific congresses as we have presented them at the Society of Neuroscience in San Diego (CA, USA) in 2018. Health care professionals can contribute to these studies by incorporating precise evaluation of symptoms, biomarkers, and resting-state connectivity patterns for TRD in patient care. For patient organizations its valuable information and reassuring to see that advances in the treatment of TRD are still being made.

To advance current methods of neuromodulation, we hope that various research groups will implement nanoparticle-mediated neuromodulation. In particular we would like to encourage the usage of mDBS in different animal models in order to restore physiological brain functions and to define its therapeutic value. We will engage with different groups conducting nanoparticle-mediated neuromodulation experiments. For clinicians, such as neurosurgeons, it is good to know that non-invasive remote neuromodulation through nanoparticles holds potential in the future. Since at the school for Mental Health and Neuroscience (MHeNS), the department of Neurosurgery has both researchers and clinicians, such knowledge transfer takes place on the spot.

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CURRICULUM VITAE

Milaine Roet was born on October 27, 1988 in The Hague. After graduating from high school in 2007, she started to study Pharmacology in Utrecht followed by Medical school at the Erasmus MC in Rotterdam. During medical school she attended the Erasmus MC Honours class of 2009 where medicine touches science and society. In 2010 she started her second master degree in Neuroscience where she studied the sensory system in mice. In 2015, she started working as a PhD candidate at the Department of Neurosurgery of the school for Mental Health and Neuroscience under supervision of prof. dr. Yasin Temel and dr. Ali Jahanshahi. During her PhD she focused on stimulating microcircuits in depression and incorporating a new advanced technique of deep brain stimulation using magnetic nanoparticles. During her PhD she went abroad to the Massachusetts Institute of Technology in Boston (USA) under supervision of prof. dr. Polina Anikeeva to learn and implement this new technique called magnetothermal deep brain stimulation in a variety of animal models. Next to her PhD trajectory, she started working as a medical doctor in Dordrecht.

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