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Direktor: Professor Dr. med. Dr. phil. Andreas Heinz

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Using the maternal immune stimulation model of schizophrenia to investigate the therapeutic efficacy of neuromodulation techniques.

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Frau Dr. rer. nat. Ravit Hadar

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Dekan: Prof. Dr. med. Axel R. Pries
1. Gutachter: Prof. Dr. Johannes Thome, Rostock
2. Gutachter: Prof. Dr. Frank Jessen, Köln

Contents

1. Introduction…….. 3

1.1 Background…….. 3

1.2 Using the maternal immune stimulation rodent model of schizophrenia to study the efficacy of focal neuromodulation in the form of deep brain stimulation on existing behavioral deficits and altered neuro-circuitry…… 4

1.3 Using the maternal immune stimulation rodent model of schizophrenia to investigate novel preventive approaches via neuromodulation……. 5

2. Own previous work……. 6

2.1 Could focal neuromodulation improve behavioral deficits and altered neuro-circuitry in the maternal immune stimulation rodent model of schizophrenia?..... 6

2.1.1 Mapping brain regions in which deep brain stimulation normalizes schizophrenia-relevant behavioral deficits…….. 6

2.1.2 Deep brain stimulation improves behavior and modulates altered neural circuits in a rodent model of schizophrenia…… 18

2.2 Using the maternal immune stimulation model of schizophrenia to trace the development of schizophrenia and to investigate preventive approaches via neuromodulation…… 29

2.2.1 Using a maternal immune stimulation model of schizophrenia to study behavioral and neurobiological alterations over the developmental course……. 29

2.2.2 Early neuromodulation prevents the development of brain and behavioral abnormalities in a rodent model of schizophrenia……. 41

2.2.3 Deep brain stimulation during early adolescence prevents microglial alterations in a model of maternal immune stimulation……. 51

2.2.4 Prevention of schizophrenia deficits via non-invasive adolescent frontal cortex stimulation in rats…… 62

3. Discussion…….. 73

3.1 Acute and focal neuromodulation reverses schizophrenia-related behavioral deficits and affect altered neuro-circuitry……. 73

3.2 Preventing the development of schizophrenia via neuromodulation…… 74

4. Summary……. 77

5. References……. 78

6. Acknowledgements……. 82

1. Introduction

1.1 Background

Schizophrenia, considered to be one of the most complex neuro-psychiatric afflictions, is a severe, highly disabling and chronic disease with a life prevalence of ~1% worldwide (Ross, Margolis, Reading, Pletnikov, & Coyle, 2006; Tamminga & Holcomb, 2005). The disorder is characterized by grave disruptions in affect, cognition and behavior and has a typical onset time in adolescence or young adulthood (Immonen, Jaaskelainen, Korpela, & Miettunen, 2017; Owen, Sawa, & Mortensen, 2016; Rapoport, Giedd, & Gogtay, 2012). In recent years, converging data from both human and animal studies suggest that schizophrenia is actually a neurodevelopmental disorder in which neuropathological processes gradually accumulate over the developmental-span, finally leading to psychosis outbreak (Rapoport, Addington, Frangou, & Psych, 2005; Rapoport et al., 2012). Schizophrenia introduces immense socio-economic burden as a result of direct treatment and hospitalization costs along with indirect costs resulting from loss of employment and the need for social support (Cloutier et al., 2016; Jin & Mosweu, 2017). Whereas antipsychotics constitute the first line of treatment, studies indicate that around 10–30% of the patients poorly respond or do not respond at all and an additional 30% of the patients gain only partial relief (Falkai et al., 2005). Further, while being effective against the positive symptoms, antipsychotics fail to substantially improve negative symptoms and cognitive deficits (Millan, Fone, Steckler, & Horan, 2014). Altogether, this points to the necessity of profounder understanding of the neuropathology underlying schizophrenia in an effort to develop novel therapeutic treatments for this disorder. Further, the neurodevelopmental nature of schizophrenia together with its devastating outcomes, encourage the development and testing of prevention, rather than intervention, options. Human studies are of high necessity but obviously lack controlled and systematic settings and face ethical and practical boundaries. Given these limitations, the use of pre-clinical animal studies is crucial. To this end, an adequate and valid animal model of schizophrenia that captures the neurodevelopmental disease course is mandatory. The maternal immune stimulation (MIS) model of schizophrenia meets these requirements. In this model, the exposure of pregnant rodents to the viral mimic polyriboinosinic–poly-ribocytidylic acid (poly I:C) gives rise to schizophrenia-relevant behavioral abnormalities observed in the adult offspring (Meyer & Feldon, 2010, 2012; Piontkewitz, Arad, & Weiner, 2011a, 2012). Notably, neurobiological alterations in relevant brain circuits precede the behavioral abnormalities and hence the model recapitulates the maturational delay observed in schizophrenia (Piontkewitz et al., 2011a, 2012). In the present work, the MIS model was utilized to trace behavioral and neurobiological

alterations relevant to schizophrenia disease-progression in an effort to test novel therapeutic intervention and prevention strategies.

1.2 Using the MIS rodent model of schizophrenia to study the efficacy of focal neuromodulation in the form of Deep brain stimulation (DBS) on existing behavioral deficits and altered neuro-circuitry.

DBS constitutes a novel neuromodulation technique that selectively affects specific brain regions and its related circuitries. DBS is an invasive, however reversible and safe procedure, involving the intracerebral implantation of stimulating electrodes which allow for the delivery of electrical current to regions of interest (Miocinovic, Somayajula, Chitnis, & Vitek, 2013). The Federal Drug administration (FDA) has approved DBS as a therapeutic technique for various neurological conditions belonging to movement disorders as essential tremor, dystonia and Parkinson's disease (Bari, Thum, Babayan, & Lozano, 2018). The overwhelming efficacy of DBS in otherwise treatment resistant neurological disorders has kindled an interest in this method as a therapeutic option for pharmacotherapy-refractory psychiatric disorders and ample trials have been conducted with regard to various diseases among which depression, addiction and obsessive-compulsive disorder (OCD) (Hamani et al., 2014; Holtzheimer et al., 2012; Malone et al., 2009; Muller et al., 2013; Staudt, Herring, Gao, Miller, & Sweet, 2019; Voges, Muller, Bogerts, Munte, & Heinze, 2013). However, as for today, the only psychiatric disorder for which DBS had been approved under a humanitarian device exemption is OCD (Hamani et al., 2014) resulting in persistent and ongoing efforts to evaluate the efficacy of DBS in the field of neuro-psychiatry using various animal models (Hamani & Nobrega, 2010, 2012; Hardenacke et al., 2013; Reznikov, Binko, Nobrega, & Hamani, 2016). Nevertheless, DBS is more than a therapeutic procedure as with regard to pre-clinical studies it also serves as an investigative tool (i.e. (Casquero-Veiga et al., 2016; Hamani et al., 2010; Mundt et al., 2009; Rea et al., 2014; Rummel et al., 2016; Toda, Hamani, Fawcett, Hutchison, & Lozano, 2008; Winter et al., 2015; Winter et al., 2008) and it allows identifying neuro-circuitries in both pathological and healthy brains (Klein et al., 2011).

The initial part of the work presented here sought to investigate the efficacy of DBS in the context of schizophrenia using the MIS rodent model. Considering the elusive etiology of schizophrenia along with its neuropathological complexity, the first milestones were to use DBS in order to 1. Identify brain regions whose electrical modulation will result in improvement of schizophrenia-relevant behavioral deficits 2. Trace the most effective stimulation parameters to be used 3. Finally, implement the most potent DBS protocols to investigate its effects on the underlying neuro-circuitry.

1.3 Using the MIS rodent model of schizophrenia to investigate novel preventive approaches via neuromodulation.

In recent years, due to the growing acceptance that schizophrenia constitutes a severe neurodevelopmental disorder, some attempts have been made to interfere early in disease progression in an effort to halt or even to prevent future manifestation of this disorder (Smesny et al., 2014; Smesny et al., 2017). Among the first attempts is a small number of randomized control trials in which antipsychotics were chronically applied to young individuals identified at high risk to develop psychosis episode or schizophrenia in the future (McGlashan et al., 2006; Woods et al., 2003). In this line of studies, antipsychotics were administered during the pre-symptomatic period of adolescence and the generally positive results stimulated researchers to test the efficacy and mechanism of action of various antipsychotics in preventing schizophrenia using the MIS rodent model for schizophrenia (Meyer, Spoerri, Yee, Schwarz, & Feldon, 2010; Piontkewitz, Arad, & Weiner, 2011b; Piontkewitz, Assaf, & Weiner, 2009). The capacity of the MIS model to accurately capture the neurodevelopmental course of schizophrenia is manifested on the behavioral as well as neuropathological level; with this regard, in accordance with the clinical progression of schizophrenia, in the MIS model deficits reflecting the positive symptomatology of schizophrenia first appear in adulthood whereas various neuropathologies either temporally precede the behavioral deficits or accompany its outbreak (Piontkewitz et al., 2011a, 2012). Whereas the current limited number of clinical controlled trials do not allow for a decisive conclusion, the overall approach, namely attempting to halt or prevent schizophrenia's development, has gained acceptance within the scientific community and introduced new lines of research (Heinssen & Insel, 2015).

The second part of the work presented here used the MIS rodent model of schizophrenia to investigate novel approaches to minimize or even prevent the development of schizophrenia. To this end, the studies gathered in this part tested two different approaches as prevention measurements for schizophrenia-relevant deficits and as such were all chronically administered prior to the full-blown of schizophrenia-relevant behavioral deficits. The studies include the chronic application of: 1. High frequency DBS of the medial prefrontal cortex 2. Anodal or cathodal transcranial direct current stimulation (tDCS) of the frontal cortex. Common to these tested approaches were the complementary investigations of neurobiological or brain-structural alterations.

2. Own previous work

2.1 Could focal neuromodulation improve behavioral deficits and altered neuro-circuitry in the Maternal Immune Stimulation (MIS) rodent model of schizophrenia?

2.1.1 Mapping brain regions in which deep brain stimulation affects schizophrenia-like behavior in two rat models of schizophrenia

Klein, J., Hadar, R., Gotz, T., Manner, A., Eberhardt, C., Baldassarri, J., Schmidt, T.T., Kupsch, A., Heinz, A., Morgenstern, R., Schneider, M., Weiner, I., Winter, C

Brain Stimulation, 2013

As for today, a major challenge in the management of schizophrenia is the partial efficacy of antipsychotics drugs, underlying the need for the development of novel treatment strategies. The present study used two rodent models of schizophrenia, namely the MIS and the pubertal cannabinoid administration rat models, to test the hypothesis that deep brain stimulation is capable of normalizing behavioral deficits apparent in these models. For the MIS model, pregnant dams were administered with the immune activating agent poly I:C (4 mg/kg) or saline and its male offspring (poly I:C n=50, saline n=50) were used to test the effects of DBS on schizophrenia-relevant behavioral deficits. Adult rats were subjected to bilateral stereotactic electrode implantation into one of the following regions: subthalamic nucleus (STN, n = 12/10), entopeduncularis nucleus (EP, n = 10/11), globus pallidus (GP, n = 10/10), medial prefrontal cortex (mPFC, n = 8/8), or dorsomedial thalamus (DM, n = 10/11). For the pubertal cannabinoid administration rat model, adult male rats were treated with the CB1 receptor agonist WIN 55,212-2 (WIN, n = 16) or saline (n = 12) during puberty. At adulthood rats were subjected to bilateral stereotactic electrode implantation into either the mPFC (n = 8/6) or the DM (n = 8/6). Following surgeries, all rats received one-week recovery period before behavioral testing. Behavioral testing consisted of the pre-pulse inhibition (PPI) of the acoustic startle reflex (ASR) paradigm, a well-established cross-species phenomenon that is disrupted in schizophrenia and in both models, PPI was tested without DBS and under DBS at different stimulation parameters. Results indicate that deficits in PPI of the ASR following maternal poly I:C application were normalized upon DBS. The therapeutic effects of DBS depended on both stimulation target and stimulation parameters. DBS delivered to the mPFC and DM at high frequencies yielded the most prominent results. These effects were replicated in the pubertal cannabinoid administration rat model of schizophrenia. Collectively, these results suggest that brain regions, in which DBS was successful in normalizing PPI deficits, might be of

therapeutic relevance when considering a neuromodulation approach for the treatment of schizophrenia.

Mapping brain regions in which deep brain stimulation affects schizophrenia-like behavior in two rat models of schizophrenia

Klein, J., Hadar, R., Gotz, T., Manner, A., Eberhardt, C., Baldassarri, J., Schmidt, T.T., Kupsch, A., Heinz, A., Morgenstern, R., Schneider, M., Weiner, I., Winter, C

Brain Stimul, 2013; 6(4), 490-499.

https://doi.org/10.1016/j.brs.2012.09.004

2.1.2 Deep brain stimulation improves behavior and modulates neural circuits in a rodent model of schizophrenia

Bikovsky, L.*, Hadar, R.*, Soto-Montenegro, M.L. *, Klein, J., Weiner, I., Desco, M., Pascau, J., Winter, C., Hamani, C.,

Experimental Neurology, 2016

Based on the demonstration that DBS at high frequencies is successful in normalizing sensorimotor gating deficits in the MIS roent model of schizophrenia, the current study was designed to extend this line of investigation. This study hence tested the efficacy of high frequency DBS to the mPFC and to the nucleus accumbens (Nacc) on sensorimotor gating and on attentional selectivity processes. In addition, the effects of DBS on neurocircuitries were studied. Pregnant dams were injected to the tail vein with the immune activating agent poly I:C (4 mg/kg) or saline and its male offspring was used to test the effects of DBS on behavior and neurocircuitries. When reaching adulthood rats were subjected to bilateral stereotactic electrode implantation into the mPFC or Nacc. Following 1-2 weeks of rest either behavioral or imaging testing took place. For studying sensorimotor gating function the PPI paradigm was used (mPFC-DBS: n = 5 saline, n = 6 poly I:C, Nacc-DBS: n = 8 saline, n = 8 poly I:C). For studying attentional selectivity the latent inhibition (LI) paradigm was used (mPFC- DBS: n=55; Nacc- DBS: n=89). In addition, using glucose uptake positron emission tomography (PET) imaging the effects of DBS on neurocircuitries were also investigated. On the behavioral level, both mPFC- and Nacc-DBS were successful in alleviating abnormalities in PPI and LI observed in MIS offspring. Importantly, saline offspring treated with Nacc-DBS exhibited deficits in PPI and LI, whereas this phenomenon was not observed in mPFC-DBS treated saline offspring. Generally, the effects of DBS on metabolism was profounder in saline offspring when compared to the MIS group; increased metabolism was observed following mPFC-DBS in the parietal cortex, ventral hippocampus, striatum and Nacc, while reduction was found in the cerebellum, brainstem, hypothalamus and periaqueductal gray. On the other hand, Nacc-DBS led to increased activity in the olfactory bulb and ventral hippocampus whereas reduced activity was observed in the septal area, periaqueductal gray, brainstem and hypothalamus. In MIS offspring differences in metabolism levels following mPFC-DBS were similar to those observed in saline offspring, apart from a reduced activity in the hypothalamus and brainstem. In contrast to that, Nacc-DBS induced no statistical changes in brain metabolism in MIS offspring. Altogether, this study shows that DBS of either mPFC or Nacc delivered to the adult MIS progeny improves

behavioral deficits in PPI and LI. Despite shared behavioral results, stimulation delivered to these two targets induced different metabolic responses.

Deep brain stimulation improves behavior and modulates neural circuits in a rodent model of schizophrenia

Bikovsky, L.*, **Hadar, R.***, Soto-Montenegro, M.L. *, Klein, J., Weiner, I., Desco, M., Pascau, J., Winter, C., Hamani, C.,

Exp Neurol. 2016; 283(Pt A), 142-150

https://doi.org/10.1016/j.expneurol.2016.06.012

2.2 Using the MIS model of schizophrenia to trace the development of schizophrenia and to investigate preventive approaches via neuromodulation.

2.2.1 Using a maternal immune stimulation model of schizophrenia to study behavioral and neurobiological alterations over the developmental course

Hadar, R., Soto-Montenegro, M.L., Gotz, T., Wieske, F., Sohr, R., Desco, M., Hamani, C., Weiner, I., Pascau, J., Winter, C.

Schizophrenia Research, 2015

Epidemiological, neuroimaging and post mortem studies indicate that schizophrenia is a neurodevelopmental disorder and as such characterized by impairments to early brain development that later interfere with brain maturation processes over the peri-adolescence period, ultimately giving rise to psychosis-outbreak typically during late adolescence or young adulthood. Whereas the postnatal delay of psychosis-outbreak is a well-documented feature of schizophrenia, the exact neurobiological trajectories accompanying the aberrant behavioral course are not fully understood. Using the MIS rodent model of schizophrenia, the current study hence sought to compare the adolescence period of schizophrenia with the adult period, characterized by symptoms' manifestation, at the behavioral as well as the neurobiological level. Pregnant dams were injected to the tail vein with the immune activating agent poly I:C (4 mg/kg) or saline and its male offspring was used for investigations. For studying the pre-symptomatic period of adolescence, animals were tested on the PPI paradigm at post-natal day (PND) 35 and PND 60, and again in adulthood at PND 100 (n=10 in both MIS and saline groups). Abnormal brain activity patterns were measured using 18 fluoro desoxyglucose (FDG) PET and using *post mortem* HPLC changes in neurotransmitter levels were measured; this was performed on brains derived from PND 35 and PND 100 rats (PND 35: n = 10 MIS and n = 10 saline, PND 100: n = 16 MIS and n = 21 saline groups). The longitudinal assessment of PPI revealed that PPI deficits in MIS offspring first emerged post-puberty, i.e. there were no differences in PPI levels between offspring of MIS and controls on PND 35 and PND 60 but MIS offspring showed lower PPI levels than controls on PND 100. Looking at changes in neurotransmission, the most interesting finding relates to the dopaminergic-system; MIS offspring exhibited higher levels of DA in the Nacc and lower levels of DOPAC in the mPFC when compared to control offspring. FDG-PET results strengthen these findings as MIS offspring exhibited lower glucose metabolism in the cortex, PFC and ventral hippocampus whereby higher glucose metabolism was found in the Nacc and amygdala.

Maturation further affected changes in neurotransmission and metabolic activity across brain structures. MIS offspring exhibited aberrant neurotransmission and metabolic activity prior to and with the development of PPI deficits in adolescence as well as in adulthood, pointing to early impairments due to MIS status. Altogether, these results indicate that neurochemical and metabolic changes following MIS are neurodevelopmental in nature, eventually leading to behavioral deficits when these abnormalities increase.

Using a maternal immune stimulation model of schizophrenia to study behavioral and neurobiological alterations over the developmental course

Hadar, R., Soto-Montenegro, M.L., Gotz, T., Wieske, F., Sohr, R., Desco, M., Hamani, C., Weiner, I., Pascau, J., Winter, C.,

Schizophr Res. 2015; 166, 238-247

https://doi.org/10.1016/j.schres.2015.05.010

2.2.2 Early neuromodulation prevents the development of brain and behavioral abnormalities in a rodent model of schizophrenia

Hadar, R., Bikovski, L., Soto-Montenegro, M.L., Schimke, J., Maier, P., Ewing, S., Voget, M., Wieske, F., Gotz, T., Desco, M., Hamani, C., Pascau, J., Weiner, I., Winter, C.

Molecular Psychiatry, 2017

The view that schizophrenia is a brain disorder in which neuropathologies appear early in brain-development and further evolve over the developmental course insinuates that there is a potential time-window for therapeutic intervention. In this study the rodent MIS model of schizophrenia was utilized to test whether early neuromodulation in the form of DBS could affect disease progression and its severity. Based on the known involvement of the prefrontal cortex and its related circuitries in the development and manifestation of schizophrenia and previous findings demonstrating that high frequency DBS to the mPFC is capable of normalizing behavioral deficits in the MIS model this region was targeted in this study. For generating the experimental groups, pregnant dams were injected to the tail vein with the immune activating agent poly I:C (4 mg/kg) or saline and its male offspring was used for investigations. On PND 33 -34, offspring were subjected to bilateral stereotactic electrode implantation into the mPFC and continuous high frequency stimulation (or sham) was delivered from PND 35 to 47. Behavioral and neurobiological assessments were conducted at adulthood, i.e. PND > 90. Adult animals were tested in the PPI paradigm following that neurochemical assessments were carried out (saline-sham: n = 8; saline-DBS: n = 10; MIS-sham: n = 8; MIS-DBS: n = 9). Other animals were tested in the LI paradigm (saline-sham: n = 20; saline-DBS: n = 15; MIS-sham: n = 17; MIS-DBS: n = 16) and one week later a portion of these animals were tested in the discrimination reversal (DR) paradigm (saline-sham: n = 8; saline-DBS: n = 8; MIS-sham: n = 8; MIS-DBS: n = 8). Thereafter, brains were used for ex vivo MRI (saline-sham: n = 10; saline-DBS: n = 7; MIS-sham: n = 13; MIS-DBS: n = 8). Using FDG-PET, the effects of mPFC-DBS on brain-metabolic changes were tested in another 29 rats (saline-sham: n = 6; saline-DBS: n = 8; MIS-sham: n = 8; MIS-DBS: n = 7). The most striking results of adolescence mPFC-DBS relate to its efficacy in successfully preventing the emergence of PPI, LI and DR deficits. The prevention of these behavioral deficits was accompanied by the prevention of the otherwise enlarged lateral ventricles (LV) volumes in MIS offspring. Further, preventive mPFC-DBS reduced excessive dopamine content in the GP of MIS animals. Interestingly, early mPFC-DBS was found to induce only minimal effects on brain metabolism, in both MIS and control animals. Collectively, this study demonstrated that targeted neuromodulation applied at an early disease stage, could prevent the development of schizophrenia-relevant behavioral and neurobiological deficits in the MIS model.

Early neuromodulation prevents the development of brain and behavioral abnormalities in a rodent model of schizophrenia

Hadar, R., Bikovski, L., Soto-Montenegro, M.L., Schimke, J., Maier, P., Ewing, S., Voget, M., Wieske, F., Gotz, T., Desco, M., Hamani, C., Pascau, J., Weiner, I., Winter, C.

Mol Psychiatry. 2017; 23(4), 943-951.

https://doi.org/10.1038/mp.2017.52

2.2.3 Deep brain stimulation during early adolescence prevents microglial alterations in a model of maternal immune activation

Hadar, R. *, Dong, L. *, Del-Valle-Anton, L., Guneykaya, D., Voget, M., Edemann-Callesen, H., Schweibold, R., Djodari-Irani, A., Goetz, T., Ewing, S., Kettenmann, H., Wolf, S.A., Winter, C.

Brain, Behavior and Immunity, 2016

The neurodevelopmental MIS model of schizophrenia provides an excellent experimental platform for studying the longitudinal and progressive nature of this disease. As such, it has been used for studying the efficacy of various therapeutic approaches, among which also novel preventive avenues in an effort to interfere with disease progression. Further, it was demonstrated that in the MIS model an association between neuroinflammation and schizophrenia-relevant behavior exist and that therapeutic intervention using minocycline, an anti-inflammatory drug, normalized altered behavior and abnormal microglia activation in adult MIS offspring. Since mPFC-DBS during adolescence was shown to prevent the development of behavioral and structural deficits in the MIS model, the current study was designed to complement these findings and test whether DBS during adolescence alters microglia properties in adulthood. For generating the experimental groups, pregnant dams were injected to the tail vein with the immune activating agent poly I:C (4 mg/kg) or saline and its male offspring was used for investigations. Behaviorally inconspicuous adolescent MIS offspring and its controls were randomly assigned to either high frequency mPFC-DBS or Nacc-DBS and surgeries for bilateral stereotactic electrode implantation were conducted on PND 33 - 34. DBS was delivered continuously from PND 35 to 47. Behavioral testing followed by *post mortem* immunohistochemical assessments were conducted at adulthood, i.e. PND > 90 on non-stimulated animals as well as on animals from the mPFC- and Nacc- DBS groups. For behavior, adult animals were tested in the PPI paradigm (saline: n = 18; MIS: n = 20) and results indicated a decrease in PPI in the MIS offspring. Microglia density and soma size were studied in the MIS and control rats (6-10 animals from each group: (MIS: n = 6; saline, n = 9; MIS sham, n = 8; MIS DBS, n = 10; saline control, n = 9; saline sham, n = 10; saline DBS, n = 10)). Results showed that MIS animals exhibited increased microglia density and soma size in the hippocampus and Nacc. In the MIS model DBS to both mPFC and Nacc prevented the increase in both microglia density and soma size in the related projection areas. This study shows that in addition to the preventive effects of continuous electrical brain stimulation during adolescence on neuropathological and behavioral deficits, this approach also holds promise to prevent neuro-inflammatory components in the MIS model. Overall, these results support the approach of early intervention in neurodevelopmental disorders, pointing to the possibility of preventing behavioral abnormalities along with neuropathologies in the form of altered neuroinflammation.

Deep brain stimulation during early adolescence prevents microglial alterations in a model of maternal immune activation

Hadar, R. *, Dong, L. *, Del-Valle-Anton, L., Guneykaya, D., Voget, M., Edemann-Callesen, H., Schweibold, R., Djodari-Irani, A., Goetz, T., Ewing, S., Kettenmann, H., Wolf, S.A., Winter, C.

Brain Behav Immun. 2016; 63, 71-80

https://doi.org/10.1016/j.bbi.2016.12.003

2.2.4 Prevention of schizophrenia deficits via non-invasive adolescent frontal cortex stimulation in rats

Hadar, R., Winter, R., Edemann-Callesen, H., Wieske, F., Habelt, B., Khadka, N., Felgel-Farnholz, V., Barroeta-Hlusicka, E., Reis, J., Tatarau, C.A., Funke, K., Fritsch, B., Bernhardt, N., Bikson, M., Nitsche, M.A., Winter, C.,

Molecular Psychiatry, 2019

The remarkable efficacy of early mPFC-DBS to prevent a battery of behavioral deficits and neuropathologies in the MIS model prompted the current line investigation. Whereas the application of DBS to young individuals at risk of developing psychosis is, due to its invasive nature, not applicable, the promotion of other non-invasive neuromodulation techniques as preventive measures is of clinical relevance. The current study sought to test the potential of prefrontal cortex (PC) transcranial direct current stimulation (tDCS), a safe and well-tolerated neuromodulation technique, as a preventive strategy to halt schizophrenia-related deficits in the MIS model. Pregnant dams were injected to the tail vein with the immune activating agent poly I:C (4 mg/kg) (for the MIS group) or saline (for the control group) and its male offspring was used for investigations. Offspring was subjected to tDCS-electrode placement during PND 33 – 34 and tDCS in either anodal or cathodal polarities (and sham) was delivered twice a day over the preventive time-window of adolescence (PND 35-47) (saline sham: n = 15; saline anodal: n = 14; saline cathodal: n = 13; MIS sham: n = 14; MIS anodal: n = 12; MIS cathodal: n = 11). Animals were then left undisturbed until reaching adulthood when behavioral testing followed by *post mortem* structural and immunohistological assessments were performed. Behavioral testing included PPI, DR, amphetamine-induced activity (AIA), social interaction (SI) and sucrose consumption test (SCT). Results indicate that anodal tDCS delivered through adolescence was successful in preventing the development of reduced PPI, abnormal rapid DR as well as elevated AIA levels in the MIS model. Interestingly, both anodal and cathodal adolescence tDCS were able to prevent structural deficits in the form of enlarged LV. Whereas MIS offspring exhibited deficits in SI and SCT, tDCS in both polarities yielded no effect on these behaviors. Further, tDCS did not affect the observed reduction of parvalbumin-expressing cells in the mPFC of MIS animals. The current study demonstrates that the application of non-invasive tDCS during adolescence, prior to the manifestation of schizophrenia-relevant behavioral abnormalities, is capable of preventing the development of positive symptoms and related neuropathologies in the MIS model. Overall, the results of this study introduce a novel approach for the prevention of schizophrenia-development via non-invasive neuromodulation in the form of tDCS. Altogether, tDCS provides an intervention modality that could be rather easily translated into the clinic for verification.

Prevention of schizophrenia deficits via non-invasive adolescent frontal cortex stimulation in rats.

Hadar, R., Winter, R., Edemann-Callesen, H., Wieske, F., Habelt, B., Khadka, N., Felgel-Farnholz, V., Barroeta-Hlusicka, E., Reis, J., Tatarau, C.A., Funke, K., Fritsch, B., Bernhardt, N., Bikson, M., Nitsche, M.A., Winter, C.,

Mol Psychiatry. 2019; 25(4):896-905

https://doi.org/10.1038/s41380-019-0356-x

3. Discussion

The body of work presented here was conceptualized within the contemporary neuro-psychiatric climate, which suggests that the development of novel therapeutic avenues is of great interest and need (Millan et al., 2016; Millan, Goodwin, Meyer-Lindenberg, & Ove Ogren, 2015). This is due to the general consensus among leading clinicians and scientists that existing treatments for neuro-psychiatric disorders are imperfect (Millan, Goodwin, Meyer-Lindenberg, & Ogren, 2015; Millan, Goodwin, Meyer-Lindenberg, & Ove Ogren, 2015). With this regards schizophrenia is no exception; despite the availability of different classes of antipsychotics ('typical' and 'atypical'), bearing affinity to a variety of neurotransmitter systems along with different receptor profiles, social, cognitive and emotional deficits are hardly normalized following its administration, let alone the grave side effects often observed (historically reviewed and thoughtfully discussed by Millan et al., 2015b).

For that the MIS model of schizophrenia, developed following epidemiological human studies pointing to an association between a variety of maternal infections during pregnancy and the heightened risk of schizophrenia in the offspring, was used (Brown, 2006; Brown & Patterson, 2011; Brown & Susser, 2002; Jones, Rantakallio, Hartikainen, Isohanni, & Sipila, 1998; Mednick, Machon, Huttunen, & Bonett, 1988). Consequently, the work presented here sought first to investigate a novel therapeutic approach for treating already existing symptomatology of schizophrenia, namely neuromodulation, and then to extend the investigations and test the possibility of preventing the development of schizophrenia-symptomatology.

3.1 Acute and focal neuromodulation reverses schizophrenia-related behavioral deficits and affect altered neuro-circuitry.

This line of research utilized cross-species phenomena known to be disrupted in the human schizophrenia condition as well as in offspring of dams subjected to the viral mimic poly I:C during pregnancy (Meyer, 2014; Zuckerman, Rehavi, Nachman, & Weiner, 2003; Zuckerman & Weiner, 2003, 2005). Specifically, the pre-pulse inhibition (PPI) of the acoustic startle response paradigm which allows to measure deficits in sensorimotor gating and the latent inhibition (LI) paradigm which allows to probe attentional selectivity deficits were used here as behavioral output criteria. Since reduced PPI and disrupted LI have both been linked to a variety of neuropsychiatric disorders and are consistently found in schizophrenia, these behavioral outputs were chosen to serve as a proof of concept for the therapeutic potential of DBS for schizophrenia. As a first step DBS at different, clinically relevant,

stimulation parameters was acutely delivered to chief loci in the basal ganglia-thalamo-cortical-circuitry and its effect on PPI was measured in parallel (Klein et al., 2013). Results indicated that DBS indeed has the capacity to normalize PPI deficits when delivered to the medial prefrontal cortex (mPFC), the dorsmedial thalamus (DM) or the globus pallidus (GP). Collectively these results also pointed to the advantageous effects of DBS delivered at high frequencies. The following experiment hence utilized only high frequency stimulation protocols and added to the behavioral output the LI paradigm. Here, the acute effects of DBS delivered to the mPFC as well as the nucleus accumbens (Nacc) were examined and by using glucose uptake positron emission tomography (PET) the neurocircuitry influenced by DBS was investigated (Bikovsky et al., 2016). As in the aforementioned investigation, also here when targeting the mPFC and Nacc, the acute application of DBS was successful in alleviating PPI deficits in the MIS offspring; additionally, DBS normalized LI deficits. Surprisingly, metabolic effects following DBS application were more prominent in control animals than in MIS offspring. Moreover, DBS to the mPFC altered the same neurocircuitries in control and MIS animals with both activation and deactivation patterns whereas DBS to the Nacc yielded no significant alterations in brain glucose metabolism. These results underline the fact that DBS is capable of altering both behavior and brain network activity; however the output is dependent on the underlying neurobiological properties. Jointly, the results from this part lay the groundwork for further investigations into the potency of DBS to reverse existing deficits in sensorimotor gating and attentional selectivity in schizophrenia. Further, these results indicate that in animals electrical stimulation of selected brain regions can improve behavioral deficits.

3.2 Preventing the development of schizophrenia via neuromodulation.

Though the exact etiology of schizophrenia is unknown, in recent years a wealth of evidence suggests that schizophrenia is the result of a complex interplay of genetic, epigenetic and environmental factors that hamper the normal neurodevelopmental course, ultimately resulting in the emergence of this disorder (Insel, 2010; Rapoport et al., 2012). The notion that schizophrenia is a neurodevelopmental disorder underlies the second part of the work presented here. All different lines of investigations conducted in this part used the MIS model of schizophrenia in an effort to test preventive avenues to this disorder, along with delineating its neuropathological development.

As a first step the MIS model was used to compare the adolescence period, also known as the pre-symptomatic period of schizophrenia, with the period of adulthood in which symptoms are fully present by looking at the behavioral as well as the neurobiological levels. Specifically the adolescence period was observed at its beginning, namely postnatal day (PND) 35 and at late stage i.e. PND 60, thus

capturing both poles of adolescence; adulthood was examined at one time point, namely PND 100. Tracing the development of sensory gating deficits in the form of PPI in the MIS model revealed that these deficits first emerge at adulthood (Hadar et al., 2015). No differences in PPI performance between MIS offspring and controls were seen in early or late adolescence therefore this study could add and compliment previous data indicating the same longitudinal progression of deficits in attentional selectivity in the form of LI (Piontkewitz et al., 2011a, 2012). Combining FDG-PET with neurochemical investigations using *post mortem* HPLC this longitudinal study revealed that MIS offspring had decreased levels of DOPAC in the mPFC and lower glucose uptake in the PFC as well as heightened levels of DA in the Nacc along with higher metabolism in this region. Interestingly, alterations in neurochemical and metabolic activity in the mPFC of MIS offspring were apparent prior to the manifestation of the behavioral deficits, i.e. during adolescent, and remained through adulthood state. This finding of an early impairment in the mPFC in MIS offspring is significant on both theoretical and practical levels; on the one hand it strengthen the notion that schizophrenia is a neurodevelopmental disorder, at least as here reflected in the MIS model and it also implied that modulating mPFC neuronal activity at an early stage might alter disease progression. This was practically tested as the next step. A few human studies focusing on individual at high risk to develop psychosis or at a prodromal stage identified a therapeutic time window during which the application of antipsychotic drugs was shown to ease the transition into psychosis (McGlashan et al., 2006; Woods et al., 2003). This line of investigations was successfully back translated using the MIS model and revealed the homologous period for preventive interventions in the form of antipsychotics in rats, which corresponds to PND 34-47 i.e. the early adolescence period (Meyer et al., 2010; Piontkewitz et al., 2011b; Piontkewitz et al., 2009). Using this time-window, targeted neuromodulation of the mPFC of MIS rats was hence tested as a preventive strategy by delivering high frequency chronic DBS throughout rats' early adolescence (Hadar et al., 2017; Hadar et al., 2016). Assessing the prevention of schizophrenia-relevant abnormalities using the MIS rodent model could be studied on different levels, not all applicable in the human situation; besides studying cross-species phenomenon (as for example the aforementioned PPI and LI) and brain-structural alterations, animal models also allow investigating neuropathological processes using *post mortem* techniques. By combining different levels of scrutiny, the effects of chronic preventive DBS to the mPFC in MIS animals revealed striking alterations in behavior, brain-structure and metabolic, neuroimmunology and neurochemistry. Specifically mPFC-DBS to MIS animals during adolescence prevented deficits in PPI, LI and normalized abnormal rapid discrimination reversal (DR), a phenomenon reflecting the positive symptoms of schizophrenia. Further, increased lateral ventricles (LV) volume, a brain-structural hallmark of schizophrenia that is also apparent in the MIS model, was prevented following mPFC-DBS. Pursuing previous own findings in the MIS model pointing to alterations in microglia activation (Mattei et al., 2014), the effects of

preventive mPFC-DBS on microglia was also investigated and also here early stimulation was successful in normalizing microglia abnormalities (Hadar et al., 2016) along with other neurochemical and metabolic changes. Taken together, these investigations pointed to the feasibility of targeting the mPFC at an early stage in preventing the development of schizophrenia-relevant abnormalities. Obviously, an invasive procedure as DBS is not applicable as a preventive measure for individuals at (high) risk to develop psychosis, for most as long as there is no biological marker that can reliably predict the transition to psychosis. Alternatively, however, the use of non-invasive neuromodulation procedures as a preventive measure is more feasible; transcranial direct current stimulation (tDCS) is a considerably safe, well tolerated and non-invasive technique that was hence used as a second step and applied to the prefrontal cortex (PFC) of MIS animals during the preventive time-window of adolescence (Hadar et al., 2019). Interestingly, when applied chronically to the PFC during adolescence, (anodal) tDCS, though lacking the spatial precision of DBS, was also able to prevent behavioral deficits relevant to the positive symptoms of schizophrenia as well as LV increment and enhanced mesolimbic dopaminergic neurotransmission. It had however no effect on alterations relevant to the negative symptomatology of schizophrenia, as social interaction and anhedonia.

Altogether, the bulk of work presented and discussed in this part suggests that the prevention of schizophrenia-development via neuromodulation is plausible. The exact translation into the clinic obviously necessitates complementary line of human studies; to this end, tDCS presents itself as a realistic approach due to its safety and tolerability.

4. Summary

The present work used a neurodevelopmental animal model of schizophrenia, namely the MIS rodent model, to study the capacity and potency of neuromodulations to ameliorate schizophrenia-relevant behavioral and neurobiological abnormalities. Acute and focal DBS to the mPFC was found to be therapeutically relevant as it successfully normalized deficits in sensorimotor gating and attention selectivity apparent in the adult MIS animals. Using a longitudinal approach the development of sensorimotor deficits in the MIS model was traced and was found to exhibit a maturational delay, in accordance with the clinical situation. Further, this approach revealed aberrant neurochemistry profile in the mPFC during the pre-symptomatic period of adolescence, prior to the outbreak of the behavioral deficits. As a result, chronic DBS to the mPFC of adolescent MIS animals was tested and revealed that this approach could prevent the development of deficits in sensorimotor gating, attentional selectivity and reversal learning. Along with these effects, DBS was able to prevent increased LV volume and to normalize neurochemical alterations. Finally, a non-invasive neuromodulation technique in the form of tDCS was chronically applied during adolescence and revealed that tDCS could prevent behavioral deficits belonging to the positive-symptomatology of schizophrenia, along with abnormal LV volumes.

Taken together, this pre-clinical, translational-directed work points to the plausible efficacy of early, non-invasive, neuromodulation approach as a preventive measure for the development of schizophrenia.

5. References

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Erklärung

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,
- die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden,
- mir die geltende Habilitationsordnung bekannt ist.

Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

……. ……..

Datum Unterschrift