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

Lior Bikovsky^{b,1}, Ravit Hadar^{a,1}, María Luisa Soto-Montenegro^{d,e,1}, Julia Klein^f, Ina Weiner^{b,c}, Manuel Desco^{d,e,g}, Javier Pascual^{d,e,g}, Christine Winter^{a,*}, and Clement Hamani^{h,i,j}

^aDepartment of Psychiatry and Psychotherapy, University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany

^bSchool of Psychological Sciences, Tel Aviv University, Tel Aviv, Israel

^cSagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel

^dInstituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain

^eCIBERSAM, Madrid, Spain

^fDepartment of Psychiatry and Psychotherapy, Charité University Medicine Berlin, Campus Charité Mitte, Berlin, Germany

^gDepartamento de Bioingeniería e Ingeniería Aeroespacial, Universidad Carlos III de Madrid, Spain

^hBehavioural Neurobiology Laboratory, Centre for Addiction and Mental Health, 250 College Street, Toronto, ON, M5T 1R8, Canada

ⁱCampbell Family Mental Health Research Institute, CAMH, Canada

^jDivision of Neurosurgery, Toronto Western Hospital, 399 Bathurst Street, Toronto, ON, M5T 2S8, Canada

Abstract

Schizophrenia is a debilitating psychiatric disorder with a significant number of patients not adequately responding to treatment. Deep brain stimulation (DBS) is a surgical technique currently investigated for medically-refractory psychiatric disorders. Here, we use the poly I:C rat model of schizophrenia to study the effects of medial prefrontal cortex (mPFC) and nucleus accumbens (Nacc) DBS on two behavioral schizophrenia-like deficits, i.e. sensorimotor gating, as reflected by disrupted prepulse inhibition (PPI), and attentional selectivity, as reflected by disrupted latent inhibition (LI). In addition, the neurocircuitry influenced by DBS was studied using FDG PET. We found that mPFC- and Nacc-DBS alleviated PPI and LI abnormalities in poly I:C offspring, whereas Nacc- but not mPFC-DBS disrupted PPI and LI in saline offspring. In saline offspring, mPFC-DBS increased metabolism in the parietal cortex, striatum, ventral hippocampus and Nacc, while reducing it in the brainstem, cerebellum, hypothalamus and periaqueductal gray. Nacc-DBS, on the other hand, increased activity in the ventral hippocampus

*Corresponding author. christine.winter@uniklinikum-dresden.de (C. Winter).

¹Authors have equally contributed to the manuscript.

and olfactory bulb and reduced it in the septal area, brainstem, periaqueductal gray and hypothalamus. In poly I:C offspring changes in metabolism following mPFC-DBS were similar to those recorded in saline offspring, except for a reduced activity in the brainstem and hypothalamus. In contrast, Nacc-DBS did not induce any statistical changes in brain metabolism in poly I:C offspring. Our study shows that mPFC- or Nacc-DBS delivered to the adult progeny of poly I:C treated dams improves deficits in PPI and LI. Despite common behavioral responses, stimulation in the two targets induced different metabolic effects.

Keywords

Schizophrenia; Maternal immune activation rat model; Deep brain stimulation; Prepulse inhibition; Latent inhibition; FDG-PET

1. Introduction

Schizophrenia is a debilitating psychiatric disorder with a lifetime prevalence of approximately 1% (Tamminga and Holcomb, 2005). Despite the development of new medications, about 30% of patients do not adequately respond to pharmacological treatment (Falkai et al., 2005, 2006). Deep brain stimulation (DBS) is a surgical technique that involves the delivery of electrical current to the brain parenchyma through implanted electrodes. In psychiatry, DBS has been approved for treatment-resistant obsessive-compulsive disorders (Hamani et al., 2014b) and is currently being investigated for depression (Holtzheimer et al., 2012; Lozano et al., 2008; Malone et al., 2009; Mayberg et al., 2005), anorexia (Lipsman et al., 2013), Alzheimer's disease (Kuhn et al., 2015; Laxton et al., 2010) and drug addiction (Muller et al., 2013; Stelten et al., 2008; Voges et al., 2013). So far, though different targets have been proposed (Ewing and Winter, 2013; Mikell et al., 2009), no clinical trials have been reported on the use of DBS in schizophrenia.

Schizophrenia is a complex and multi-symptomatic disorder. The well-documented association between intrauterine inflammatory/immunologic responses (e.g. to infectious agents) and the susceptibility for the development of schizophrenia promoted the establishment of the so-called "maternal immune activation" (MIA) models (Baharnoori et al., 2012; Piontkewitz et al., 2012a). These involve administering pregnant dams with viruses, bacterial products (i.e. lipo-polysaccharides) or viral mimics, such as polyinosinic-polycytidilic acid (poly I:C) (Meyer, 2014). The progeny born after such insults develop neuropathological and behavioral changes that somewhat mimic those observed in schizophrenia. Some of these include deficits in sensorimotor gating, as reflected by a decrease in prepulse inhibition (PPI), attentional selectivity deficits, as reflected in disrupted latent inhibition (LI), decreased hippocampal and prefrontal cortex and increased ventricular volumes, and reduced hippocampal neurogenesis (Ozawa et al., 2006; Piontkewitz et al., 2011, 2012a; Piontkewitz et al., 2009; Piontkewitz et al., 2012b). Notably, the full spectrum of behavioral abnormalities only emerges after the offspring have reached late adolescence or early adulthood (Hadar et al., 2015; Piontkewitz et al., 2011; Zuckerman et al., 2003), which is consistent with the post-pubertal emergence of full-blown phenotype in schizophrenia (Tandon et al., 2009).

In a previous report, we have shown that DBS at high frequencies applied to the medial prefrontal cortex (mPFC) and dorsomedial thalamus of the adult offspring of poly I:C injected dams normalized PPI deficits (Klein et al., 2013). In this study, we extend that line of research by investigating the behavioral effects of mPFC and nucleus accumbens (Nacc) DBS applied to adult rats on both the PPI and LI paradigms. Based on the fact that DBS is known to influence structures at a distance from the stimulated target (Hamani and Temel, 2012) and given the strong anatomical and functional links between mPFC and Nacc (Hamani et al., 2011; Vertes, 2004) we hypothesize that both mPFC and Nacc stimulation will affect the characteristic behavioral deficits of poly I:C offspring in both PPI and LI by specifically modulating neurocircuits involved in schizophrenia. To study the neurocircuitry influenced by DBS we have used glucose uptake positron emission tomography (PET) imaging (Klein et al., 2011).

2. Materials and methods

2.1. Animals

Rats were housed 2–4/cage in a temperature and humidity controlled vivarium with a 12-h light–dark cycle and with food and water ad-libitum. Experiments were performed during day time (lights on for PPI and PDG-PET studies, and lights off for LI studies), according to the guidelines of the European Union Council Directive 2010/63/EU for care of laboratory animals and were approved by the local ethic committee (Senate of Berlin, Germany for PPI experiments, Animal Care and use Committee, Tel Aviv University, Israel for LI experiments and Ethics Committee for Animal Experimentation of Hospital Gregorio Marañón Madrid, Spain for FDG-PET studies and the Centre for Addiction and Mental Health).

2.2. Experimental design

Wistar rats (Harlan Laboratories, Germany, Israel and Spain) were mated and the first day after copulation was defined as day 1 of pregnancy. On gestation day 15, pregnant dams were given a single i.v. injection to the tail vein of either poly I:C (4 mg/kg; SIGMA, Germany) dissolved in saline, or saline alone under light isoflurane anesthesia (Hadar et al., 2015). On post-natal day (PND) 21, pups were weaned and housed according to sex and litter. Experimental groups consisted of male offspring derived from multiple independent litters. Surgery was performed on adult offspring (PND > 90), bilateral electrodes were implanted into either the mPFC or the Nacc shell. Behavioral and imaging experiments commenced 1–2 weeks *post-surgery*. During the last 5 days of recovery, rats were handled for about 10 min daily. Handling comprised habituation to the investigator, the stimulation procedure, i.e. connection of the electrodes to the cable system, and if applicable, the testing chamber. DBS was conducted during PPI testing for 40 min, during pre-exposure and conditioning stages of the LI paradigm for 30 and 15 min and during FDG uptake periods for 45 min at 130 Hz, 150 μ A and a pulse duration of 90 μ sec (biphasic stimulation mode). These parameters were chosen as they were found to be the most effective in our previous experiments with this model (Klein et al., 2013). DBS was performed with an isolated stimulator (MultichannelSystems; Coulbourn Instruments, Allentown, PA, USA) connected

to the electrodes via an isolated cable system hanging from the ceiling of the behavioral apparatus and a swivel.

2.3. Surgery

Surgery was conducted under general anesthesia (PPI experiments: fentanyl (0.005 mg/kg i.m., Janssen-Cilag, Germany), midazolam (2 mg/kg i.m., Hameln, Germany), medetomidine dihydrochloride (0.135 mg/kg i.m., Elanci Animal Health, Germany), antagonized upon completion with naloxone (0.12 mg/kg i.m. Inresa Arzneimittel, Germany), flumazenil (0.2 mg/kg i.m. HEXAL Germany) and atipamezolhydrochlorid (0.75 mg/kg s.c. Elanco Animal Health, Germany); LI experiments: isoflurane (Nicholas Piramal, Northumberland, United Kingdom) followed by avertin (20 ml/kg i.p.); imaging: ketamine (100 mg/kg i.p., Pfizer, Ortaköy-Istanbul, Turkey) and xylazine 1 mg/kg i.p., Calier, Barcelona, Spain). Cathodes (monopolar platinum–iridium electrodes with connector; Plastics One, Roanoke, USA) were bilaterally implanted into the mPFC (more specifically into the ventral prefrontal cortex; anteroposterior (AP) +3.5, mediolateral (ML) 0.6, dorsoventral (DV) -3.4) or the Nacc shell (AP +1.2, ML 1.5, DV -8.2), as previously described (Klein et al., 2013). Anodes were wrapped around anchor screws implanted in close proximity to the stimulating electrode. Electrode placement was confirmed in cresyl violet stained sections or imaging. For the former, rats were anaesthetized and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde (PFA). For the latter, computed tomography (CT) scans were obtained and co-registered to a reference MRI study (anatomical MRI template obtained from a non-operated animal). Only animals with correct electrode placement were included in the study (Fig. 1a + b).

2.4. Behavior

2.4.1. Prepulse inhibition (PPI)—PPI of the acoustic startle reflex (ASR) was measured in three sessions given 24 h apart. Session 1 (baseline 1) was conducted without stimulation. Session 2 was conducted while animals received either mPFC or Nacc-DBS. Session 3 (baseline 2) was carried out with no DBS applied to assess whether the observed effects in Session 2 were permanent or transient, i.e. effect not noticed 24 h after DBS. During test sessions, animals were placed in a wire mesh cage mounted on a transducer-platform. Loudspeakers on both sides of the cage were used for acoustic stimulation. Sessions began with a 5 min acclimatization phase, which was followed by the PPI test. During acclimatization, animals were exposed to background noise followed by 10 startle stimuli (20 ms each). The test session consisted of 7 different types of trials administered in a pseudorandom order: 1) pulse alone (100 dB sound pressure levels (SPL) white noise, 20 ms duration); 2) control (no stimulus); 3–4) prepulse alone (72 dB or 68 dB, pure tone, 10 Hz, 20 ms duration); 5–7) prepulse (72 dB, 68 dB, or 64 dB) followed by pulse (100 ms interstimulus interval). Animals received a total of 10 presentations of each type. Background noise intensity during the whole experiment was 60 dB SPL. PPI was calculated according to the formula $100 - 100\% \times (PPx/PA)$, in which PPx is the ASR of the 10 PPI trials for each individual prepulse intensity and PA is the mean ASR of the pulse alone trials. The average PPI response over the three pre-pulse intensities was analyzed (Klein et al., 2013; Mattei et al., 2014).

2.4.2. Latent inhibition (LI)—LI was measured in a thirst-motivated conditioned emotional response (CER) procedure by comparing the suppression of drinking to a tone previously paired with a foot-shock in rats that received non-reinforced exposure to the tone before conditioning (pre-exposed, PE) and in rats for whom the tone was novel (non-pre-exposed, NPE). Each LI experiment included eight experimental groups in a $2 \times 2 \times 2$ design with main factors of pre-exposure (NPE, PE), maternal immune stimulation (poly I:C, saline) and treatment (DBS, sham). LI was measured in rodent test chambers with a retractable bottle (Campden Instruments). Rats were handled (5 days), water-deprived (23 h/day; five days) and trained to lick in the experimental chamber (5 days). Following training, surgery took place and ad libitum water was reinstated until the animals recovered from the procedure (1–2 weeks). Thereafter, water restriction was reinstated and rats were again re-trained to drink in the experimental chamber for 5 days. On the next 4 days, LI was conducted, consisting of the following stages given 24 h apart: *Pre-exposure*: PE rats were placed in the chamber with the bottles removed and presented with 40 tones (10 s, 80 dB, 2.8 kHz) given 40 s apart. NPE animals were placed in the chamber but were not exposed to tones. *Conditioning*: With the bottles removed, all rats were placed in the chamber and received 2 tone-shock (0.5 mA, 1 s duration) pairings given 5 min apart. *Lick retraining* *Test*: Rats were placed in the chamber and allowed to drink from the bottle. Upon completing 75 licks, a tone was presented for 5 min. Time to complete licks 51–75 (before the tone) and licks 76–100 (after the tone) was recorded. Time to complete licks 76–100 was logarithmically transformed for parametric analysis of variance. Longer log times indicate a stronger drinking suppression. LI was defined as significantly shorter log times to complete licks 76–100 of the PE compared with NPE rats. DBS was administered during pre-exposure and conditioning only. In the pre-exposure session, rats were continuously stimulated. In the conditioning session, stimulation was halted for the duration of each shock.

2.4.3. Neuroimaging—For each rat, two PET scans were acquired 48 h apart. Animals were injected with the [^{18}F]-FDG (~1 mCi) into the tail vein. 45 min later scans were obtained. DBS was conducted during the uptake period. For baseline measures, rats were attached to the stimulation system but received no current. Imaging was obtained under isoflurane anesthesia (3% induction and 1.5% maintenance in 100% O_2) using a small-animal PET/CT scanner (ARGUS PET/CT, SEDECAL, Madrid). Images were reconstructed using a 2D OSEM (2D ordered subsets expectation maximization algorithm) with a voxel size of $0.3875 \times 0.3875 \times 0.7750 \text{ mm}^3$. Energy window was 400–700 keV and decay and dead time corrections were applied. CT scans were acquired with the following parameters: 320 mA, 45 KV, 360 projections, 8 shots, and 200 μm of resolution. Images were reconstructed using a Feldkamp algorithm (isotropic voxel size of 0.121 mm). In addition, an MRI study of a non-operated animal was acquired in a 7-Tesla Biospec 70/20 scanner (Bruker, Ettlingen, Germany) under sevoflurane anesthesia (4.5% for induction and 2.5% for maintenance in 100% O_2). A T2-weighted spin echo sequence was acquired, with TE = 33 ms, TR = 3732 ms, and a slice thickness of 0.8 mm (34 slices). The matrix size was 256×256 and a FOV of $3.5 \times 3.5 \text{ cm}$. This single-animal study was used as an anatomical template in order to display the results of the SPM study.

PET data were analyzed as previously described (Soto-Montenegro et al., 2014). Briefly, CT studies were co-registered with a random reference CT scan using an automatic rigid registration method based on mutual information (Pascau et al., 2009) and the spatial transformation obtained for each CT image was subsequently applied to the corresponding PET. The single MRI study was spatially co-registered to the reference CT scan. A brain mask was segmented on this MRI study and applied to all registered PET images to eliminate voxels outside the brain. The resulting images were smoothed with an isotropic Gaussian filter (2 mm FWHM) to minimize the effect of registration errors and ensure normality of data. Voxel values were normalized to the average brain intensity in order to obtain the regional characterization of metabolic changes circumventing overall differences in animal brain metabolism.

2.5. Statistical analysis

PPI data were analyzed using two way repeated measurements ANOVA with maternal immune stimulation (saline, poly I:C) and treatment (test session 1–3) as factors. LI data was analyzed with a three-way ANOVA (maternal immune stimulation $\times$ DBS $\times$ pre-exposure). Significant interactions were followed by least significant difference post hoc comparisons. PET data were analyzed using SPM12 (Statistical Parametric Mapping, version 6225, Wellcome Trust Centre for Neuro-imaging, London, UK). Groups were compared using a multifactorial ANOVA with a significance threshold of $p < 0.01$ (corresponding to a $T = 2.6$), uncorrected for multiple comparisons. Factors of study were immune stimulation (saline, poly I:C) and treatment (sham, DBS). To reduce type I error, a 50-voxel clustering threshold (spatial-extent) was applied and no significant regions smaller than 50 connected activated voxels were accepted.

3. Results

3.1. Behavior

3.1.1. Prepulse inhibition—Experiments were conducted in 27 rats (vmPFC-DBS: $n = 5$ saline, $n = 6$ poly I:C, Nacc-DBS: $n = 8$ saline, $n = 8$ poly I:C). In animals receiving mPFC-DBS we found significant main effects of maternal immune stimulation ($F(1.18) = 6.390$, $p = 0.032$) and treatment ($F(2.18) = 5.340$, $p = 0.015$), as well as a significant maternal immune stimulation $\times$ treatment interaction ($F(2.18) = 4.538$, $p = 0.025$, Fig. 2a). In animals given Nacc-DBS, a significant maternal immune stimulation $\times$ treatment interaction was recorded ($F(2.23) = 28.023$, $p < 0.001$) without significant immune stimulation ($F(1.23) = 3.235$, $p = 0.093$) or treatment effects ($F(2.23) = 1.086$, $p = 0.345$, Fig. 2). At baseline and in session 3, post hoc analysis revealed that in both experiments (mPFC and Nacc) the offspring of poly I:C dams had a significant decrease in prepulse inhibition when compared to offspring of their saline treated counterparts. This deficit was normalized after DBS of either the mPFC or the Nacc (Fig. 2). In addition, Nacc-DBS (Fig. 2b) but not mPFC-DBS (Fig. 2a) induced a deficit in PPI in the offspring of saline treated dams.

3.1.2. Latent inhibition—Experiments were conducted in 153 rats, 97 Nacc DBS and 56 mPFC DBS. Nine of these rats (8 in the former and 1 in the latter treatment) were excluded from the study due to postoperative death or inaccurate electrode placements. At the end,

Nacc groups had $n = 10\text{--}12$ rats, whereas those in mPFC experiments had $n = 6\text{--}8$ animals each. In both experiments (mPFC and Nacc), there were no differences in the time to complete licks 51–75 before the presentation of the tone (all p 's > 0.05 ; overall mean A periods = 8.57 sec for mPFC and Nacc). Figs. 3a and b present the mean log times to complete licks 76–100 (after tone) in mPFC/sham and Nacc/sham stimulated rats, respectively. LI was present in the sham offspring of saline-treated dams but was absent in the sham offspring of poly I:C-treated dams. Nacc-DBS restored LI in the offspring of poly I:C-treated dams but disrupted LI in the saline offspring (significant main effect of pre-exposure ($F(1.81) = 5.973$, $p < 0.02$); significant pre-exposure $\times$ maternal treatment $\times$ stimulation interaction ($F(1, 81) = 7.845$, $p < 0.01$; significant difference between the PE and NPE groups, i.e., presence of LI, in the saline-sham ($p < 0.04$) and in the poly I:C-DBS groups ($p < 0.01$), but not in the saline-DBS and poly I:C-sham groups) in post hoc comparisons). mPFC-DBS restored LI in poly-I:C offspring without affecting LI in controls (main effect of pre-exposure ($F(1.47) = 25.36$, $p < 0.001$); significant pre-exposure $\times$ maternal treatment $\times$ stimulation interaction ($F(1, 47) = 8.196$, $p < 0.01$); significant difference between the PE and the NPE groups, i.e., presence of LI, in the saline-sham ($p < 0.01$), saline-DBS ($p < 0.01$) and the poly I:C-DBS groups ($p < 0.01$), but not in the saline-DBS and poly I:C-sham groups in post hoc comparisons).

3.1.3. Neuroimaging—After exclusion of 7 rats due to electrode misplacement, analysis was conducted in a total of 33 animals (mPFC: $n = 7$ saline, $n = 9$ poly I:C; Nacc: $n = 9$ saline, $n = 8$ poly I:C). In the offspring of saline treated dams, mPFC-DBS increased activity in the parietal cortex and ventral hippocampus (R: $t = 13.28$, $p < 0.001$; L: $t = 6.09$, $p < 0.001$), striatum and NAcc (R: $t = 9.06$, $p < 0.001$; L: $t = 6.09$, $p < 0.001$) and decreased it in brain stem regions including the PAG ($t = 6.12$, $p < 0.001$), vestibular nuclei/locus coeruleus ($t = 8.47$, $p < 0.001$), the hypothalamus ($t = 3.27$, $p = 0.003$) and cerebellum ($t = 4.28$, $p < 0.001$, Fig. 4a). Nacc-DBS increased activity recorded in the left ventral hippocampus ($t = 4.86$, $p < 0.001$) and olfactory bulb ($t = 4.64$, $p < 0.001$). Nacc-DBS reduced metabolism in the septal area ($t = 3.51$, $p = 0.002$), hypothalamus ($t = 3.16$, $p = 0.003$) and, similar to mPFC-DBS, in the brainstem, including the PAG and the right vestibular nuclei/locus coeruleus ($t = 3.41$, $p = 0.002$, Fig. 4b). In the offspring of poly I:C treated dams, effects were less pronounced: mPFC-DBS increased activity in the same structures than in the offspring of saline treated dams, namely the parietal cortex and ventral hippocampus (R: $t = 6.99$, $p < 0.001$; L: $t = 5.31$, $p < 0.001$), striatum and NAcc (R: $t = 6.55$, $p < 0.001$; L: $t = 5.31$, $p < 0.001$) and OB ($t = 6.55$, $p < 0.001$). mPFC-DBS decreased activity in the left brainstem ($t = 4.67$, $p < 0.001$) and hypothalamus ($t = 3.28$, $p = 0.003$, Fig. 5a) We did not find any significant decrease or increase in brain glucose metabolism in poly I:C rats given Nacc-DBS (Fig. 5b).

4. Discussion

As previously reported, offspring of dams exposed to poly-I:C showed deficits in sensorimotor gating (i.e. disrupted PPI) and attentional selectivity (i.e. disrupted LI). DBS applied to either the mPFC or the Nacc alleviated LI and PPI deficits. These findings extend previous observations as we have recently demonstrated that mPFC-DBS transiently

improved PPI in the adult offspring of poly I:C dams (Klein et al., 2013). Ma and Leung (2014) found that rats given stimulation to the Nacc at 130 Hz had an attenuation in ketamine-induced PPI deficits and hyper-locomotion, while Angelov and colleagues found that Nacc DBS was not effective in rats with breeding-induced low PPI (Angelov et al., 2014). When considered in light of our present data, these findings suggest that mPFC and Nacc may be a suitable DBS targets for schizophrenia-like behavior. The equivalent effects following DBS in different targets suggest a similar mechanism of action in phenotypic rats. In contrast, DBS of the Nacc and the mPFC exerted different effects in controls. While the latter did not affect the offspring of saline treated dams, Nacc-DBS disrupted both PPI and LI in these animals. This discrepancy is similar to what has been reported for pharmacological treatments with antipsychotics, which also improve behavior in schizophrenia-like animals while impairing it in controls (Barak and Weiner, 2011; Meyer et al., 2010). This suggests that the effects of therapeutic interventions may be dependent on the neurobiological properties of activated brain circuits, previously found to be abnormal in poly I:C rats.

DBS at the high frequencies conducted in our study is known to reduce activity of neuronal populations in the targeted region while activating axons in the vicinity of the electrodes (Florence et al., 2015; Hamani and Temel, 2012). If the former mechanism was the main driver of our DBS results, one could expect lesions of PFC or Nacc to induce similar behavioral effects. In fact, both PFC lesions and DBS are ineffective in naive rats (Joel et al., 1997; Lacroix et al., 1998), whereas Nacc shell lesions and DBS disrupt LI (Jongen-Relo et al., 2002; Weiner et al., 1996). Studies comparing brain lesions and DBS have not been conducted in phenotypic poly I:C offspring. Here, we used FDG-PET as an in vivo technique to investigate the functional consequences of delivering DBS (Klein et al., 2013) in structures at a distance from the target. When looking at the effects of DBS in the offspring of saline-treated rats, we were basically assessing the metabolic pattern influenced by stimulation in awake, freely moving animals. We found that, in the offspring of saline treated dams, both mPFC and Nacc-DBS reduced activity in brainstem regions, particularly the regions including the PAG and vestibular nuclei/locus coeruleus. Projections from mPFC to the brainstem are far more pronounced than those from the ventral striatum (Gabbott et al., 2005). As the Nacc and mPFC have strong reciprocal connections (particularly the PL and Nacc) (Hamani et al., 2011), this may suggest that the recruitment of brainstem pathways after DBS in both targets could have occurred due to the modulation of similar circuits, probably involving the mPFC. Besides this common effect on brainstem activity, we found that DBS in the mPFC and Nacc recruited different brain structures and circuits in offspring of saline treated dams. This is similar to previous descriptions in which metabolic mapping was recorded with in situ hybridization (Hamani et al., 2014a). In our study, stimulation of the mPFC increased activity in the parietal cortex, striatum, ventral hippocampus and Nacc, structures that are inter-connected. Of those, however, the ventral hippocampus and the Nacc receive a strong direct input from the mPFC (Gabbott et al., 2005), suggesting that the activation of the remainder areas was likely polysynaptic. Nacc-DBS on the other hand, increased activity in the ventral hippocampus and olfactory bulb, while reducing metabolism in the septal area and hypothalamus. Such effects were likely resultant from the activation of either mono or polysynaptic pathways. These differences in

network pattern may help to explain why behavior in LI and PPI worsens in control animals receiving Nacc but not mPFC-DBS.

To our surprise, the metabolic effects of DBS were less striking in the offspring of poly I:C treated dams than in the saline group. After mPFC-DBS, activity was modulated in almost the same regions as those in the offspring of saline treated dams, but the effects were less pronounced, with increases in the parietal cortex, ventral hippocampus, striatum, olfactory bulb and Nacc and decreases in the brainstem and hypothalamus. Nacc stimulation, on the other hand, did not result in any significant changes (activation/deactivation) in brain glucose metabolism. These findings highlight that DBS of the same target affects not only behavior but also brain network activity, depending on the neurobiological properties. In the present study, DBS was given to the adult progeny of poly I:C treated dams – that is – animals that already present a schizophrenia-like phenotype. As we have previously published, these animals have an abnormal pattern of glucose metabolism with lower activity in the ventral hippocampus, cortex and PFC and higher metabolism in the amygdala and Nacc in comparison to controls (Hadar et al., 2015). Though not direct conclusions may be derived from our data, one possibility is that mPFC-DBS may exert its beneficial behavioral effects in poly I:C offspring by normalizing abnormal activity in the ventral hippocampus and frontal area. That said, Nacc DBS improved behavior but did not induce these same metabolic effects. In this study, we could not isolate a common metabolic pattern that could explain the equivalent behavioral effects of mPFC- and Nacc-DBS. The fact that Nacc-DBS did not significantly affect brain metabolism in poly I:C offspring strengthens the notion that mPFC and Nacc-DBS not only operate different neurobiological circuits but also different neurobiological mechanisms.

The goal in this study was to assess whether stimulation applied while the animals were performing the LI and PPI tasks could reverse impaired behavioral and underlying neurobiological patterns. Such design was selected as it would mimic a clinical scenario in which schizophrenic patients with overt clinical symptoms received DBS. From a translational perspective, results from our study are not without limitations. Clinical symptoms of the disease are fairly complex and broad, ranging from disruptions in thought process, perception, memory, and mood. Deficits in the PPI and LI as measured here only indicate an inability of the animals in sensorimotor gating and attentional selectivity. That said, maternal immune stimulation models have many features that resemble those of schizophrenia-like states. Animals undergo neurodevelopmental changes following the immune stimulation of their mothers by the administration of bacterial/viral-like products (Meyer et al., 2010; Piontkewitz et al., 2011). The offspring of poly I:C rats develop volumetric changes in brain structures that are similar to those compromised in schizophrenia (e.g. decrease in hippocampal and increase in ventricular volumes) (Piontkewitz et al., 2011; Piontkewitz et al., 2009). Animals have a decrease in neurogenesis (Piontkewitz et al., 2012b) and high levels of dopamine in some parts of the brain (Hadar et al., 2015). Behavioral and neurochemical deficits cannot be appreciated in adolescent animals, only being observed during late adolescence/adulthood (Meyer et al., 2010; Piontkewitz et al., 2011). Treatment with antipsychotics improves performance in the PPI and LI and counters some of the neurochemical abnormalities recorded in these animals (Meyer et al., 2010; Piontkewitz et al., 2011; Piontkewitz et al., 2009; Piontkewitz et al.,

2012b). If one considers that the behavioral effects of DBS parallel those of antipsychotics in poly I:C rats, a question that emerges is whether stimulation could be useful in humans. In different psychiatric applications of DBS (e.g. depression, obsessive-compulsive disorder, anorexia nervosa, drug addiction) stimulation of the nucleus accumbens and subgenual cingulum (region that is analogous to the rodent ventral mPFC) have been proven to be safe (Hamani et al., 2014b; Kuhn et al., 2014; Lipsman et al., 2013; Mayberg et al., 2005; Schlaepfer et al., 2008). In clinical trials though, patients with treatment resistant psychiatric disorders are included, while our study was carried out in animals not previously selected for therapy-resistance. The chosen strategy consequently more closely resembles a scenario in which DBS is offered as a first line intervention. The issue of treatment resistance is complex to be address in animals with not many valid preclinical models available to mimic this scenario (especially in schizophrenia). In our particular case, an alternative would have been to treat poly I:C offspring with antipsychotics, select those that did not respond, and have them treated with DBS. This, however, would require a very large number of animals (as most rats would in theory present some degree of response to the drugs) and faces the challenge of defining “treatment-responsive” vs. “treatment-resistant” rats at the level of individual animals. In addition, selected behavioral measurements would have to be suitable for test/re-test, as they would be serially recorded in the same rats over time. While treatment-resistance models are certainly needed, they would be premature at a stage when the potential capacity of a new intervention to affect a specific pathology needs to be tested, as was the case here. Now that we have established that DBS of mPFC and Nacc is capable of targeting and modulating the neural pathways/substrates of PPI and LI and reversing their disruptions, conducting more challenging studies in treatment-resistant rats is a reasonable step for future investigation. Despite the caveats described above, our results suggest that DBS could be useful for treating deficits in sensorimotor gating and attentional selectivity that characterize the disease. Whether further symptoms relevant to schizophrenia may respond to stimulation needs to be tested.

In summary our study shows that mPFC or Nacc-DBS delivered to the adult progeny of poly I:C treated rats improves deficits in PPI and LI. Despite common behavioral responses, stimulation in these two targets induces different metabolic effects. This study adds to the growing literature suggesting that electrical stimulation of specific brain regions improves behavioral deficits in animal models of psychiatric disorders.

Acknowledgments

We thank Renate Winter and Christiane Koelske for excellent technical assistance. This research was conducted under the EraNet Neuron framework (DBS_F20rat) and supported by the Federal Ministry of Education and Research, Germany (BMBF 01EW1103), the Ministry of Economy and Competitiveness ISCIII-FIS grants (FIS PI14/00860, CPII14/00005) co-financed by ERDF (FEDER) Funds from the European Commission, “A way of making Europe”, Fundación Mapfre and Comunidad de Madrid (S2013/ICE-2958), Spain, the Chief Scientist Office of the Ministry of Health, Israel (3-8580) and the Canadian Institutes of Health Research (FRN 110068). RH was financed by the German Research Foundation (DFG PAK 591 (WI 2140/2-1).

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Fig. 1.

Electrode placements. (a) Schematic reconstruction of electrode placements in either the mPFC (left) or Nacc (right), histologically verified in rats undergoing PPI (grey circles) and LI (black circles: PE; white circles: NPE) experiments. (b) Representative CT scan showing electrode positioned in either the mPFC (upper) or Nacc (bottom).

Fig. 2.

Effects of DBS on PPI with averaged PPI values across three different prepulse intensities in the offspring of saline and poly I:C treated rats: For each brain area (mPFC (a) and Nacc (b)), animals underwent three test sessions 24 h apart. Session 1 (baseline 1) was conducted with no stimulation applied. Session 2 was conducted while animals were receiving active DBS. Session 3 (baseline 2) was carried out with DBS turned off to assess whether the observed effects in Session 2 were permanent or transient. DBS in either the mPFC or Nacc significantly decreased PPI deficits in poly I:C offspring. In controls, while no changes were observed after mPFC-DBS, stimulation of the Nacc has induced significant PPI deficits. (*) Significantly different from baseline 1 within groups. (§) Significant differences between groups (saline vs. poly I:C). Results are presented as means $\pm$ S.E.M.; *, § $p < 0.05$.

Fig. 3.

Effects of DBS on LI in the offspring of saline and poly I:C treated dams: (a) mPFC-DBS reversed prenatal poly I:C-induced deficits in LI without affecting LI in controls. (b) Nacc-DBS improved prenatal poly I:C-induced LI deficits while deteriorating this behavior in controls. Data represent mean $\pm$ S.E.M of log times to complete licks 76–100 (after tone onset) of pre-exposed (PE) and non-pre-exposed (NPE) offspring of saline- or poly I:C treated dams that did (DBS) or did not receive DBS (sham). (*) Significant differences between PE and NPE groups, namely presence of LI.

Fig. 4. Effects of DBS on brain metabolism in the offspring of saline treated dams. Colored PET overlays on MR reference indicate increased ^{18}F -FDG uptake (hot colors) or decreased (cold colors) glucose metabolism for DBS treated rats compared to sham rats. (a) mPFC-DBS in saline rats increased metabolic activity in the striatum (ST), ventral hippocampus (vHipp), parietal cortex (PC) and Nacc, and reduced it in the brainstem (BS), periaqueductal gray matter (PAG) hypothalamus (hypoth) and cerebellum (Cb). (b) Nacc-DBS in saline rats increased metabolic activity in the vHipp and olfactory bulb (OB), and decreased FDG-uptake in the septal area (SA), hypoth, BS and PAG. (c) Table summarizes statistical group differences for the respective regions of interest (ROI) and brain sides (right: R, left: L).

Fig. 5.

Effects of DBS on brain metabolism in the offspring poly I:C treated dams. Colored PET overlays on MR reference indicate increased ^{18}F -FDG uptake (hot colors) or decreased (cold colors) glucose metabolism for DBS treated rats compared to sham rats. (a) mPFC-DBS in poly I:C rats increased metabolic activity in the straitum (ST), ventral hippocampus (vHipp), parietal cortex (PC) and Nacc, and decreased FDG-uptake in the brainstem (BS) and hypothalamus (hypoth). (b) Nacc-DBS in poly I:C rats did not induce any significant metabolic change in the brain. (c) Table summarizes statistical group differences for the respective regions of interest (ROI) and brain sides (right: R, left: L).