This is a postprint version of the following published document:

Hadar, R., … et al. (2018). Early neuromodulation prevents the development of brain and behavioral abnormalities in a rodent model of schizophrenia. *Molecular Psychiatry*, 23(4), 943–951.

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

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

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Abstract

The notion that schizophrenia is a neurodevelopmental disorder in which neuropathologies evolve gradually over the developmental course indicates a potential therapeutic window during which pathophysiological processes may be modified to halt disease progression or reduce its severity. Here we used a neurodevelopmental maternal immune stimulation (MIS) rat model of schizophrenia to test whether early targeted modulatory intervention would affect schizophrenia's neurodevelopmental course. We applied deep brain stimulation (DBS) or sham stimulation to the

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medial prefrontal cortex (mPFC) of adolescent MIS rats and respective controls, and investigated its behavioral, biochemical, brain-structural and -metabolic effects in adulthood. We found that mPFC-DBS successfully prevented the emergence of deficits in sensorimotor gating, attentional selectivity and executive function in adulthood, as well as the enlargement of lateral ventricle volumes and mal-development of dopaminergic and serotonergic transmission. These data suggest that the mPFC may be a valuable target for effective preventive treatments. This may have significant translational value, suggesting that targeting the mPFC before the onset of psychosis via less invasive neuromodulation approaches may be a viable preventive strategy.

INTRODUCTION

The neurodevelopmental model of schizophrenia postulates that an interplay between environmental insults and genetic factors might lead to the initiation of pathological processes that result in a cascade of neural abnormalities. These might eventually give rise to profound disruptions in cognition and emotion, which typically first emerge in late adolescence.[1,2] This post-natal delay is a marked feature of schizophrenia; however, the exact course of neural mal-development and its relation to disease outbreak are not fully understood. As a result, there is a growing interest in studying schizophrenia-specific pathophysiological processes in neurodevelopmental animal models. One such model is the maternal immune stimulation (MIS) model in which the exposure of pregnant rodents to the viral mimic polyriboinosinic–poly-ribocytidylic acid (poly I:C) results in the emergence of schizophrenia-relevant behavioral abnormalities. Some of these include deficits in sensorimotor gating, as reflected in decreased pre-pulse inhibition (PPI), attentional selectivity, as reflected in disrupted latent inhibition (LI) and executive function as measured in the discrimination reversal (DR) paradigm. Importantly, behavioral abnormalities recapitulate the maturational delay of schizophrenia and are preceded by neuropathological alterations in schizophrenia-relevant brain circuits.[3–5]

Taken together, human and animal data indicate that schizophrenia is a neurodevelopmental disorder in which maturational abnormalities during adolescence eventually lead to overt symptomatology. This in turn implies the existence of a potential therapeutic window during which pathophysiological processes may be modified to either halt disease progression or reduce its severity. Consequently, research has focused on the prodromal or high-risk stage for psychosis to identify biological markers, and evaluate preventive intervention avenues. To this end, the pre-symptomatic administration of antipsychotic drugs (APDs) has yielded encouraging results in humans and in MIS rats:[6–10] In a placebo controlled double-blind study, the administration of the atypical antipsychotic olanzapine over a period of 8 weeks to individuals at high risk for psychosis resulted in a significantly greater symptomatic improvement as measured by scale of prodromal symptoms and positive and negative syndrome scale rating scales.[6] When tested for a much longer period, that is, 1 year, the likelihood of conversion to psychosis was reduced in olanzapine treated individuals with a nearly significant difference from the control group.[7] Using the MIS model various agents were given during the pre-symptomatic stage, that is, between post-natal days 35–47 in rats and 35–65 in mice. These studies demonstrated that the administration of APDs haloperidol, clozapine and risperidone as well as the antidepressant fluoxetine prevented the emergence

of multiple psychosis-related behavioral, pharmacological and anatomical abnormalities.[9–11] Given the anatomical characteristics of schizophrenia pathology, a question arises as to whether spatial-specific interventions that modulate specific anomalous neural circuits would attain the same effect and in turn promote the development of treatment strategies that target the underlying pathophysiological mechanisms of schizophrenia.

Electro-magnetic brain stimulation techniques modulate brain activity facilitating anatomically targeted therapeutic strategies. Among these, deep brain stimulation (DBS) has the greatest specificity. It is approved for the treatment of various movement disorders and under investigation for the treatment of different psychiatric disorders. In addition, DBS provides a powerful investigative tool to study the interrelation between the neurobiological state within a specific circuit and behavioral outcome. In this context, altered prefrontal cortex (PFC)-circuits are considered fundamental to the development of schizophrenia.[12–14] Previously, we have shown that medial PFC (mPFC)-DBS applied to phenotypic MIS rats normalizes behavioral deficits with relevance to schizophrenia.[15,16] Thus, here we sought to study whether mPFC-DBS given to behaviorally inconspicuous adolescent MIS rats would prevent the manifestation of relevant behavioral and neurobiological deficits in adulthood.

MATERIALS AND METHODS

Animals

Rats were housed in a temperature- and humidity- controlled vivarium with a 12-h light–dark cycle with food and water *ad libitum* (unless otherwise stated). Experiments were performed according to the guidelines of the European Union Council Directive 2010/63/EU for care of laboratory animals and after approval by the local ethic committees (Regierungspräsidium Dresden, Germany (PPI, high performance liquid chromatography); Tel Aviv University, Israel (LI, DR, magnetic resonance imaging (MRI)); Ethics Committee for Animal Experimentation of Hospital Gregorio Marañón, Madrid, Spain (fluorodeoxyglucose positron emission tomography (FDG-PET)).

Experimental design

Wistar rats (Harlan Laboratories, Venray, The Netherlands and Jerusalem, Israel) were mated and the first day after copulation was defined as day 1 of pregnancy. On gestation day 15, dams were given a single injection to the tail vein of either poly I:C (4 mg/kg; Sigma, Munich, Germany) dissolved in saline, or saline alone under isoflurane anesthesia.[3,4] On post-natal day (PND) 21, pups were weaned and housed by sex and litter. On PND 33–34, electrodes were implanted. DBS/sham-stimulation began on PND 35 and was delivered continuously until PND 47. Behavioral and neurobiological analyses were conducted at PND > 90. Three experiments were designed with each experimental group comprising male offspring from multiple independent litters. Experiment 1 tested the effects of mPFC-DBS on PPI of the acoustic startle response followed by post-mortem neurochemical assessment in 35 rats (saline-sham: $n = 8$; saline-DBS: $n = 10$; poly-sham: $n = 8$; poly-DBS: $n = 9$) derived from 15 litters (8 saline, 7 poly). Experiment 2 studied the effects of mPFC-DBS on LI in 69 rats (saline-sham pre-exposed (PE): $n = 12$; saline-sham non-pre-exposed (NPE): $n = 8$; saline-DBS-PE: $n = 8$; saline-DBS-NPE: $n = 7$; poly-sham-PE: $n = 10$; poly-sham-NPE:

$n = 8$; poly-DBS-PE: $n = 9$; poly-DBS-NPE: $n = 7$) and 1 week later on DR in 32 rats (saline-sham: $n = 8$; saline-DBS: $n = 8$; poly-sham: $n = 8$; poly-DBS: $n = 8$) derived from 26 litters (12 saline, 14 poly). Thereafter, brains (saline-sham: $n = 10$; saline-DBS: $n = 7$; poly-sham: $n = 13$; poly-DBS: $n = 8$) were subjected to *ex vivo* MRI. Experiment 3 tested the effects of mPFC-DBS on brain-metabolic changes using FDG-PET in 29 rats (saline-sham: $n = 6$; saline-DBS: $n = 8$; poly-sham: $n = 8$; poly-DBS: $n = 7$) derived from 13 litters (6 saline, 7 poly).

In all experiments the allocation of animals to their experimental groups was done randomly in an a priori design manner. Experimenters were blind to the identity of animals when conducting the experiments.

Early continuous DBS

Stereotactic surgeries were conducted under balanced anesthesia (Fentanyldihydrogencitrat 0.005 mg/kg, Midazolamhydrochlorid 2.00 mg/kg, Medetomidin 0.15 mg/kg) or with isoflurane (Nicholas Piramal, Northumberland, UK) followed by an i.p. injection of avertin (20 ml/kg). Monopolar platinum iridium electrodes (E363/6/SP, Plastics1) were bilaterally implanted at AP: +3.2 mm, *L*: 0.7 mm, *V*: − 3.3 mm (Paxinos and Watson, 1998)[17] and plugged into a socket together with a screw ground electrode (E363/20/SP, Plastics1). The assembly was fixed using dental acrylic cement (Technovit Heraeus-Kulzer, Hanau, Germany). Upon completion, rats were dressed with rodent jackets. On PND 35, electrode pedestals were connected to a microstimulator,[18] devices were attached to the jackets, stimulation was initiated and continuously delivered for 12 days at 130 Hz, 150 μA biphasic pulses, 90 μs pulse duration. On PND 47, stimulators and jackets were removed. Sham-stimulated rats were treated the same way as DBS-rats, including being connected to the devices and wearing jackets, without receiving electrical stimulation.

Behavior

Pre-pulse inhibition—PPI is a cross-species phenomenon measuring sensorimotor gating. Reduced PPI reflects gating deficits seen in and relevant to schizophrenia.[19] PPI of the acoustic startle response was measured in a sound-attenuated chamber (Startle Response System, TSE, Bad Homburg, Germany) equipped with a wire mesh cage mounted on a transducer-platform and two loudspeakers.[3,15,20] Experiments consisted of a 5 min acclimatization phase and the test session. Throughout the experiment, background noise was set at 60 dB sound pressure level. During acclimatization, animals received 10 initial startle stimuli (100 dB sound pressure level, white noise, 20 ms). The test session consisted of seven different trial types delivered each 10 times in a pseudorandom order with an inter-trial interval of 20 to 30 s: (1) startle-pulse alone (100 dB sound pressure level white noise, 20 ms), (2) control (no stimulus), (3 and 4) pre-pulse alone (72/68 dB, pure tone, 10 kHz, 20 ms); (5–7) pre-pulse (72/68/64 dB) each followed by a startle-pulse with an inter-stimulus interval of 100 ms. PPI was calculated according to the formula 100–100% × (mean acoustic startle response of PPI-trials/mean acoustic startle response of pulse-alone-trials). For analysis, the average PPI response over the three pre-pulse intensities was taken.[3,15,20,21]

Latent inhibition—LI is a cross-species selective attention phenomenon, reflecting the normal attentional bias to ignore stimuli that were experienced as irrelevant in the past. Disrupted LI reflects attentional overswitching and distractibility considered relevant to positive symptoms of schizophrenia.[22] LI was measured in a thirst-motivated conditioned emotional response procedure by comparing the suppression of drinking to a tone previously paired with a foot-shock in rats that either received non-reinforced exposure to the tone before conditioning (PE) or for which the tone was novel (NPE). Water-deprived rats (23 h/ day; 5 days) were trained to lick in experimental chambers (5 days). LI procedure consisted of four stages given 24 h apart: Pre-exposure: bottles were removed from chambers. PE rats were presented with 40 tones (10 s, 80 dB, 2.8 kHz) given 40 s apart while NPE rats were not exposed to tones. Conditioning: bottles were removed from chambers. Rats received two tone-shock (0.5 mA, 1 s duration) pairings given 5 min apart. Lick retraining test: rats were allowed to drink from the bottle in chambers. Upon completing 75 licks, a tone was presented for 5 min. Time to complete licks 51–75 (before tone) and licks 76–100 (after tone) was recorded. The latter was logarithmically transformed for parametric analysis of variance. LI was defined as significantly shorter log times to complete licks 76–100 of PE compared with NPE rats.

Discrimination reversal—DR is a cross-species phenomenon reflecting the ability of an organism to change behavior in the face of changing contingencies. Abnormally rapid DR is a manifestation of excessive switching to respond according to the current stimulus-reinforcement contingency/ies and therefore considered relevant to positive symptoms of schizophrenia.[23] DR was assessed in a T-maze that had a hidden platform (15.5 × 15.5 cm) in one of the arms and was submerged in a swimming pool.[23] On the first day (position discrimination) rats were trained to acquire left–right position discrimination with the platform consistently positioned in one of the arms. Rats were allowed to choose between arms. Once it entered an arm, a door was lowered. If the correct arm was chosen, the rat was allowed to remain on the platform for 5 s, if the wrong one was chosen, the rat was confined to the arm for 5 s. Thereafter rats were taken to a holding cage for a 10 s inter-trial interval. Training continued until a criterion of five consecutive correct trials was reached. On the next day (reversal), rats were first retrained until criterion on the position discrimination of the first day was reached, and then trained until reaching the criterion on the reversal of this discrimination, that is, with the platform located in the opposite arm. The number of trials to reach the criterion was recorded in both stages.

Post-mortem neurochemistry

After completion of PPI experiments, biochemical properties were investigated using post-mortem high performance liquid chromatography.[3,24,25] Rats were decapitated and micropunches were taken from 0.5–1 mm thick brain slices from mPFC, nucleus accumbens (Nacc), caudate putamen (CPu), globus pallidus (GP) and hippocampus (Hipp) at the following coordinates with reference to Bregma: mPFC: +3.2 to +2.2; Nacc and CPu: +1.7 to +0.7; GP: − 0.8 to − 1.3; Hipp: − 2.1 to − 3.1.[3,25] Monoamines (dopamine (DA), serotonin (5-HT)) and their metabolites (DOPAC, 5-HIAA)[3,25] were separated on a column (ProntoSil 120-3-C18-SH; Bischoff Analysentechnik und -geräte GmbH, Germany) and

electrochemically detected (41000, Chromsystems Instruments & Chemicals, Gräfelfing, Germany).

Ex vivo MRI

After completion of LI and DR experiments, rats were perfused transcardially with 4% paraformaldehyde. MRI was performed on a 7.0T/30 spectrometer (Bruker, Rheinstetten, Germany) using a volume coil for excitation and a rat quadrature coil for acquisition. Axial T2-weighted images were acquired with RARE sequence with the following parameters: repetition time (TR)/echo time (TE) = 5400/12.5 ms, RARE factor = 8, number of averages (NA) = 4, flip angle = 180, field of view of 36×27 mm^2, in-plane resolution of 0.281×0.281 mm^2 and 22 axial slices of 0.281 mm thickness. Anatomical borders[26] used to draw the contour around lateral ventricles (LV) are presented in Figure 1. The area of the LV was extracted using manual segmentation (Medical Image Analysis version R2013b MATLAB) from 10 consecutive slices. LV volumes were calculated by combining areas of all slices multiplied by the resolution (voxel size: 0.281 [ref. 3]). The acquisition and analysis of the MRI data were performed by two experienced and blinded external operators.

FDG-PET

[^{18}F] FDG was injected into the tail vein and, after a 45 min uptake period, animals were scanned for 45 min under isoflurane anesthesia using a small-animal PET/computed tomography (CT) scanner (ARGUS PET/CT, SEDECAL, Madrid, Spain). Images were reconstructed using a 2D OSEM (ordered subset expectation maximization algorithm) with a spatial resolution of 1.45 mm full width at half maximum, a voxel size of $0.3875 \times 0.3875 \times 0.7750$ mm and an energy window of 400–700 keV. CT studies were acquired with the following parameters: 320 mA, 45 KV, 360 projections, 8 shots, and 200 μm of resolution and reconstructed using a Feldkamp algorithm (isotropic voxel size: 0.121 mm). One un-operated rat was additionally scanned using a 7-Tesla Biospec 70/20 MRI scanner (Bruker) to provide anatomical templates. A T2-weighted spin echo sequence was acquired (TE = 33 ms, TR = 3732 ms, 34 slices of 0.8 mm, matrix size: 256×256 pixels at an field of view of 3.5×3.5 cm^2). PET images were co-registered for voxel-by-voxel comparisons statistical parametric maps. A random reference CT scan was selected (CT$_{ref}$) and all studies were co-registered with it using an automatic rigid registration method. The spatial transformation obtained for each CT was applied to the corresponding PET image. The MRI study was spatially co-registered to the CT$_{ref}$. A brain mask segmented on this MRI study was applied to PET images. Resulting images were smoothed and voxel values were normalized to the average brain intensity.[27] The acquisition of PET data was random across groups, investigators were blinded to treatment, sham and DBS animals were always scanned on the same day, data analysis was automated and images processed at the same time.

Statistical analysis

PPI and biochemical data were analyzed using two way analysis of variance (ANOVA; factors: MIS × neuromodulation) followed by Holm-Sidak *post hoc* tests. LI and DR data were analyzed with three-way ANOVA (factors: MIS × neuromodulation × pre-exposure for LI/repeated factor of stage for DR) followed by least significant difference *post hoc* comparisons. Significance was set at $P < 0.05$. PET data were analyzed using SPM12

(Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, London, UK). Groups were compared using a multifactorial ANOVA[28,29] with a significance threshold set at $P < 0.05$. In compliance with the ethical demand to minimize the number of animals used, statistics were not corrected for multiple comparisons. Although this leads to a reduction in power, it prevents an underestimation of the statistical significance. To reduce type I error, a 50-voxel clustering threshold (spatial-extent) was applied.

RESULTS

mPFC-DBS in adolescent MIS rats prevented the manifestation of PPI deficits in adulthood

Rats showed no measurable reaction in pre-pulse alone trails and no differences in startle reaction in startle pulse-alone-trials across groups (saline_sham: 563.58 ± 169.22, saline_DBS: 322.33 ± 69.79, poly_sham: 640.13 ± 186.29, poly_DBS: 449.85 ± 189.86; MIS: $F_{(1,31)} = 0.42$, $P = 0.51$, neuromodulation: $F_{(1,31)} = 1.90$, $P = 0.17$, MIS × neuromodulation: $F_{(1,31)} = 0.02$, $P = 0.87$). In contrast, disrupted PPI was found in sham-stimulated poly I:C-offspring. Disruption was prevented following mPFC-DBS. MIS × neuromodulation interactions ($F_{(1,31)} = 14.84$, $P < 0.001$) and *post hoc* comparisons revealed significant differences between sham-stimulated poly I:C- and saline-offspring as well as between sham and DBS conditions in poly I:C-offspring ($P < 0.05$; Figure 2a).

mPFC-DBS in adolescent MIS rats prevented LI disruption in adulthood

LI was absent in the sham-stimulated poly I:C-offspring. mPFC-DBS prevented disruption of LI in poly I:C-offspring. ANOVA yielded significant main effects for pre-exposure ($F_{(1,61)} = 36.74$, $P < 0.001$) and neuromodulation ($F_{(1,61)} = 5.26$, $P = 0.025$), as well as a significant pre-exposure × MIS × neuromodulation interaction ($F_{(1,61)} = 4.21$, $P = 0.045$). *Post hoc* comparisons confirmed a significant difference between PE and NPE groups in saline-sham ($P < 0.01$), saline-DBS ($P < 0.01$) and in poly-DBS groups ($P < 0.01$), but not in poly-sham groups (Figure 2b).

mPFC-DBS in adolescent MIS rats prevented abnormally rapid reversal in adulthood

No difference between groups on *position discrimination* was found. *Reversal* slowed performance in all groups but the poly-sham group. Abnormally rapid reversal was prevented by mPFC-DBS. ANOVA yielded significant main effects of MIS ($F_{(1,56)} = 8.84$, $P = 0.006$), neuromodulation ($F_{(1,56)} = 6.96$, $P = 0.013$), and stage ($F_{(1,56)} = 87.06$, $P < 0.001$), and a significant MIS × neuromodulation × stage interaction ($F_{(1,56)} = 3.23$, $P = 0.047$). *Post hoc* comparisons confirmed that the poly-sham group required fewer trials to reach the reversal criterion than the other groups ($P < 0.0001$; Figure 2c).

mPFC-DBS in adolescent MIS rats partially prevented the development of biochemical abnormalities

When compared to sham-stimulated saline-offspring, sham-stimulated poly I:C-offspring showed increased DA contents in the Nacc and GP, decreased DA and DOPAC contents in the Hipp, decreased 5-HT contents in the mPFC, Hipp, CPu and GP as well as decreased 5-HIAA contents in the mPFC and Hipp.[3,25] mPFC-DBS affected neurotransmitter contents in all regions except the Nacc. In the mPFC, DBS increased DOPAC contents in saline- and

poly I:C-offspring ($F(1,24) = 4.20$, $P = 0.05$). Significant MIS × neuromodulation interactions and subsequent *post hoc* analyses further revealed decreased contents of 5-HT ($F(1,23) = 4.48$, $P = 0.045$) and 5-HIAA ($F(1,23) = 5.46$, $P = 0.029$) in the mPFC of poly-sham but not poly-DBS groups when compared to their respective saline-controls. A similar MIS × neuromodulation interaction was found for 5-HT ($F(1,31) = 5.22$, $P = 0.029$) and 5-HIAA ($F(1,31) = 4.57$, $P = 0.041$) in the Hipp. In the CPu, mPFC-DBS increased DA contents ($F(1,31) = 14.68$, $P = 0.011$) and in the GP it increased 5-HT contents ($F(1,25) = 12.27$, $P = 0.002$) in both saline- and poly I:C-offspring. A significant MIS × neuromodulation interaction ($F(1,24) = 4.71$, $P = 0.04$) and consecutive *post hoc* analysis further indicated that in the GP mPFC-DBS normalized pathologically high contents of DA in poly I:C-offspring (Figure 3, Table 1).

mPFC-DBS in adolescent MIS rats prevented the enlargement of LV volumes in adulthood

While sham-stimulated poly I:C-offspring had larger LV volumes than sham-stimulated saline-offspring, no differences in LV volumes were seen between poly I:C- and saline-offspring that received DBS. ANOVA yielded main effects of MIS ($F(1,34) = 6.755$, $P = 0.014$) and neuromodulation ($F(1,34) = 5.675$, $P = 0.023$). *Post hoc* comparisons yielded significant differences between the poly I:C-sham-stimulation and the other three conditions (all $P < 0.005$). This data, however, needs to be interpreted with caution as MIS × neuromodulation interaction was not significant.

mPFC-DBS in adolescent MIS rats did not have lasting effects on brain metabolism

In the offspring of saline treated dams, mPFC-DBS increased brain activity in the right thalamus ($T = 2.45$, $P < 0.011$) and decreased it in the left insular-piriform cortex ($T = 3.00$, $P = 0.003$). In the offspring of poly I:C treated dams, mPFC-DBS increased brain activity in the temporal cortex ($R: T = 2.67$, $P = 0.006$; $L: T = 2.07$, $P = 0.024$) (Figure 4).

DISCUSSION

To the best of our knowledge, this is the first study testing the feasibility of early targeted neuromodulation to halt the development of neuropathological development and behavioral deficits following prenatal insult. We studied how mPFC-DBS applied continuously during a sensitive period in adolescence affects deficits in selective attention, executive function and sensorimotor gating that emerge in adult rats following prenatal poly I:C exposure.[3,30] We found that mPFC-DBS successfully prevented the emergence of all of these deficits. Along with this prevention of behavioral deficits, mPFC-DBS also prevented the enlargement of LV volumes in poly I:C-offspring. Enlarged LVs is one of the structural hallmarks of schizophrenia[31] and its parallel manifestation coupled with the emergence of cross-species behavioral deficits in poly I:C-offspring contributes to the strong construct validity of this translational model.[30] Thus, the therapeutic efficacy of early neuromodulation of mPFC-circuits via mPFC-DBS substantiates the major pathophysiological relevance of the mPFC in the development of behavioral deficits phenotypic of schizophrenia.

Executive functions and sensorimotor gating are thought to be dependent on the mPFC[32,33] and indeed studies point to the involvement of the PFC in LI, DR and PPI. To this effect, it

was shown that regulation of LI depends on normal PFC GABAergic transmission[34] and that excitotoxic mPFC-lesions potentiate LI.[35] Further, pathologically enhanced reversal learning was shown to be rescued/normalized following brain-derived neurotrophic factor (BDNF) infusions into the mPFC[36] and facilitation of reversal learning was observed after excitotoxic mPFC-lesions.[36,37] Finally, excitotoxic mPFC-lesions and MK-801 microinjections into the mPFC were shown to disrupt PPI.[38–40] Adding on to these findings, we previously reported reduced brain metabolism and altered neurotransmission in the PFC of adolescent and adult MIS rats that point to an early and lasting hypo-frontality.[3] Likewise, decreased PFC-volumes were found in poly I:C-offspring that first emerged at late adolescence and continued into adulthood.[5] Together, these and our present data suggest that behavioral deficits in MIS rats result from an early impairment of the mPFC and that targeted interference with this early impairment rescues manifestation of behavioral and structural deficits.

On a brain-metabolic level, we previously showed that MIS rats display lower glucose uptake in the ventral Hipp and PFC and higher glucose uptake in the amygdala and Nacc.[3] Applying mPFC-DBS to adult MIS rats in an acute manner, that is, shortly before PET scanning, resulted in increased metabolic activity in the striatum, ventral hippocampus, Nacc and parietal cortex while decreasing it in the brainstem and hypothalamus.[16] In contrast, our present data indicate that early mPFC-DBS induces only minor effects on brain metabolism in both saline- and MIS- offspring; increased thalamic and decreased insular-piriform cortex activity were observed in saline offspring whereas only increased activity in the temporal cortex was found in MIS-offspring. The findings from the current study suggest that DBS effects on brain metabolism are transient in nature and may not be captured by FDG-PET conducted two months following neuromodulation.

On a biochemical level, adult poly I:C-offspring most prominently displayed increased levels of DA in the Nacc and the GP, and decreased levels of 5-HT in the mPFC, Hipp, CPu and GP. This corresponds to previous findings in this model[3,25] and is in agreement with the longstanding view of dopaminergic dysregulations contributing to schizophrenia-pathophysiology[41,42] as well as with numerous human studies pointing to deficits in serotonergic function in schizophrenia patients.[43] The most distinct biochemical effect of preventive DBS was related to DA in the GP such that it reduced pathologically high contents in poly I:C-offspring to the level found in saline-offspring. This is in line with the ameliorating effects of DBS on PPI, LI and DR, three behavioral paradigms reflecting the positive pole in schizophrenia which has been associated with hyperactivity of the subcortical dopaminergic system.[44] Furthermore, adult poly I:C-offspring that underwent early mPFC-DBS did not display decreased contents of 5-HT in the mPFC and the Hipp as their sham-stimulated counterparts did. It is known that DBS affects neurotransmission in the DBS-target itself as well as in projection sites[25,44–46] and that these effects depend on the neurobiological state.[43] Neurochemical effects of mPFC-DBS have so far been described for the Hipp and Nacc; *in vivo* measurements via microdialysis revealed increments of serotonergic transmission in both projection sites as a consequence of mPFC-DBS.[45,47] Together these findings imply that the remarkable behavioral effectiveness of early mPFC-DBS could be related to an early interference of PFC-neuromodulation with the mal-development of dopaminergic and serotonergic transmission in the mPFC, Hipp and GP.

Notably, the inefficacy of DBS in restoring the poly I:C-induced augmentation of dopamine in the nucleus accumbens may imply that such dopaminergic imbalances only play a minor mechanistic role in driving the behavioral abnormalities in this model. However, striatal dopamine dysfunction is well documented in poly I:C offspring and it has been shown to begin already in-utero.[48] An alternative possibility is that such augmentation does not drive the behavioral abnormalities on its own but rather in combination/interaction with additional dysfunctions, such as that of PFC, which fails to modulate the DA system throughout development.[5,30] We can further speculate that DBS given during an asymptomatic developmental stage reduces/prevents the PFC abnormality while not interfering with striatal/mesolimbic hyper-dopaminergia that on its own is insufficient for producing poly-I:C-induced behavioral abnormalities.

This notion is further corroborated by previous studies[9,10] which found that administration of atypical APDs during adolescence prevented structural and behavioral deficits similar to those found here, suggesting that systemic and targeted preventive interventions share a final common pathway that most likely comprises interferences with and restoration of pathological processes in the PFC-circuitry. As APDs have been shown to reduce intracellular DA contents,[49] one such common pathway may comprise a reduction of pathologically high DA in the GP. Considering the pharmacological profile of atypical APDs entailing a blockade of 5-HT receptors,[50] another pathway could be allied to 5-HT–DA interactions.

Further mechanisms mediating the preventive potency of mPFC-DBS in the context of schizophrenia might include synaptic plasticity and neuroprotection within the PFC-circuitry as these are considered to be influenced by DBS and relevant to schizophrenia pathology.[50–52] Pre-clinical studies using both primate and rat parkinsonian models in which protracted nigrostriatal degeneration is induced were able to demonstrate that DBS improved dopaminergic cell survival.[53–56] Other studies suggested that DBS might exert its neuroprotective potency via the induction of BDNF as well as the modulation of glial cell line-derived neurotrophic factor-family receptor gene expression.[57,58] A positive effect of DBS on neurogenesis was also found, resulting in an increment of hippocampal neurogenesis in the intact brain as well as reversing suppressed neurogenesis in corticosterone treated animals.[59] And finally, evidence for synaptic plasticity following DBS includes the induction of short-term potentiation, long-term potentiation and long-term depression.[60] All things considered, future experiments should aim to elucidate the exact underlying mechanisms of preventive mPFC-DBS in the context of schizophrenia.

Irrespective of the mechanisms underlying the present effects and given the central role of the PFC in schizophrenia neuropathology[61] and its involvement in cognitive impairments before onset of psychosis,[62] the therapeutic effectiveness of early neuromodulation of PFC-circuitries suggests that the PFC may be a relevant target for preventive therapeutic interventions in individuals at high risk for psychosis.

In summary, the goal of this work was to study the feasibility of targeted preventive treatment strategies for schizophrenia. The use of a well validated animal model enabled a controlled examination. Our findings suggest that the mPFC may be a suitable target for

effective preventive treatment. To this end, our findings may have significant translational value, suggesting that targeting the mPFC and related circuitries before the onset of psychosis via less invasive neuromodulation approaches may be a viable preventive strategy. Attempts have been made to modulate neural circuitry in schizophrenia patients using transcranial direct current stimulation.[63] The question now arises as to whether such non-invasive PFC-neuromodulation in high-risk individuals would similarly affect symptom emergence and therefore constitute a practicable approach for the preventive interference with schizophrenia neuropathological processes. Given the complexity of schizophrenia, its entire behavioral and neurobiological spectrum cannot be fully captured using a single animal model. For the present study, we have decided to work with the poly I:C MIS model given its strong construct and predictive validity as well as previous successes to achieve effective prevention in this model. Future studies using additional pre-clinical models will be necessary to even draw sounder conclusions.

Acknowledgments

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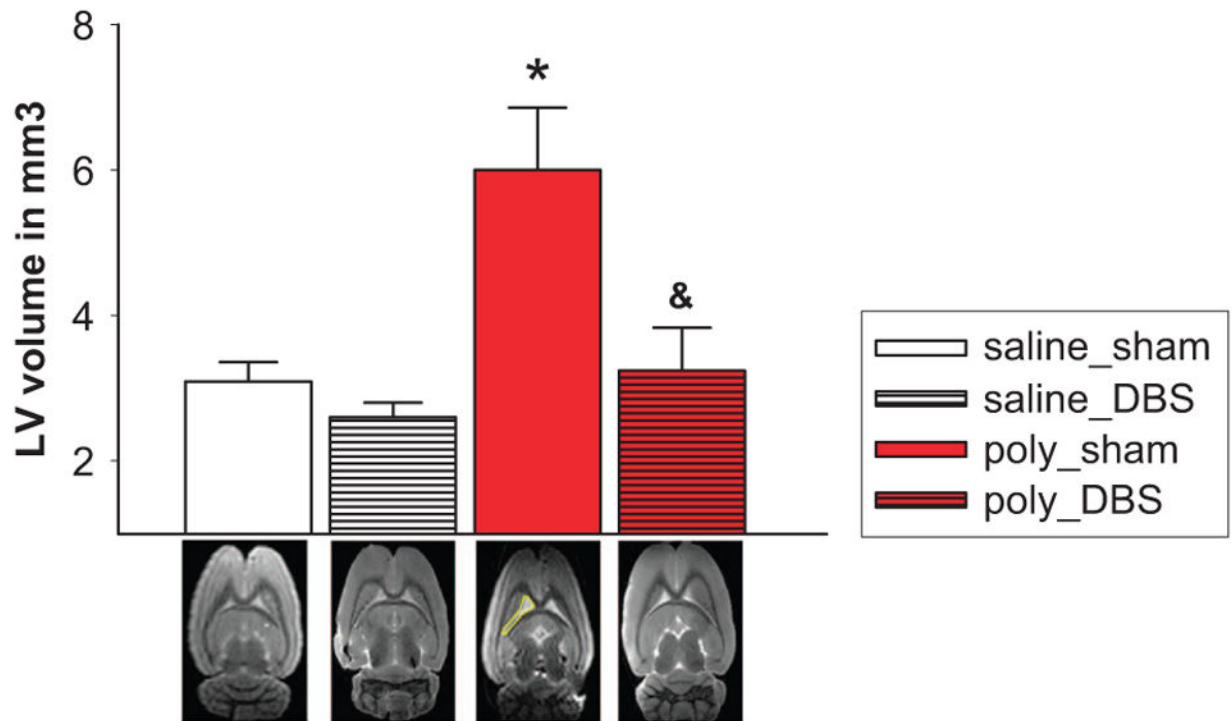
Figure 1.
Effects of mPFC-DBS in adolescent rats on LV volumes in adulthood. Bar plots show LV volumes of saline (saline) or poly-I:C (poly) offspring that received sham-stimulation (sham) or mPFC-DBS (DBS; saline-sham: $n = 10$; saline-DBS: $n = 7$; poly-sham: $n = 13$; poly-DBS: $n = 8$). Below, representative T2-weighted images at the level of the LV for each group. Results are expressed as mean values ± s.e.m; *vs respective saline group; &vs respective sham-stimulation group; *,&$P < 0.05$. DBS, deep brain stimulation; LV, lateral ventricular; mPFC, medial prefrontal cortex.
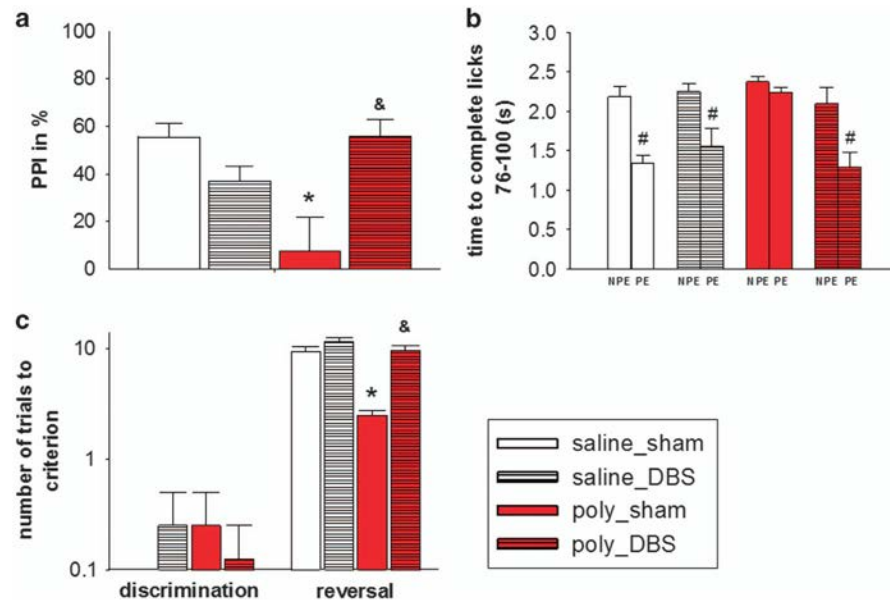
Figure 2.
Effects of mPFC-DBS in adolescent rats on behavioral performance in adulthood. mPFC-DBS prevented (**a**) development of PPI deficits (saline-sham: $n = 8$; saline-DBS: $n = 10$; poly-sham: $n = 8$; poly-DBS: $n = 9$), (**b**) disruption of LI (saline-sham PE: $n = 12$; saline-sham NPE: $n = 8$; saline-DBS-PE: $n = 8$; saline-DBS-NPE: $n = 7$; poly-sham-PE: $n = 10$; poly-sham-NPE: $n = 8$; poly-DBS-PE: $n = 9$; poly-DBS-NPE: $n = 7$) and (**c**) abnormally rapid reversal in adult poly I:C-offspring (poly) (saline-sham: $n = 8$; saline-DBS: $n = 8$; poly-sham: $n = 8$; poly-DBS: $n = 8$). Results are expressed as mean values ± s.e.m;. *vs respective saline group; &vs respective sham-stimulation group; #vs respective NPE group; *,&,#$P < 0.05$. DBS, deep brain stimulation; LI, latent inhibition; mPFC, medial prefrontal cortex; NPE, non-pre-exposed; PE, pre-exposed; PPI, pre-pulse inhibition.
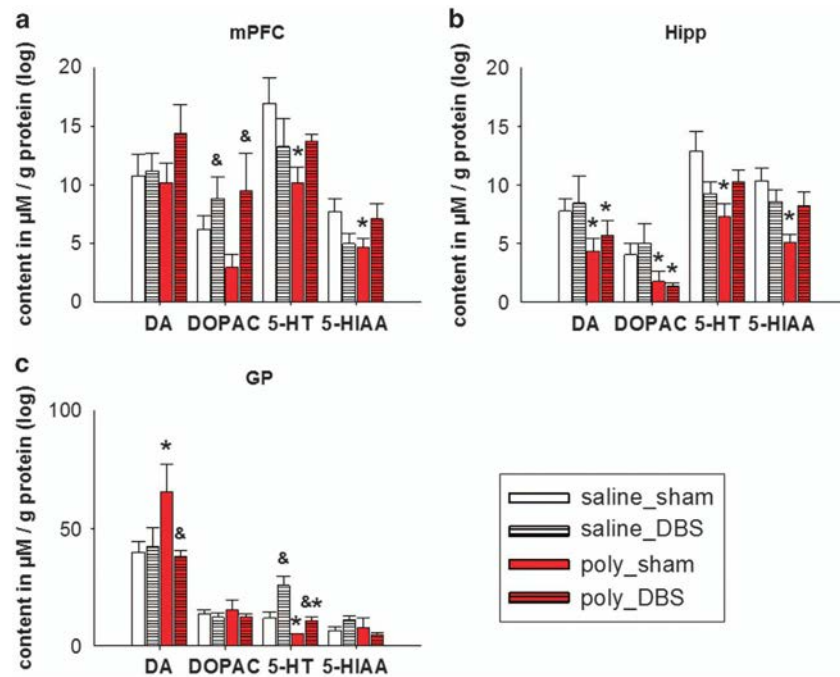
Figure 3.
Effects of mPFC-DBS in adolescent rats on neurotransmitter contents in adulthood.
Neurochemical contents were examined in adult saline- (saline) and poly I:C-offspring
(poly), that received sham-stimulation (sham) or mPFC-DBS (DBS; saline-sham: $n = 8$;
saline-DBS: $n = 10$; poly-sham: $n = 8$; poly-DBS: $n = 9$). mPFC-DBS affected contents of
DA, DOPAC, serotonin (5-HT) and 5-HIAA in the mPFC (**a**), Hipp (**b**), and globus pallidus
(GP) (**c**). Results are expressed as mean values ± s.e.m.; *vs respective saline group; &vs
respective sham-stimulation group; *, &$P < 0.05$. DA, dopamine; DBS, deep brain
stimulation; Hipp, hippocampus; mPFC, medial prefrontal cortex.
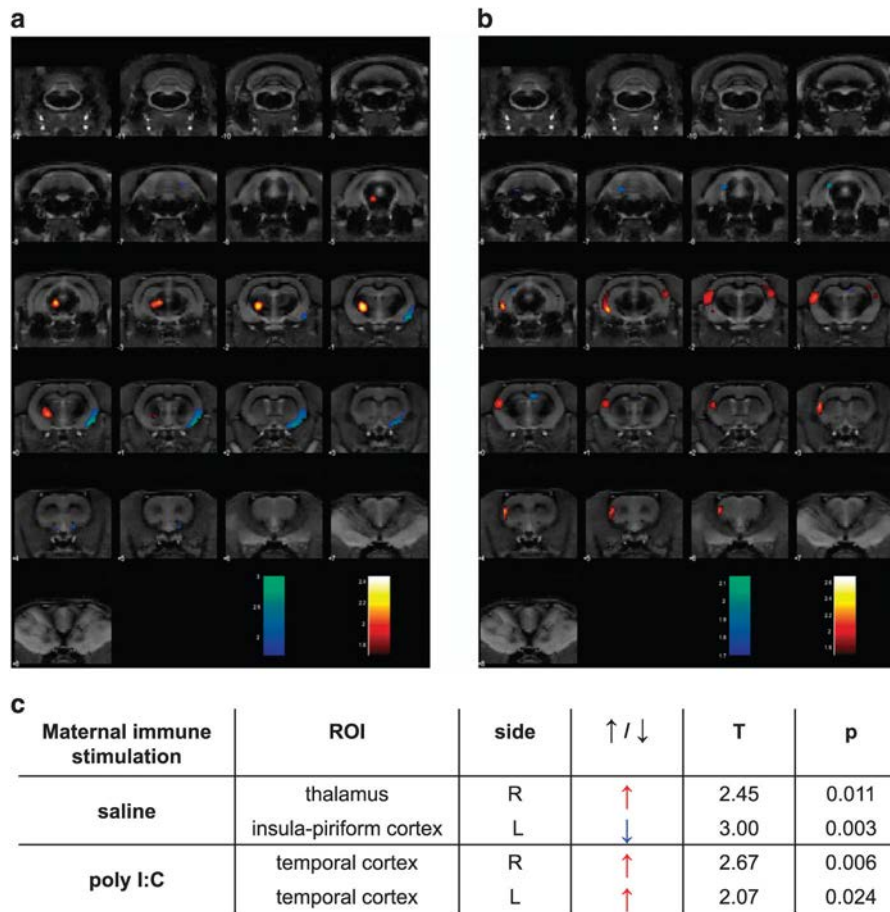
Figure 4.
Effects of mPFC-DBS in adolescent rats on brain-metabolic activity in adulthood. Colored PET overlays on MR reference indicate increased (hot colors) or decreased (cold colors) ^{18}F-FDG uptake for DBS treated rats compared to sham-stimulated rats (saline-sham: $n = 6$; saline-DBS: $n = 8$; poly-sham: $n = 8$; poly-DBS: $n = 7$). mPFC-DBS (**a**) increased brain activity in the right thalamus and decreased it in the left insular-piriform cortex in adult saline-offspring and (**b**) increased brain activity in the temporal cortex of adult poly I:C-offspring. (**c**) Table summarizes statistical group differences for the respective ROI and brain sides (right: R, left: L). DBS, deep brain stimulation; mPFC, medial prefrontal cortex; MR, magnetic resonance; PET, positron emission tomography; ROI, regions of interest.

Table 1

Effects of mPFC-DBS in adolescent rats on neurotransmitter contents in adulthood

Region	Transmitter	MIS	Stim	Content in µM/g protein	Two-way ANOVA effects	DF	F-value	P-value
mPFC	DA	Saline	Sham	10.78 ± 1.78	MIS	(1,24)	0.447	0.51
			DBS	11.16 ± 1.49	Stim	(1,24)	1.412	0.25
		Poly I:C	Sham	10.15 ± 1.72	Interaction	(1,24)	0.985	0.33
			DBS	14.40 ± 2.39				
	DOPAC	Saline	Sham	6.21 ± 1.14	MIS	(1,24)	0.343	0.56
			DBS	8.80 ± 1.83	Stim	(1,24)	4.198	0.05 *
		Poly I:C	Sham	2.97 ± 1.11	Interaction	(1,24)	0.77	0.39
			DBS	9.45 ± 3.22				
	5-HT	Saline	Sham	16.94 ± 2.18	MIS	(1,23)	3.477	0.08
			DBS	13.24 ± 2.37	Stim	(1,23)	0.002	0.96
		Poly I:C	Sham	10.14 ± 1.34	Interaction	(1,23)	4.484	0.05 *
			DBS	13.67 ± 0.65				
	5-HIAA	Saline	Sham	7.71 ± 1.04	MIS	(1,23)	0.188	0.67
			DBS	4.97 ± 0.90	Stim	(1,23)	0.026	0.87
		Poly I:C	Sham	4.67 ± 0.80	Interaction	(1,23)	5.459	0.03 *
			DBS	7.07 ± 1.33				
Hipp	DA	Saline	Sham	7.76 ± 1.01	MIS	(1,31)	5.239	0.03 *
			DBS	8.44 ± 2.31	Stim	(1,31)	0.571	0.46
		Poly I:C	Sham	4.36 ± 1.03	Interaction	(1,31)	0.06	0.81
			DBS	5.70 ± 1.20				
	DOPAC	Saline	Sham	4.04 ± 0.99	MIS	(1,31)	8.236	0.01 *
			DBS	5.05 ± 1.63	Stim	(1,31)	0.086	0.77
		Poly I:C	Sham	1.77 ± 0.89	Interaction	(1,31)	0.457	0.5
			DBS	1.37 ± 0.27				
	5-HT	Saline	Sham	12.86 ± 1.63	MIS	(1,31)	2.498	0.12
			DBS	9.22 ± 0.99	Stim	(1,31)	0.064	0.8
		Poly I:C	Sham	7.32 ± 1.07	Interaction	(1,31)	5.217	0.03 *

Region	Transmitter	MIS	Stim	Content in μM/g protein	Two-way ANOVA effects	DF	F-value	P-value
Nacc	5-HIAA		DBS	10.23 ± 1.05				
		Saline	Sham	10.32 ± 1.14	MIS	(1,31)	6.084	0.02 *
			DBS	8.56 ± 1.04	Stim	(1,31)	0.346	0.56
		Poly I:C	Sham	5.10 ± 0.66	Interaction	(1,31)	4.571	0.04 *
			DBS	8.20 ± 1.18				
	DA	Saline	Sham	383.69 ± 19.86	MIS	(1,25)	4.497	0.04 *
			DBS	438.84 ± 22.69	Stim	(1,25)	0.557	0.46
		Poly I:C	Sham	474.57 ± 50.01	Interaction	(1,25)	1.789	0.19
			DBS	459.20 ± 17.85				
	DOPAC	Saline	Sham	82.63 ± 10.15	MIS	(1,25)	1.278	0.27
			DBS	70.30 ± 3.55	Stim	(1,25)	0.121	0.73
		Poly I:C	Sham	58.95 ± 4.59	Interaction	(1,25)	3.816	0.06
			DBS	76.72 ± 6.94				
	5-HT	Saline	Sham	29.50 ± 4.12	MIS	(1,25)	1.797	0.19
			DBS	28.97 ± 5.48	Stim	(1,25)	0.022	0.88
		Poly I:C	Sham	23.82 ± 4.64	Interaction	(1,25)	0	0.98
			DBS	23.06 ± 2.84				
CPu	5-HIAA	Saline	Sham	13.71 ± 2.47	MIS	(1,25)	1.824	0.19
			DBS	12.97 ± 2.55	Stim	(1,25)	0.014	0.9
		Poly I:C	Sham	9.81 ± 1.55	Interaction	(1,25)	0.218	0.65
			DBS	11.07 ± 1.35				
	DA	Saline	Sham	528.38 ± 11.41	MIS	(1,31)	2.683	0.11
			DBS	596.84 ± 34.89	Stim	(1,31)	7.334	0.01 *
		Poly I:C	Sham	569.27 ± 29.42	Interaction	(1,31)	0.002	0.96
			DBS	640.33 ± 26.38				
	DOPAC	Saline	Sham	75.42 ± 9.78	MIS	(1,31)	2.109	0.16
			DBS	60.67 ± 4.80	Stim	(1,31)	0.649	0.43
		Poly I:C	Sham	56.31 ± 5.23	Interaction	(1,31)	1.913	0.18
			DBS	60.21 ± 3.31				
	5-HT	Saline	Sham	12.61 ± 2.14	MIS	(1,31)	7.895	0.01 *

Region	Transmitter	MIS	Stim	Content in µM/g protein	Two-way ANOVA effects	DF	F-value	P-value
			DBS	14.67 ± 2.73	Stim	(1,31)	0.175	0.68
		Poly I:C	Sham	8.67 ± 1.09	Interaction	(1,31)	0.475	0.5
			DBS	8.17 ± 0.68				
	5-HIAA	Saline	Sham	12.97 ± 4.08	MIS	(1,31)	3.201	0.08
			DBS	9.77 ± 1.61	Stim	(1,31)	0.27	0.61
		Poly I:C	Sham	6.37 ± 1.06	Interaction	(1,31)	0.461	0.5
			DBS	6.80 ± 1.40				
GP	DA	Saline	Sham	39.94 ± 4.39	MIS	(1,24)	2.427	0.13
			DBS	42.43 ± 8.10	Stim	(1,24)	3.264	0.08
		Poly I:C	Sham	65.66 ± 11.86	Interaction	(1,24)	4.705	0.04*
			DBS	38.22 ± 2.58				
	DOPAC	Saline	Sham	13.79 ± 1.56	MIS	(1,26)	0.112	0.74
			DBS	12.21 ± 1.86	Stim	(1,26)	0.896	0.35
		Poly I:C	Sham	15.12 ± 4.25	Interaction	(1,26)	0.063	0.8
			DBS	12.40 ± 1.21				
	5-HT	Saline	Sham	11.92 ± 2.51	MIS	(1,25)	15.336	0.00*
			DBS	25.70 ± 3.98	Stim	(1,25)	12.274	0.00*
		Poly I:C	Sham	5.09 ± 0.23	Interaction	(1,25)	2.118	0.16
			DBS	10.78 ± 1.42				
	5-HIAA	Saline	Sham	6.48 ± 1.52	MIS	(1,26)	1.373	0.25
			DBS	11.09 ± 1.54	Stim	(1,26)	0.145	0.71
		Poly I:C	Sham	7.83 ± 4.01	Interaction	(1,26)	3.297	0.08
			DBS	4.82 ± 0.95				

Abbreviations: ANOVA, analysis of variance; CPu, caudate putamen; DF, degrees of freedom; GP, globus pallidus; Hipp, hippocampus; MIS, maternal immune stimulation; mPFC-DBS, medial prefrontal cortex-deep brain stimulation; Nacc, nucleus accumbens. Neurochemical contents were examined in adult saline-(saline) and poly I:C-offspring (poly), that received either sham-stimulation (sham) or mPFC-DBS (DBS; saline-sham: $n = 8$; saline-DBS: $n = 10$; poly-sham: $n = 8$; poly-DBS: $n = 9$). DA, DOPAC, serotonin (5-HT) and 5-HIAA were measured in the mPFC, Hipp, Nacc, CPU, and GP and are expressed as mean values ± s.e.m. The presence of MIS- and DBS-effects are depicted by MIS, stim and interaction, DF, F-/P-values.

*
$P < 0.05$.