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

Ravit Hadar[1,2] · Rebecca Winter[2] · Henriette Edemann-Callesen[1,2,3] · Franziska Wieske[1] · Bettina Habelt[1,2] ·
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Cristian Alexandru Tatarau[1] · Klaus Funke[6] · Brita Fritsch[5] · Nadine Bernhardt [2] · Marom Bikson[4] ·
Michael A. Nitsche[7,8] · Christine Winter[1,2]

Abstract

Schizophrenia is a severe neurodevelopmental psychiatric affliction manifested behaviorally at late adolescence/early adulthood. Current treatments comprise antipsychotics which act solely symptomatic, are limited in their effectiveness and often associated with side-effects. We here report that application of non-invasive transcranial direct current stimulation (tDCS) during adolescence, prior to schizophrenia-relevant behavioral manifestation, prevents the development of positive symptoms and related neurobiological alterations in the maternal immune stimulation (MIS) model of schizophrenia.

Introduction

Characterized by severe alterations in emotion, behavior, and cognition, schizophrenia constitutes one of the most devastating psychiatric afflictions [1] affecting approximately one percent of the population worldwide. Current treatment options include antipsychotic medications as first-line therapy [1]. Unfortunately, not only do these medications often introduce severe side-effects which might even worsen over time, but also a significant proportion of patients fails to respond or only partially responds to available pharmacotherapy [2], highlighting the need for improved treatment options. In this context, also preventive avenues capable of reducing or even completely abolishing the development of schizophrenia need consideration. The growing acceptance that schizophrenia constitutes a neurodevelopmental disorder, in which neuropathological processes gradually evolve over the developmental course and eventually result in behavioral manifestation in the form of psychosis outbreak [3], supports this approach. As for today, few randomized control trials sought to explore prevention options for individuals at high-risk to develop psychosis. Psychological, pharmacological, and nutritional prevention approaches were tested with generally positive outcomes suggesting that a preventive approach might be fruitful [4]; interestingly, results do not support the use of

✉ Christine Winter
christine.winter@charite.de

1 Department of Psychiatry and Psychotherapy, Charité University Medicine Berlin, Campus Mitte, Berlin, Germany

2 Department of Psychiatry and Psychotherapy, Medical Faculty Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany

3 International Graduate Program Medical Neurosciences, Charité Universitätsmedizin Berlin, Berlin, Germany

4 Department of Biomedical Engineering, The City College of New York, CUNY, New York, NY 10031, USA

5 Department of Neurology, University Medical Center, Albert Ludwigs University Freiburg, Freiburg, Germany

6 Department of Neurophysiology, Medical School of the Ruhr-Universität Bochum, Bochum, Germany

7 Department Psychology and Neurosciences, Leibniz Research Centre for Working Environment and Human Factors at TU Dortmund, Dortmund, Germany

8 Department Neurology, University Hospital Bergmannsheil, Ruhr-Universität Bochum, Bochum, Germany
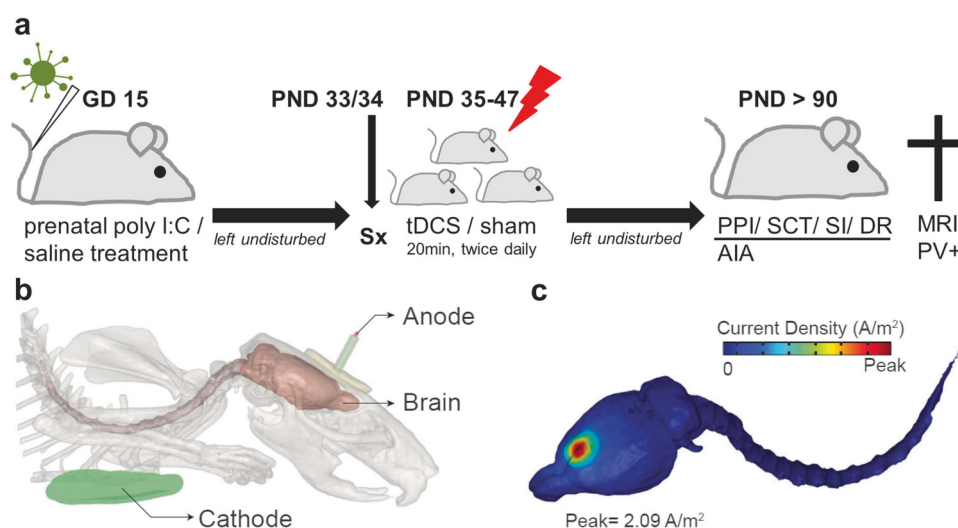
Fig. 1 General experimental design and computational finite element method (FEM). **a** Pregnant rats were i.v. injected with either *polyriboinosinic-polyribocytidilic acid* (poly I:C) (4 mg/kg) or saline on gestation day (GD) 15. Male offspring derived from both groups were left undisturbed until postnatal day (PND) 33–34 when they were randomly divided into three stimulation groups (sham, anodal, cathodal) and surgeries (Sx) for tDCS electrodes placement were conducted. From PND 35 to PND 47 rats were subjected twice daily to either sham, anodal, or cathodal tDCS to their frontal cortices twice a day for 20 min. Rats were left undisturbed until reaching PND 90 when they were tested for: pre-pulse inhibition (PPI), sucrose consumption (SCT), social interaction (SI), discrimination reversal (DR), and amphetamine-induced activity (AIA). Rats were then sacrificed and brains were subjected to ex-vivo MRI followed by immune-detection for parvalbumin (PV+) neurons. **b** Three-dimensional (3D) image of segmented brain, spinal cord, and electrode placement over frontal cortex (anode electrode- in accordance with bregma, AP +3.0) and thoracic region (cathode electrode). **c** Predicted current density over the frontal cortex. Peak current density was 2.09 A/m^2

antipsychotic medications as a first-line preventive treatment [4]. When considering the potential side-effects of antipsychotics introduced at a highly sensitive period during adolescence during which emotional, social, and hormonal shifts take place, it becomes clear that other, less aggressive preventive avenues would be desirable.

Previously, using the neurodevelopmental maternal immune stimulation (MIS) model of schizophrenia, we demonstrated that continuous electrical stimulation delivered directly to the medial prefrontal cortex (mPFC) of young adolescent rats via invasive deep brain stimulation (DBS) prevented behavioral, brain structural, and neurobiological manifestation of schizophrenia [5]. These remarkable findings pointed to the pivotal role of the prefrontal cortex in the progression of schizophrenia and suggested it might serve as a viable locus for the implementation of preventive neuromodulation approaches/techniques. Obviously, due to its invasive nature, the application of DBS as a preventive measure in the clinical situation, prior to the development of psychosis, is not feasible. To this end, applying neuromodulation to individuals at high-risk of developing psychosis in an attempt to interfere with schizophrenia neuropathological processes would necessitate a non-invasive and safe technique. This prompted us to explore the preventive effects of transcranial direct current stimulation (tDCS) delivered to the PFC. tDCS modulates cortical excitability and neuroplastic processes in a polarity-dependent manner via weak electrical current application [6–9].

We hence subjected offspring of saline and MIS rats between postnatal day (PND) 35 to 47—a period equivalent to human early adolescence—twice a day to 20 min of tDCS delivered to the frontal cortex (FC). Rats from both groups were exposed to either anodal tDCS, cathodal tDCS or sham-stimulation and then left undisturbed until adulthood, i.e., PND 90, when behavioral and neurobiological testing took place (Fig. 1a).

Materials and methods

Computational finite element method (FEM) of young rat tDCS model and a predicted current density/field intensity of frontal cortex stimulation

FEM model analysis predicted a peak current density of 2.09 A/m^2 and peak field intensity of 4.17 V/m. As expected, average current density and average power dissipation (scaled to 50% of the peak) was higher under the electrode and its periphery. However, spreading was slightly more prominent in the caudal direction—towards the bregma than

the rostral direction. Maximum average current density (scaled) and average power dissipation (scaled) at the frontal cortex region was ~1.34 A/m^2 and ~10.30 W/m^3, respectively (Fig. 1b and Suppl. Fig. 1).

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 committee (Regierungspräsidium Dresden, Germany).

Experimental design

Wistar rats (Harlan Laboratories) were mated and the first day after copulation was defined as day one 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, Germany) dissolved in saline, or saline alone under isoflurane anesthesia [10, 11]. Altogether 12 Batches of saline and 11 Batches of poly I:C rats were generated with an average of 9.75 offspring of which 4.5 were males and an average of 10.72 offspring of which 4.4 were males, respectively. On postnatal day (PND) 21, pups were weaned and housed by sex and litter. On PND 33–34, poly I:C and saline male offspring were randomly divided into three stimulation groups (2 x 3 design): anodal tDCS (with the anode placed over the frontal cortex (FC)), cathodal tDCS (with the cathode placed over the FC) or sham-stimulation (i.e., sham electrode placed over FC) and then surgeries for tDCS electrodes placement were conducted (saline_sham: $n = 15$; saline_anodal: $n = 14$; saline_cathodal: $n = 13$; poly_sham: $n = 14$; poly_anodal: $n = 12$; poly_cathodal: $n = 11$). Anodal/cathodal/sham-stimulation began on PND 35 and was delivered twice a day for 20 min until PND 47. Behavioral and neurobiological analyses were conducted at PND > 90. Behavioral testing was done in the following order: pre-pulse inhibition, sucrose consumption test, social interaction, reversal learning, and amphetamine-induced hyperactivity. Experimenters were blind to the identity of animals when conducting experiments and analysis. Experiments and analyses were conducted by independent researchers.

tDCS electrode-placement and current delivery

On PND 33–34 rats received subcutaneous (s.c.) balanced anesthesia (fentanyldihydrogencitrat 0.005 mg/kg, midazolamhydrochlorid 2.00 mg/kg, medetomidin 0.15 mg/kg) and were then placed in stereotactic frames. Skulls were carefully exposed at the frontal area and a round epicranial electrode holder was placed above the frontal cortices (at 3 mm anterior to Bregma, on the midline) and fixed using dental acrylic cement (Technovit® Heraeus-Kulzer, Hanau, Germany) and superglue. Upon completion, an antagonist cocktail was applied s.c. (naloxone (0.12 mg/kg, Inresa Arzneimittel, Germany), flumazenil (0.2 mg/kg, HEXAL Germany), and atipamezol (0.75 mg/kg, Elanco Animal Health, Germany)). For stimulation, an electrode (3.5 mm^2) was connected into the electrode holder filled with 0.5 µl NaCl (0,9%) as conductive medium. Electrodes were connected via cables to an external computer-interfaced constant current generator (STG4008, MultiChannelSystems GmbH, Reutlingen, Germany). Anodal and cathodal stimulation were delivered at an intensity of 50 µA (Current density 14.3 A/m²) twice a day for 20 min in between PND 35 and PND 47. In order to avoid discomfort, stimulation begun and ended with current slowly ramping up and down, respectively, for 10 s. Stimulation continuity was monitored using an amperemeter. Both duration and current density were chosen to approximate parameters commonly used in human conditions proven to be below the threshold for inflammation and neurodegeneration (see refs. [12, 13]).

Computational model and solution method

Segmentation of an exemplary magnetic resonance imaging (MRI) scan of a template young rat head into nine tissue masks namely scalp, skull, cerebro-spinal fluid (csf), gray matter, white matter, cerebellum, hippocampus, thalamus, and air was performed using ScanIP software (Simpleware, Exeter, UK) to develop a high resolution (~0.1 mm) MRI derived FEM model. Conductivity values were assigned as, scalp: 0.465 S/m; skull: 0.01 S/m; csf: 1.65 S/m; air: 1x10–15; gray matter: 0.276 S/m; cerebellum: 0.276 S/m; hippocampus: 0.126 S/m; white matter: 0.126 S/m; thalamus: 0.276 S/m, electrodes: 5.99 x 107 S/m, saline 4 S/m, plastic cannula 1x10–15 S/m. All tissue values were based on prior literature [14, 15]. Computer aided design (CAD) models of epicranial electrode, plastic cannula, and glass isomer cement geometries with exact dimensions (from experiment) were first modeled in SolidWorks 2016 (Dassault Systemes Americas Corp., MA, USA), imported into the rat head model, and positioned based on the locations as in the experiment (Fig. 1b, c). The resulting volumetric meshes were later imported into COMSOL Multiphysics 5.1 (COMSOL Inc., MA, USA) to solve the model. The final FEM model was solved for greater than 5,950,000 tetrahedral elements. Electrical simulation was carried out using quasistatic approximation (Laplace equation, $\nabla(\sigma \nabla V) = 0$, where V = potential and σ = conductivity) and the boundary conditions were applied as normal current density at the

exposed boundary of the gold electrode (anode = 50 μA) and ground at the exposed surface of the cathode (adhesive gel based electrode). Other remaining external surfaces of the model were electrically insulated. Predicted current density/field intensity plots and slices were generated, and the 50% of the peak values of average current density and average power dissipation at each slice from bregma to frontal cortex were reported.

Behavior

Pre-pulse inhibition

PPI is a cross-species phenomenon that measures sensorimotor gating. Reduced PPI reflects gating deficits seen in and relevant to schizophrenia [16]. PPI of the ASR 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 [10, 17, 18]. 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 (SPL). During acclimatization, animals received five initial startle stimuli (100 dB SPL, white noise, 20 ms). The test session consisted of four different trial types delivered each ten times in a pseudorandom order with an inter-trial interval of 20 to 30 s: startle-pulse alone (100 dB SPL white noise, 20 ms) and three pre-pulses (81/73/69 dB, 20 ms) each followed by a startle-pulse with an inter-stimulus interval of 100 ms. PPI was calculated according to the formula 100–100%x (mean ASR of PPI-trials/mean ASR of pulse-alone-trials). For analysis, the following responses were calculated and presented: acoustic startle response (ASR) alone, PPI for each of the pre-pulse intensities separately (81/73/69) and an average PPI response over the three pre-pulse intensities (mean).

Discrimination reversal (DR)

DR is a cross-species phenomenon that reflects the ability of an organism to change its behavior in the face of changing contingencies. Abnormally rapid DR is relevant to the positive symptoms of schizophrenia as it indicates an excessive switching [19]. DR was assessed in a T-maze that had a hidden platform (15.5 x 15.5 cm) in one of the arms and was submerged in a swimming pool [5, 19]. 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 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 the criterion of the position discrimination of the first day was reached, and then trained until reaching the criterion of the reversal of this discrimination, i.e., with the platform located in the opposite arm. The number of trials to reach the criterion was recorded in both stages.

Sucrose consumption test (SCT)

The SCT is based on the natural preference of rodents for a sweet solution over water, and decreased preference is considered to mimic anhedonia (decreased ability to experience pleasure/ reduced sensitivity to reward), a core affective state associated with negative symptoms of schizophrenia. 48 h prior to testing, animals were habituated to the individual testing cages and bottles (containing water). Twenty-four hours thereafter, animals were habituated to the sucrose solution (Nestlé, Milchmädchen gezuckerte Kondensmilch, (1:3)) for 30 min in their home cage and subsequently food restricted until time of testing (15 g food available per animal). On the day of testing, animals were placed in the individual cages with free access to the sucrose solution for 15 min. Bottles were weighed before and after testing. The amount of sucrose solution consumed over a period of 10 min after 21 h food restriction was normalized to the individual body weight [20, 21].

Social interaction

Social interaction deficits are considered to represent social withdrawal, a core feature of schizophrenia. Social interaction with an unfamiliar social partner was assessed in a dimly lit transparent box measuring 60 x 40 x 40 cm (*l* x *w* x *h*). Sessions were recorded using a video camera while the experimenter remained outside the test room during interactions. One day prior to testing rats were first habituated to the testing room and then after to the test arena (for 30 min). Following each session the aquarium was thoroughly cleaned to avoid any odor remains. On the testing day rats were brought into the testing room and following a habitation period of 30 min testing begun. Unfamiliar social partners were sex-, age-, and weight-matched Wistar rats. An experimental rat and its unfamiliar social partner (painted with ink to allow distinction) were placed in the arena facing each other. Sessions lasted for 10 min. Social behavior was subsequently quantified offline by an experimenter blind to the experimental

condition. The frequency of the following social behaviors was quantified for the experimental rat [22]: Social exploration, divided in anogenital and non-anogenital investigation of the partner (sniffing or licking the anogenital or any part except for the anogenital area, respectively) and approaching the social partner throughout the test arena.

Neurobiological assessments

Amphetamine-induced activity

Increased locomotor response to amphetamine is considered to mimic the exacerbation of psychotic symptoms in response to amphetamine in schizophrenia [11]. Amphetamine-induced activity was measured in boxes measuring 60 x 40 x 40 cm^3. Sessions were recorded using a video camera while the experimenter remained outside the test room during interactions. Analysis was done using EnthoVision software. Tests begun with a habituation period to the box, after which rats were injected with amphetamine (Sigma, Germany; 1 mg/kg/mL), and activity was further recorded for 60 min Animals were tracked using EthoVision (Noldus). Distance moved in cm was recorded and analyzed over 10 min time bins.

Preparation of brains for post mortem neurobiological assessments

Rats were deeply anaesthetized with a single i.p. injection of pentobarbital (60 mg/kg) and perfused transcardially with cold 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Brains were dissected from the skulls and postfixed overnight.

Ex-vivo MRI

MRI acquisitions were performed on a 7.0 Tesla rodent scanner (Bruker BioSpin MRI GmbH, Ettlingen, Germany) at Charité (Berlin, Germany). The acquisition protocol consisted of a multi slice localizer (field of view (FOV) 50 x 50mm) and T2-weighted contrast images with a rapid acquisition with relaxation enhancement (RARE) sequence (imaging parameters: TR/TE = 4050/30 ms, RARE factor 8, NEX 6, FOV 30 x 30 mm, MD 256x256) resulting in 42 slices á 0.5 mm. To improve quality, perfused rat heads were scanned for 13 min per animal. The axial view proved to be the most useful for manual segmentation. 15 slices from −0.1 (first view of the anterior part of the anterior commissure) to + 1.7 mm from Bregma were analyzed for each animal (in accordance to the rat brain atlas of Paxinos & Watson [23]). For best image quality, contrast was adjusted using MRIcron. The lateral ventricles were

outlined using ImageJ and special attention was paid on distinguishing the ventricular area from adjoining areas through identifying differences in contrast by eye. Volumes were obtained by multiplying the summed up amount of pixels times pixel area with slice thickness (ventricular volume = (sum(measured amount of pixels × 0.117 mm)) × 0.12 mm).

Immunohistochemistry of parvalbumin-expressing GABAergic interneurons

Following perfusion brains were subsequently cryoprotected in a solution of 30% sucrose in 0.1 M phosphate buffer, pH 7.4 at 4 °C for 72 h before being snap-frozen in methyl butane with liquid nitrogen between −60 °C and −80 °C and stored at −80 °C until sectioning. Brains were sectioned into 40 µm thick coronal slices. Every 3rd free-floating slice was rinsed several times in Phosphate-buffered saline (PBS) and incubated with a polyclonal rabbit anti-parvalbumin antibody (Oncogene Research Products, San Diego, CA, USA, 1:500) in PBS with 0.3% Triton X-100 and 5% normal goat serum overnight at 4 °C as described earlier [24]. Sections were further washed in PBS and incubated overnight at 4 °C with a biotinylated secondary antibody (goat anti rabbit, Vector Laboratories, Burlingame, CA, USA, 1:1,000) and visualized by 3,3'-diaminobenzidine (DAB) after incubation with Vectastain Elite ABC HRP Kit (Vector Laboratories, Burlingame, CA, USA).

Parvalbumin-immunoreactive cells of the right medial prefrontal cortex (prelimbic and infralimbic cortices) between Bregma + 3.2 to + 2.7 (according to Paxinos and Watson [23] rat brain atlas) were quantified stereologically by a blinded investigator using a Leica DMRA microscope equipped with a Retiga-2000R Color camera and the Stereo Investigator System (MicroBrightField). To enumerate cells, the optical fractionator was used with the following sampling parameters: serial section interval = 3; section thickness = 40 µm; counting frame size = 250,000 µm²; grid size = 250,000 µm²; the coefficient of error value (Gundersen, $m = 1$) reached by the counting was 0.046 ± 0.005.

Statistical analysis

Rats from both poly I:C and saline dams were randomly assigned to treatments (anodal, cathodal, and sham), no more than 2–3 rats from the same litter were used for each experimental condition. PPI, SCT, SI, immunohistochemistry, and MRI data were analyzed using two-way analysis of variance (ANOVA; factors: phenotype, tDCS) followed by Holm-Sidak post hoc tests if applicable. DR and AIA were analyzed with three-way ANOVA (factors: phenotype,

tDCS, stage/time) followed by Tukey-HSD post hoc tests. Significance was set at $P < 0.05$.

Results

Computational finite element method (FEM) of young rat tDCS model and a predicted current density/field intensity of frontal cortex stimulation

FEM model analysis predicted a peak current density of 2.09 A/m^2 and peak field intensity of 4.17 V/m. As expected, average current density and average power dissipation (scaled to 50% of the peak) was higher under the electrode and its periphery. However, spreading was slightly more prominent in the caudal direction—towards the bregma than the rostral direction. Maximum average current density (scaled) and average power dissipation (scaled) at the frontal cortex region were ~1.34 A/m^2 and ~10.30 W/m^3, respectively (Fig. 1b and Suppl. Fig. 1).

Schizophrenia-related behaviors and neuropathological manifestations prevented by adolescence tDCS

Pre-pulse inhibition (PPI) constitutes a cross-species behavioral phenomenon measuring sensorimotor gating. Reduced PPI is thought to reflect gating deficits observed in and relevant to schizophrenia [16]. As expected, adult offspring to MIS dams (poly_sham) exhibited reduced levels of PPI of an acoustic startle response (Fig. 2a) [5, 10]. Remarkably, adult offspring of MIS rats which received anodal tDCS during their adolescence period exhibited intact PPI (Fig. 2a), reaching inhibition levels comparable to those of control sham treated rats (saline_sham) (Fig. 2a). Interestingly, in offspring of saline rats, anodal tDCS during adolescence led to expression of PPI deficits in later adulthood (Fig. 2a). Two-way analysis of variance (factors: phenotype × tDCS) yielded a significant main effect for 81 db and significant interactions for all pre-pulse/pulse presentations (81/73/69 dB) and its mean (main effect
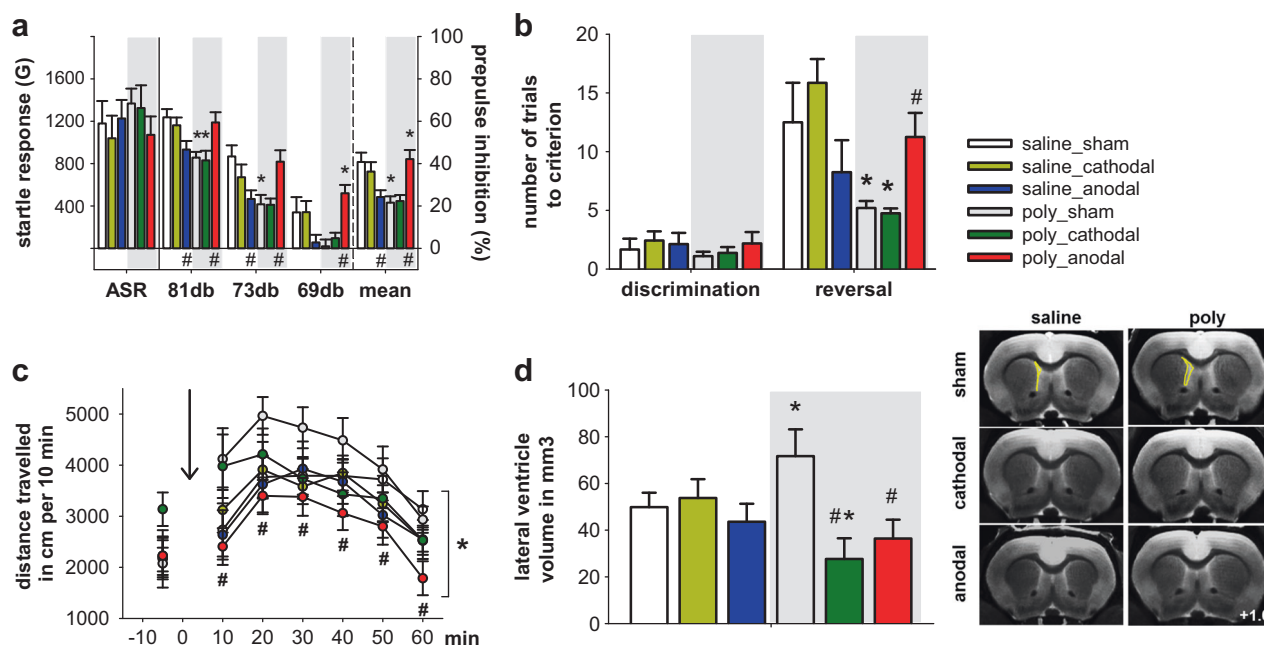
Fig. 2 Schizophrenia-related behaviors and neuropathological manifestations prevented by adolescence tDCS. **a** Reduced PPI levels in adulthood were observed in adult poly I:C-offspring (poly_sham), reflecting gating deficits seen in and relevant to schizophrenia; PPI deficits were prevented following anodal tDCS during adolescence. (*n* saline: sham = 11, cathodal = 13, anodal = 14; *n* poly: sham = 14, cathodal = 10, anodal = 12). **b** Rapid reversal was observed in adult poly I:C-offspring, reflecting excessive switching in response to altering contingencies and belonging to positive symptomatology of schizophrenia; Rapid reversal was prevented following anodal tDCS during adolescence. (*n* saline: sham = 14, cathodal = 11, anodal = 13; *n* poly: sham = 10, cathodal = 8; anodal = 11). **c** Elevated AIA levels were observed in adult poly I:C offspring, reflecting enhanced mesolimbic dopaminergic neurotransmission, as observed in schizophrenia;

Elevated AIA levels were prevented following anodal tDCS during adolescence. (*n* saline: sham = 12, cathodal = 9, anodal = 13; *n* poly: sham = 12, cathodal = 9, anodal = 10). **d** Increased LV volumes were observed in adult poly I:C-offspring (poly_sham), in line with findings from schizophrenia patients; Enlargement of LV volumes were prevented following anodal tDCS and cathodal tDCS during adolescence. (*n* saline: sham = 13, cathodal = 13, anodal = 14; *n* poly: sham = 12, cathodal = 11, anodal = 11). Left: bar plots show lateral ventricle volumes in mm^3 for each group; Right: representative T2-weighted images at the level +0.1 from bregma for each group. Results are expressed as mean values ± s.e.m.; * = significant vs. respective saline group; # = significant vs. respective sham tDCS group

phenotype: $F(1,68) = 5,48$, $P < 0,05$, interactions: $F(2,68) = 10.31$, $P < 0.001$; $F(2,68) = 9.59$, $P < 0.001$; $F(2,68) = 11.96$, $P < 0.001$; $F(2,68) = 14.53$, $P < 0.001$, respectively) and was followed by Holm-Sidak post hoc tests (n saline: sham $= 11$, cathodal $= 13$, anodal $= 14$; n poly: sham $= 14$, cathodal $= 10$, anodal $= 12$).

DR reflects the organism's ability to change behavior in the face of altering contingencies. Also here, as expected, abnormalities in DR in the form of rapid reversal were observed in adult offspring to MIS dams (poly_sham) [5] (Fig. 2b), which is indicative of excessive switching behavior typical for positive symptoms of schizophrenia. We again found that anodal tDCS during adolescence was successful in preventing excessive switching behavior development in MIS offspring, whereas cathodal tDCS yielded no effect on DR performance (Fig. 2b). Three-way analysis of variance (factors: phenotype × tDCS x stage) yielded a significant effect for main factor stage ($F(1,61) = 87.93$, $P < 0.001$) and a significant stage x phenotype interaction ($F(1,61) = 5.56$, $P < 0.05$) and phenotype × tDCS x stage interactions ($F(2,61) = 5.04$, $P < 0.01$), followed by Tukey-HSD. (n saline: sham $= 14$, cathodal $= 11$, anodal $= 13$; n poly: sham $= 10$, cathodal $= 8$; anodal $= 11$).

Reduced PPI and rapid DR are thought to reflect positive symptoms of schizophrenia resulting from a hyperactive mesolimbic-dopamine system [19, 25]. Enhanced meso-limbic dopaminergic neurotransmission is generally observed in schizophrenia [25] and in rats can be indirectly measured via elevated amphetamine-induced activity (AIA) upon low dose administration of amphetamine (1 mg/kg). We found that anodal tDCS to MIS offspring (poly_anodal) completely normalized otherwise elevated AIA levels (poly_sham). Three-way analysis of variance with repeated measurements (factors: phenotype × tDCS × time) yielded a significant effect for time ($F(6,324) = 34.79$, $P < 0.001$), and significant interactions (phenotype × time, tDCS × time, phenotype × tDCS x time; ($F(6,324) = 3.73$, $P < 0.01$), ($F(12,324) = 2.13$, $P < 0.05$), ($F(12,324) = 1.9$, $P < 0.05$), respectively) followed by Tukey-HSD (n saline: sham $= 12$, cathodal $= 9$, anodal $= 13$; n poly: sham $= 12$, cathodal $= 9$, anodal $= 10$) (Fig. 2c).

On a structural level, one acknowledged hallmark of schizophrenia—also evident in the MIS model—is the enlargement of lateral ventricles [5, 26]. Interestingly, both stimulation polarities, i.e., anodal and cathodal tDCS delivered during adolescence, prevented this structural brain characteristic of schizophrenia in the MIS offspring. Two-way analysis of variance (factors: phenotype × tDCS) yielded a significant effect for main factor tDCS ($F(2,68) = 6.46$, $P < 0.01$) and a significant interaction ($F(2,68 = 4.92$, $P < 0.05$), followed by Holm-Sidak post hoc tests (n saline: sham $= 13$, cathodal $= 13$, anodal $= 14$; n poly: sham $= 12$, cathodal $= 11$, anodal $= 11$) (Fig. 2d).

Schizophrenia-related behaviors and neuropathological manifestations unaffected by adolescence tDCS

Despite the remarkable effect of tDCS during adolescence in preventing the development of positive symptoms in the MIS offspring, behavioral deficits which belong to the negative symptom profile of schizophrenia were not affected by this intervention. Reduction in total sucrose consumption (SC), which is apparent in the MIS offspring and thought to reflect anhedonia in rodents [27] was neither affected by anodal nor cathodal stimulation. Two-way analysis of variance (factors: phenotype × tDCS) yielded a significant effect for main factor phenotype ($F(1,71) = 3.93$, $P = 0.05$) but not for the factor tDCS and no significant interaction between the factors (n saline: sham $= 15$, cathodal $= 13$; anodal $= 13$; n poly: sham $= 13$, cathodal $= 11$; anodal $= 12$) (Fig. 3a).

Similarly, tDCS did not affect deficits in sociability in the MIS offspring as measured by the social interaction (SI) paradigm [22]. Reduced numbers of SI events, i.e., number of approaches to the partner and investigation of its non-anogenitals, were observed in adult poly I:C offspring (poly_sham), reflecting deficits in social behavior. Two-way analysis of variance (factors: phenotype × tDCS) yielded a significant main effect for treatment in the number of approaches ($F(2,65) = 5.24$, $P < 0.01$) and significant interactions (($F(2,65) = 4.25$, $P < 0.05$; $F(2,65) = 4.82$, $P < 0.05$, respectively) followed by Holm-Sidak post hoc tests (n saline: sham $= 14$, cathodal $= 12$, anodal $= 12$; n poly: sham $= 13$, cathodal $= 9$; anodal $= 11$). However, neither anodal nor cathodal tDCS affected deficits in SI in the MIS offspring (Fig. 3b).

Reduction in the number of parvalbumin-expressing cells was observed in the medial prefrontal cortex (prelimbic and infralimbic) of adult poly I:C offspring, reflecting deficits in fast-spiking GABAergic interneurons. Two-way analysis of variance (factors: phenotype × tDCS) yielded a significant effect for the main factor phenotype ($F(1,30 = 4.33$, $P < 0.05$) (n saline: sham $= 6$, cathodal $= 6$, anodal $= 6$; n poly: sham $= 6$, cathodal $= 6$, anodal $= 6$). Neither anodal nor cathodal tDCS were found to significantly affect reduced levels of parvalbumin-expressing GABAergic cells in the MIS offspring [28] (Fig. 3c).

Discussion

Here, we introduce a novel approach for the prevention of schizophrenia-development, i.e., the application of adolescence anodal tDCS.

We were able to show that applying anodal tDCS to the PFC during adolescence, prior to any overt schizophrenia-
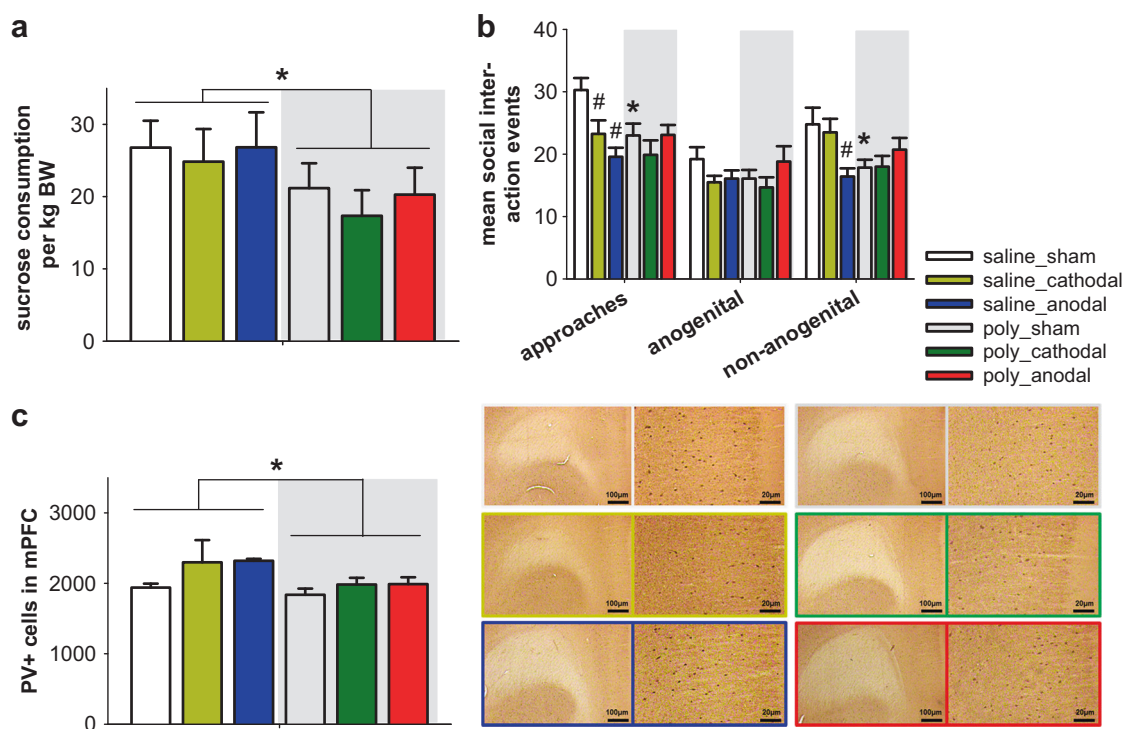
Fig. 3 Schizophrenia-related parameters unaffected by adolescence tDCS. **a** Reduction in SC levels were observed in adult poly I:C offspring, reflecting a reduction in hedonic capacity (anhedonia); Adolescence tDCS yielded no effect on the development of SC. (*n* saline: sham = 15, cathodal = 13; anodal = 13; *n* poly: sham = 13, cathodal = 11; anodal = 12). **b** Reduced numbers of SI events, i.e., number of approaches to the partner and *investigation* of its non-anogenitals, were observed in adult poly I:C offspring (poly_sham), reflecting deficits in social behavior. (*n* saline: sham = 14, cathodal = 12, anodal = 12; *n* poly: sham = 13, cathodal = 9; anodal = 11). **c** Reduction in the number of parvalbumin-expressing cells was observed in the medial prefrontal cortex (prelimbic and infralimbic) of adult poly I:C offspring, reflecting deficits in fast-spiking GABAergic interneurons. (*n* saline: sham = 6, cathodal = 6, anodal = 6; *n* poly: sham = 6, cathodal = 6, anodal = 6). Left: bar plots show mean number of parvalbumin cells in the mPFC for each group; Right: representative images of parvalbumin cells in the mPFC for each group. Results are expressed as mean values ± s.e.m.; * = significant vs. respective saline group; # = significant vs. respective sham tDCS group

related behavioral abnormalities, successfully prevented the manifestation of sensorimotor gating deficits and abnormal rapid reversal. Moreover, enhanced mesolimbic dopaminergic neurotransmission as indicated by abnormal AIA and the enlargement of lateral ventricles volumes were also prevented following focal non-invasive neuromodulation of the PFC.

For our study, we used the neurodevelopmental MIS rodent model of schizophrenia, in which a single injection of the synthetic analog of double-stranded RNA poly I:C during pregnancy elicits a virulent response via the activation of the transmembrane protein toll-like receptor 3 eventually results in neurodevelopmental pathologies in the offspring [29]. This model provides an excellent platform for the assessment of preventive strategies as—in line with the human condition—schizophrenia-relevant behavioral abnormalities first appear in adulthood, whereas brain structural and neurobiological adversities develop gradually and partially precede the occurrence of behavioral deficits [10, 30].

Given the high predictive, face and construct validity of the MIS model, the current study supports the notion that schizophrenia is a neurodevelopmental disorder entailing a critical period in brain-development during which immanent plastic changes may be modulated to affect later behavioral characteristics [3].

In fact, we here show that neuromodulation of the PFC during adolescence hindered the development of behavioral deficits related to the positive and cognitive symptoms. This is in line with previous studies [5] and suggests that during adolescence the prefrontal cortex plays a pivotal role in the development of delayed behavioral manifestation of schizophrenia. Further, the finding that neuromodulation of the PFC during adolescence prevented elevated amphetamine-induced activity suggests our intervention eventually led to normalization of the otherwise enhanced mesolimbic dopaminergic neurotransmission. This is in agreement with the demonstration that DBS to the mPFC affects and even balances subcortical neurochemical alterations [5]. Striatal dopaminergic overactivity has been associated with positive

symptoms of schizophrenia, suggesting that in this study the regulation of the former may account for the preventive effects on positive symptoms in adulthood. Future studies are necessary to unravel tDCS mechanism of action on subcortical dopaminergic neurotransmission and on behavior and to determine whether these two effects (neurobiological and behavioral) are causally related.

Interestingly, neither anhedonia nor deficits in social interaction observed in the MIS offspring were affected by adolescent tDCS. To the best of our knowledge, this is the first time that behavioral deficits related to the negative pole of schizophrenia were assessed following a preventive intervention, hence prohibiting any conclusive explanation for this effect. It might be speculated that the approach used here, i.e., repeated tDCS at 50 μA, is not sufficient to prevent negative symptomatology. Further, nevertheless not mutually exclusive, one could speculate that the region targeted, i.e., the PFC is not tightly involved in abnormalities associated with negative symptoms. Importantly, using the same time-window as in this study, Piontkewitz et al. [31] were able to demonstrate that systemic administration of the atypical antipsychotic drug risperidone prevented the otherwise reduced levels of parvalbumin cells observed in the MIS offspring. This finding suggested a putative mechanism underlying the preventive effects of intervention, specifically early normalization of altered frontal activity and inspired us to investigate parvalbumin alterations in the current study. We found however, that PFC-neuromodulation during adolescence failed to affect these GABAergic abnormalities, favoring the notions that either additional brain circuitries other than the PFC underlie the preventive effect of systemic drug application, or that tDCS per se does not suffice to prevent such abnormalities. Further, since the perceptual alterations as well as social deficits in schizophrenia are sought to be related to impaired parvalbumin signaling [32], one could speculate that the lack of effect of tDCS on SI and SC might be a consequence of its inability to affect parvalbumin-cell levels, and following this line of thought, one could further speculate that GABAergic alterations underlie negative symptomatology in schizophrenia and that an attempt to prevent the complete spectrum of this disorder necessitates also targeting abnormal GABAergic transmission. Alternatively, it might be that neuropathologies eventually resulting in the negative symptoms observed in schizophrenia take place at a different neurodevelopmental stage and not during the time-window chosen, namely adolescence. In support of the latter, negative symptoms are reported to be present prior to the diagnosis of psychosis [33] allowing to presume their underlying neurodevelopment precedes the development of positive symptoms. Identifying the precise temporal development of the underlying neuropathologies is

highly relevant for future clinical attempts to prevent schizophrenia-development. This however awaits further studies for a sounder conclusion.

A further crucial finding from this study is related to the adverse effects induced by adolescence anodal and cathodal tDCS in the control group; here rats which were subjected to anodal tDCS during adolescence exhibited deficits in PPI at adulthood, whereas both anodal and cathodal adolescence tDCS resulted in SI deficits at adulthood. These findings are important for consideration of translation of the results of this study into the clinic by applying tDCS as a preventive intervention. Since as for today, no biomarker predicting the transition to psychosis has been identified, this presymptomatic period of adolescence bears the danger of falsely diagnosing young individuals as being at high-risk for developing schizophrenia. Given the harmful effects of adolescence tDCS in the control group, the selection of subjects for such an endeavor should be done with uttermost caution.

Altogether, our results introduce a novel approach for the prevention of schizophrenia-development via tDCS. Although tDCS application should be cautiously considered due to its possible adverse effects in non-affected (sham) rats, overall it provides a non-invasive, safe, and well-tolerated brain stimulation modality that could be easily translated to and verified in the clinic.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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