Brain, Behavior, and Immunity 63 (2017) 71–80

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Special Issue on Perinatal Inflammation

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

Ravit Hadar [a,1], Le Dong [b,1], Lucia del-Valle-Anton [b], Dilansu Guneykaya [b], Mareike Voget [a,c], Henriette Edemann-Callesen [a,c], Regina Schweibold [b], Anais Djodari-Irani [b], Thomas Goetz [a], Samuel Ewing [a], Helmut Kettenmann [b], Susanne A. Wolf [b,1,*], Christine Winter [a,1]

[a] Department of Psychiatry and Psychotherapy, Medical Faculty Carl Gustav Carus, Technische Universitaet Dresden, Germany
[b] Cellular Neuroscience, Max-Delbrueck-Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany
[c] International Graduate Program Medical Neurosciences, Charité – Universitaetsmedizin Berlin, Germany

ARTICLE INFO

Article history:
Received 15 August 2016
Received in revised form 23 November 2016
Accepted 5 December 2016
Available online 7 December 2016

Keywords:
Microglia
Deep brain stimulation
Schizophrenia
Depression
Maternal immune activation

ABSTRACT

In recent years schizophrenia has been recognized as a neurodevelopmental disorder likely involving a perinatal insult progressively affecting brain development. The poly I:C maternal immune activation (MIA) rodent model is considered as a neurodevelopmental model of schizophrenia. Using this model we and others demonstrated the association between neuroinflammation in the form of altered microglia and a schizophrenia-like endophenotype. Therapeutic intervention using the anti-inflammatory drug minocycline affected altered microglia activation and was successful in the adult offspring. However, less is known about the effect of preventive therapeutic strategies on microglia properties. Previously we found that deep brain stimulation of the medial prefrontal cortex applied pre-symptomatically to adolescence MIA rats prevented the manifestation of behavioral and structural deficits in adult rats. We here studied the effects of deep brain stimulation during adolescence on microglia properties in adulthood. We found that in the hippocampus and nucleus accumbens, but not in the medial prefrontal cortex, microglial density and soma size were increased in MIA rats. Pro-inflammatory cytokine mRNA was unchanged in all brain areas before and after implantation and stimulation. Stimulation of either the medial prefrontal cortex or the nucleus accumbens normalized microglia density and soma size in main projection areas including the hippocampus and in the area around the electrode implantation. We conclude that in parallel to an alleviation of the symptoms in the rat MIA model, deep brain stimulation has the potential to prevent the neuroinflammatory component in this disease.

1. Introduction

In recent years it became evident that maternal assaults during pregnancy constitute a common risk factor for a plethora of neurodevelopmental afflictions including autism, cerebral palsy, psychosis and schizophrenia (Atladottir et al., 2010; Boksa, 2010; Brown and Susser, 2002; Vuillermot et al., 2010; Wu and Colford, 2000). To this end, prenatal immunological adversities, caused by maternal infection and/or inflammatory response, are believed to initiate a cascade of aberrant neurodevelopmental events. Their specific interaction with varied genetic and environmental factors eventually determine the nature of the disorder (Estes and McAllister, 2016; Giovanoli et al., 2013). This understanding has kindled an animal experimental approach of modelling neurodevelopmental afflictions based on the introduction of immune-activating agents to pregnant rodents in different stages of gestation.

One such approach is the maternal immune activation (MIA) model of schizophrenia in which the administration of polyriboino sinic–polyribocytidylic acid (poly I:C), a synthetic double stranded RNA, to pregnant rats on their gestational day 15 elicits a viral-like immune response via activation of Toll-like receptor 3. The progeny of poly I:C treated dams exhibit a battery of schizophrenia-relevant behavioral and neuropathological abnormalities. Among these are deficits in sensorimotor gating, latent inhibition and reversal learning (Ozawa et al., 2006; Zuckerman et al., 2003; Zuckerman and Weiner, 2003) as well as dysregulations in dopaminergic and glutamatergic neurotransmission (Hadar et al., 2015; Winter et al., 2009) structural changes (Piontkewitz et al., 2011a, 2012a), and downregulation of adult hippocampal neuroge-

* Corresponding author at: Max-Delbrück-Center of Molecular Medicine, Cellular Neuroscience, Robert-Rössle-Str. 10, 13125 Berlin, Germany.
E-mail address: susanne.wolf@mdc-berlin.de (S.A. Wolf).
[1] Equal contribution.

http://dx.doi.org/10.1016/j.bbi.2016.12.003
0889-1591/

nesis (Mattei et al., 2014; Meyer et al., 2010a; Wolf et al., 2011). In regard to the microglia phenotype in the MIA model, some studies report an increase in cell density and cytokines (Juckel et al., 2011; Li et al., 2014; Mattei et al., 2014; Van den Eynde et al., 2014; Zhu et al., 2014) while others report no change of these parameters (Giovanoli et al., 2015, 2016). The offspring of poly I:C treated dams exhibit schizophrenia-relevant behavioral abnormalities at the onset of adulthood similar as observed in humans; neurobiological and anatomical abnormalities either precede or accompany the behavioral manifestation (Hadar et al., 2015; Meyer et al., 2008; Piontkewitz et al., 2012a; Zuckerman and Weiner, 2003). The MIA model therefore provides a solid experimental paradigm for studying the progressive nature of schizophrenia. Not surprisingly this model has been used for testing a variety of therapeutic but also preventive approaches in an effort to halt or even reverse disease progression. The first preventive studies applied antipsychotic medications to MIA offspring during a presymptomatic period which is equivalent to the human presymptomatic adolescence period of schizophrenia (Meyer et al., 2010b; Piontkewitz et al., 2011b; Piontkewitz et al., 2009, 2012b). Those interventions yielded encouraging results as they were effective in preventing schizophrenia-relevant behavioral abnormalities along with brain structural and biochemical alterations observed in this model. Moreover, these animal studies conform to the scarce human studies done on individuals at "high-risk" to develop schizophrenia who in some cases were shown to benefit from an early prophylactic treatment with antipsychotics (McGlashan et al., 2006; McGorry et al., 2002; Salokangas and McGlashan, 2008). Meanwhile further interventions have been tested for their preventive potential in MIA rats: theCOX-2 inhibitor celecoxib given during puberty (postnatal days 35–46) protected adult rats from hyperlocomotion induced by MK-801 challenge (Frueh et al., 2016a). Additionally, minocycline treatment prevented behavioral abnormalities along with altered microglia in the MIA model (Mattei et al., 2014). Together with the growing acceptance of MIA as a shared risk factor for central nervous system diseases, including neuropsychiatric and neurodevelopmental disorders as well as accumulating evidence pointing to dysregulated immune pathways in these states (reviewed in (Frueh et al., 2016b), the two latter studies highlight the potential of intervening with microglia at the prodromal phase in an effort to prevent disease development.

Recently we have shown that pre-symptomatic targeted neuromodulation of the medial prefrontal cortex (mPFC) during adolescence prevented the manifestation of behavioral and structural deficits in adult MIA rats (Hadar et al., in revision). In this study we sought to investigate whether the therapeutic effect of preventive neuromodulation - similar to preventive minocycline – also involves alterations of microglia. We here studied how continuous deep brain stimulation (DBS) to the medial prefrontal cortex (mPFC) or to the nucleus accumbens (Nacc) of adolescent male MIA offspring affects microglia features in adulthood.

2. Materials and methods

2.1. Animals

Experiments were performed according to the guidelines of the European Union Council Directive 2010/63/EU for care of laboratory animals and after approval of the local ethic committees (Regierungspräsidium Dresden and Senate of Berlin, protocol numbers 24-9168.11-1/2011-51 and G 0206/14). Rats were housed in a temperature and humidity controlled vivarium with a 12-h light–dark cycle (lights on: 6 a.m. to 6 p.m.) with an access to food and water *ad libitum*. All efforts were made to minimize animal suffering and to reduce the number of animals required.

Wistar rats (Harlan Laboratories, Indianapolis, IN, USA) 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 Aldrich, St. Louis, MO, USA) dissolved in saline, or saline alone (Hadar et al., 2015; Zuckerman et al., 2003) under isoflurane anesthesia. On post-natal day (PND) 21, pups were weaned and housed according to sex and litter and maintained undisturbed until surgery. Only male rats were included for this study. On PND 33-34 surgeries for implantation of stimulating electrodes were performed on poly I:C (n = 33) and NaCl (n = 35) offspring. Electrical stimulation (or sham) began on PND 35 and was constantly delivered until PND 47. Rats were left undisturbed for 12 weeks until subsequent immunohistochemistry analysis. Each experimental group consisted of offspring derived from multiple independent litters. Male offspring was derived from 11 litters (5 saline, 6 poly I:C) for immunohistochemical analysis and from 12 litters (6 saline, 6 poly) for FACS and cytokine analysis and 6 litters (3 saline and 3 poly) for behavior and baseline analysis without DBS. The age of the animals at the day of sacrifice and subsequent preparation for analysis was 18–19 weeks (Fig. 1A).

2.2. Chronic deep brain stimulation (DBS)

Stereotactic surgeries were conducted under a balanced anesthesia (Fentanyldihydrogencitrat 0.005 mg/kg, Midazolamhydrochlorid 2.00 mg/kg, Medetomidin 0.15 mg/kg). Monopolar platinum iridium electrodes (E363/6/SP, PlasticsOne, Roanoke, VA, USA) were bilaterally implanted at apical: +3.2 mm, lateral: 0.7 mm, ventral: −3.3 mm (for mPFC) or apical: +1.0 mm, lateral: 1.2 mm, ventral: −6.5 mm (for Nacc) (according to the atlas of Paxinos and Watson, 1997). A screw ground electrode (E363/20/SP, PlasticsOne, Roanoke, VA, USA) was implanted and plugged directly into a socket (363 plug, PlasticsOne, Roanoke, VA, USA) together with the stimulating electrodes. The socket was cemented to the skull surface using dental acrylic cement (Technovit® Heraeus-Kulzer, Hanau, Germany or Popco dental, Israel). When surgeries were completed, rats were dressed with rodent jackets (Harvard Apparatus Ltd., Cambridge, UK). Electrode pedestals were connected to a microstimulator (Ewing et al., 2013) via a 3 channel 363-SL/3 cable (PlasticsOne, Roanoke, VA, USA). Devices were then attached to the jackets using an adhesive hook-and-loop tape and stimulation was initiated. Stimulation parameters were: 130 Hz with 150 μA pulses and 90 μs in duration, biphasic stimulation mode. Stimulation was continuously delivered for a period of 12 days, i.e. current was constantly delivered for 24 h over all 12 days. Thereafter stimulators were turned off and disconnected from the electrode pedestals; jackets were also removed. Brains were processed approximately 12 weeks after DBS treatment.

2.3. Pre-pulse inhibition

Pre-pulse inhibition (PPI) of the startle response (SR) 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 (Hadar et al., 2015; Klein et al., 2013; Mattei et al., 2014). Experiments consisted of a 5 min acclimatization phase and the test session. Throughout experiment, background noise was set at 60 dB sound pressure level (SPL). During acclimatization, animals received 10 initial startle stimuli (100 dB SPL, white noise, 20 ms). The test session consisted of 7 different trial types delivered each 10 times in a pseudo random order with an inter-trial interval of 20–30 s: 1) startle-pulse alone (100 dB SPL white noise, 20 ms), 2) control (no stimulus), 3&4) pre-pulse alone (72/68 dB, pure tone, 10 kHz, 20 ms); 5–7) pre-pulse (72/68 dB) each followed by a startle-