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

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ABSTRACT

A growing body of evidence sheds light on the neurodevelopmental nature of schizophrenia with symptoms typically emerging during late adolescence or young adulthood. We compared the pre-symptomatic adolescence period with the full symptomatic period of adulthood at the behavioral and neurobiological level in the poly I:C maternal immune stimulation (MIS) rat model of schizophrenia. We found that in MIS-rats impaired sensorimotor gating, as reflected in disrupted prepusle inhibition (PPI), emerged post-pubertally, with behavioral deficits being only recorded in adulthood but not during adolescence. Using post mortem HPLC we found that MIS-rats show distinct dopamine and serotonin changes in the medial prefrontal cortex (mPFC), nucleus accumbens (Nacc), caudate putamen, globus pallidus, and hippocampus. Further, FDG-PET has shown that these animals had lower glucose uptake in the ventral hippocampus and PFC and a higher metabolism in the amygdala and Nacc when compared to controls. Changes in neurotransmission and metabolic activity varied across brain structures with respect to first appearance and further development. In the mPFC and Hipp, MIS-rats showed abnormal neurochemical and metabolic activity prior to and with the development of behavioral deficits in both adolescent and adult states, reflecting an early impairment of these regions. In contrast, biochemical alteration in the Nacc and globus pallidus developed as a matter of age. Our findings suggest that MIS-induced neurochemical and metabolic changes are neurodevelopmental in nature and either progressive or non-progressive and that the behavioral deficits manifest as these abnormalities increase.

1. Introduction

Converging evidence from epidemiology, neuroimaging and post-mortem studies suggests that schizophrenia is a neurodevelopmental disorder with disruptions to early brain development interacting with peri-adolescent brain maturation and leading to aberrant behavior, typically emerging during late adolescence or young adulthood. Although this postnatal delay is a characteristic feature of schizophrenia, the exact course and neurobiological level of the maldevelopment are not fully understood. Animal models serve as important tools for identifying and studying neurobiological alterations from early age to full symptom manifestation. However, only few experimental preclinical approaches consider developmental aspects.

Based on the observations that prenatal exposure to infection constitutes a risk factor for schizophrenia, animal models implicating maternal immune stimulation (MIS) have been established. Exposing pregnant rodents to the viral mimic polyriboinosinic-polyribocytidilic acid (poly I:C) is a commonly used neurodevelopmental approach to model schizophrenia. In this model, MIS results in the emergence of myriad of behavioral, neurochemical and brain structural abnormalities in the offspring, all related to schizophrenia (Meyer and Feldon, 2012). Previous studies (Piontkewitz et al., 2012) have demonstrated that the behavioral abnormalities induced by poly I:C first emerge in adulthood, resembling the developmental delay of symptom manifestation observed in the clinic. In contrast, neuropathological alterations have been detected at different time points. While some are seen during

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adolescence and predate behavioral deficits (i.e. reduced hippocampal volumes and neurogenesis), others are first observed in adulthood (i.e. enlarged lateral ventricles and reduced prefrontal cortex volumes) (Piontkewitz et al., 2009). It was this progressive nature of brain abnormalities that led clinicians and scientists to administer atypical antipsychotic drugs prior to the full manifestation of symptoms in an attempt to halt disease progression (McGlashan et al., 2006; Piontkewitz et al., 2009).

In this study, we were interested in comparing behavioral and neurobiological characteristics of pre-symptomatic adolescents with the full symptomatic period of adulthood using offspring of MIS rats. More specifically, we sought to study the protracted emergence of a schizophrenia related behavior (i.e. deficits in sensorimotor gating as reflected in disrupted prepulse inhibition), along with the development of abnormal brain activity patterns using 18 fluoro desoxyglucose positron emission tomography (FDG-PET) and changes in neurotransmitter levels using post mortem HPLC.

2. Methods and materials

2.1. Animals

Adolescent (post natal day (PND) 35 and 60) and adult (PND100) male Wistar rats were housed 2–4/cage in a temperature and humidity controlled vivarium with a 12-h light–dark cycle and with ad lib food and water. Experiments were performed during day time, according to the guidelines of the European Union Council Directive 2010/63/EU for care of laboratory animals and were approved by the local ethic committee (Regierungspräsidium Dresden, Germany for behavioral and biochemical studies and Ethics Committee for Animal Experimentation of Hospital Gregorio Marañón Madrid, Spain for FDG-PET studies).

2.2. Prenatal Poly I:C treatment and allocation of animals to experimental groups

On gestation day 15, pregnant dams (Harlan Laboratories, Germany and Spain) were given a single i.v. injection to the tail vein of either poly I:C (4 mg/kg; SIGMA, Germany) dissolved in saline, or saline alone (Zuckerman et al., 2003; Klein et al., 2013). Behavioral phenotyping was performed on 35, 60 and 100 day old offspring ($n = 10$ in both poly I:C and saline groups). Biochemical analysis was performed on brains of 35 and 100 day old offspring (PND35: $n = 10$ poly I:C and $n = 10$ saline, PND100: $n = 16$ poly I:C and $n = 21$ saline offspring). FDG-PET analysis was carried-out in two imaging sessions at PND35 and 100 ($n = 15$ poly I:C and $n = 10$ saline offspring). Each experimental group consisted of only male offspring derived from multiple independent litters.

2.3. Behavioral phenotyping

Prepulse inhibition (PPI) of the acoustic startle response (ASR) was measured in a sound-attenuated chamber using a movement-sensitive piezoelectric measuring platform (Startle Response System, TSE, Germany). Test sessions consisted of seven different trial types delivered in pseudorandom order: 1) pulse alone (100 dB sound pressure level (SPL), white noise, 20 ms); 2) control (no stimulus); 3e4) prepulse alone (72 or 68 dB, pure tone, 10 kHz, 20 ms); 5e7) prepulse (72, 68, or 64 dB) each followed by a pulse with an inter-stimulus interval of 100 ms. A total of 10 presentations of each type was given with 20-30 s inter-trial intervals. Background noise intensity during the whole experiment was 60 dB SPL, white noise. The average PPI over the three prepulse intensities was calculated (Klein et al., 2013; Mattei et al., 2014).

2.4. Post-mortem neurochemical analyses

Rats were decapitated and micropunches were taken from 0.5–1 mm thick brain slices from the medial prefrontal cortex (mPFC), nucleus accumbens (Nacc), caudate-putamen (CPu), hippocampus (Hipp), globus pallidus (GP), thalamus (Thal) and ventral tegmental area (VTA). Monoamines (dopamine (DA), 5-HT) and their metabolites (DOPAC, HVA, 5-HIAA) were separated on a column (ProntoSil 120-3-C18-SH; Bischoff Analysentechnik und -geräte GmbH, Germany) and electrochemically detected (41,000, Chromsystems Instruments & Chemicals GmbH, Germany). Glutamate and GABA were precolumn-derivatized with *o*-phthalaldehyde-2-mercaptoethanol, separated on a column (ProntoSil C18 ace-EPS) and detected by their fluorescence at 450 nm after excitation at 330 nm (Winter et al., 2009).

2.5. Imaging

[^{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 (3% induction and 1.5% maintenance in 100% O_2) using a small-animal PET/CT scanner (ARGUS PET/CT, SEDECAL, Madrid). Images were reconstructed using a 2D OSEM (ordered subset expectation maximization algorithm) with a spatial resolution of 1.45 mm FWHM (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 rat in each group (PND35 and PND100) was additionally scanned using a 7-T Biospec 70/20 MRI scanner (Bruker, Germany) under sevoflurane anaesthesia (4.5% induction and 2.5% maintenance in 100% O_2) to provide anatomical templates for analysis of PET images. 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 FOV of 3.5×3.5 cm^2).

PET images were co-registered in order to perform voxel-by-voxel comparisons and obtain statistical parametric maps. A random reference CT scan was selected for each group (CTref35 for PND35 and CTref100 for PND100), and all CT studies were co-registered with the respective reference CT scan. A non-rigid registration transformation was calculated in order to align CTref35 with CTref100 and applied to CT studies in group PND35 already aligned with CTref35. Final CT images were aligned with CTref100. The spatial transformation obtained for each CT was then applied to the corresponding PET image. MRI studies were spatially co-registered to the reference CT scan. A brain mask was segmented and applied to PET images. Resulting images were smoothed and voxel values were normalized to the average brain intensity (Pascau et al., 2009).

2.6. Statistical analysis

One way ANOVA and one-sample *t*-test were used for behavioral analysis. Two way ANOVAs with the factors MIS and age were used for biochemical analysis. When appropriate, ANOVAS were followed by Holm Sidak post hoc test. PET data was analyzed using SPM5 software package (Wellcome Trust Centre for Neuroimaging, UK). Groups were compared using a Flexible Factorial test, uncorrected for multiple comparisons. To reduce type I error, a 50-voxel clustering threshold was applied. A p-value of 0.05 was considered statistically significant.

3. Results

3.1. PPI

A significant difference was found in saline offspring ($F(2,29) = 13.794$, $p < 0.001$) but not in poly I:C offspring ($F(2,29) = 0.059$, $p = 0.943$), such that higher levels of PPI were apparent at PND100

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