Hypersynchronicity in the default mode-like network in a neurodevelopmental animal model with relevance for schizophrenia

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Abstract

Background: Immune activation during pregnancy is an important risk factor for schizophrenia. Brain dysconnectivity and NMDA receptor (NMDAR) hypofunction have been postulated to be central to schizophrenia pathophysiology. The aim of this study was to investigate resting-state functional connectivity (resting-state functional MRI-rsfMRI), microstructure (diffusion tension imaging-DTI) and response to NMDAR antagonist (pharmacological fMRI-phMRI) using multimodal MRI in offspring of pregnant dams exposed to immune challenge (maternal immune activation-MIA model), and determine whether these neuroimaging readouts correlate with schizophrenia-related behaviour. Methods: Pregnant rats were injected with Poly I:C or saline on gestational day 15. The maternal weight response was assessed. Since previous research has shown behavioural deficits can differ between MIA offspring dependent on the maternal response to immune stimulus, offspring were divided into three groups: controls (saline, n=11), offspring of dams that gained weight (Poly I:C WG, n=12) and offspring of dams that lost weight post-MIA (Poly I:C WL, n=16). Male adult offspring were subjected to rsfMRI, DTI, phMRI with NMDAR antagonist, behavioural testing and histological assessment. Results: Poly I:C WL offspring exhibited increased functional connectivity in default mode-like network (DMN). Poly I:C WG offspring showed the most pronounced attenuation in NMDAR antagonist response versus controls. DTI revealed no differences in Poly I:C offspring versus controls. Poly I:C offspring exhibited anxiety. Conclusions: MIA offspring displayed a differential pathophysiology depending on the maternal response to immune challenge. While Poly I:C WL offspring displayed hypersynchronicity in the DMN, altered NMDAR antagonist response was most pronounced in Poly I:C WG offspring.

Keywords: diffusion MRI, MK-801, prenatal immune activation, autism spectrum disorder, biomarker

1. Introduction

Immune activation during pregnancy is an important risk factor for several neuropsychiatric disorders, including schizophrenia, which supports the neurodevelopmental hypothesis of this disorder [1, 2]. Based on this observation, offspring of rodent dams that were exposed to an immune stimulus during pregnancy, i.e. maternal immune activation (MIA), have been repeatedly and successfully used as animal models with relevance for schizophrenia, but also other neuropsychiatric disorders such as autism spectrum disorders (ASD) (reviewed in [1]). Several immune stimuli have been used to induce MIA in rodents, including the synthetic double-stranded RNA polyinosinic:polycytidylic acid (Poly I:C), which activates the Toll-like receptor 3 and thus acts as a viral mimetic. This immunostimulant is administered to pregnant dams and leads to cytokine induction in the mother, which eventually throws the cytokine equilibrium in the developing brain of the foeti off-balance. This leads to altered foetal brain development and induces long-lasting changes in brain structure and function and behaviour (reviewed in [3]). The Poly I:C model has good construct, face and predictive validity for several neuropsychiatric disorders (including schizophrenia and ASD) [3, 4]. In this study, we have evaluated the Poly I:C model primarily within the context of schizophrenia, but the results are also relevant within the context of ASD.

Several hypotheses regarding the underlying pathophysiology of schizophrenia have been proposed, including the dysconnectivity and NMDA receptor (NMDAR) hypofunction hypotheses [5, 6].

Many neuroimaging studies have reported altered functional and structural connectivity in schizophrenia patients [7]. Of particular interest is the default mode network (DMN), a network of brain regions that is active during rest. Several studies investigating DMN reported increased functional connectivity (FC) in this network in schizophrenia patients, as well as unaffected first-degree relatives and individuals at ultra-high risk for psychosis (reviewed in [8, 9]). In addition, schizophrenia patients show abnormal activity or deactivation of the DMN during several tasks (activity or deactivation dependent on the brain region and the specific task) and aberrant DMN connectivity/deactivation correlated with symptom severity (reviewed in [8]). Some studies have shown that DMN connectivity/deactivation alters with antipsychotic treatment in schizophrenia patients concurrent with symptomatic improvement [10-12]. Altogether this suggests that neuroimaging of the DMN may be a promising biomarker of disease and potentially predictive of treatment response.

A decreased structural connectivity has also been described in the DMN of schizophrenia patients, reviewed in [8]. To date, a few diffusion magnetic resonance imaging (MRI) studies have been performed in MIA offspring, focusing on microstructural changes. Changes in fractional anisotropy or apparent diffusion coefficient in white and grey matter at ages ranging from birth to adulthood have been observed [13-17]. However, no FC studies have been performed in MIA offspring.

A mounting body of evidence supports NMDAR hypofunction as a key factor of schizophrenia pathophysiology. NMDAR antagonists can produce the entire range of schizophrenia symptoms in healthy subjects [5] and anti-NMDAR antibodies can induce a limbic encephalitis characterised by severe psychotic episodes [18]. Abnormal transcript and protein expression of NMDAR subunits have been observed in

schizophrenia patients, as well as altered glutamate levels and abnormalities of modulators of the NMDAR glycine modulatory site [5]. Altered NMDAR function (through behavioural testing with NMDAR antagonists) and expression has been demonstrated in MIA models [19-26].

Interestingly, depending on the maternal weight response to immune challenge (weight loss vs. gain), offspring of polyinosinic:polycytidylic acid (Poly I:C)-treated rats have been shown to display different behavioural deficits, including different locomotive responses to NMDAR antagonists [21, 27-29]. This indicates that the maternal response to the immune stimulus is an important determinant of the long-term behavioural outcome in offspring, and likely also of the underlying pathophysiology.

Since the behavioural outcome in this animal model is variable and poorly replicable, novel predictive biomarkers are needed for evaluation of new treatments for schizophrenia at the preclinical stage. As a first step, we evaluated resting-state functional connectivity, microstructural integrity and NMDAR antagonist response using multimodal MRI in rats prenatally exposed to an immune challenge and evaluated whether changes in these *in vivo* imaging read-outs correlated with schizophrenia-like behavioural deficits.

In this study we aimed to elucidate the following research questions:

i) Is there increased FC in the default mode-like network (DMN) of adult rats exposed to prenatal immune challenge?

ii) Are FC changes associated with microstructural alterations?

iii) Do adult MIA offspring display a different hemodynamic response to an NMDAR antagonist?

iv) Do offspring of dams that lose weight post-MIA show a different pathophysiology (dysconnectivity/NMDAR antagonist response) from offspring of dams that gain weight?

v) Do FC, microstructural and NMDAR antagonist response abnormalities correlate with behavioural deficits in adult MIA offspring?

vi) Is an altered response to an NMDAR antagonist related to abnormal NMDAR levels?

2. Material and methods

2.1. Animals

Thirteen male and 13 female 10-week old Wistar Han IGS rats were purchased from Charles River Laboratories (France). Animals were single-housed in a temperature- and humidity-controlled room on a 12-hour light-dark cycle with standard food and water available ad libitum. After minimum one week of acclimatization, animals were subjected to timed mating. Male offspring were group-housed under the same conditions. Animals were treated in accordance with EU directive 2010/63/EU. Animal experiments were approved by the animal ethics committee of the University of Antwerp, Belgium (ECD 2015-77).

2.2. Study design

Study design is shown in Fig.1. Pregnant Wistar Han dams received an immune challenge (viral mimetic Poly I:C, N=9) or saline (N=4) on gestational day (GD) 15 as previously described [27]. Maternal weight and immune responses were assessed as before [27]. Offspring were divided into three groups: controls (n=11), offspring of dams that gained weight post-MIA (Poly I:C Weight Gain offspring, n=12) and offspring of dams that lost weight or showed no weight change (Poly I:C Weight Loss offspring, n=16) [27]. The Poly I:C-treated dams that gained weight gained on average 1.43±0.56 % in body weight while controls gained on average 0.47±0.26 % in body weight. This difference was not significant. Poly I:C-treated dams that lost weight or showed no weight change lost on average 1.00±0.60 % of their body weight (Fig.2). On the day of birth, litters were culled to eight pups per litter. Adult male offspring (usually three per litter) were subjected to MRI and behavioural testing during postnatal weeks (PNW) 12 and 13 (tests are described below). MRI consisted of resting-state functional MRI (rsfMRI), diffusion

tensor imaging (DTI) (scanning session 1), and pharmacological fMRI (phMRI) with the NMDAR antagonist MK-801 (dizocilpine, a non-competitive NMDAR antagonist) (10 min baseline and 30 min post-intravenous MK-801 administration) (scanning session 2). Behavioural tests included prepulse inhibition of the acoustic startle reflex, spontaneous locomotion, open field test, sucrose preference test and MK-801-induced locomotion [27]. PhMRI and locomotion with MK-801 were the last tests to be performed, in order to exclude possible effects of the psychotomimetic drug on the other imaging and behavioural read-outs. Animals were sacrificed in PNW 14 and their brains processed for GluN1 immunohistochemical staining and quantification.

2.3. Maternal immune activation (MIA) and response

Pregnant dams were injected subcutaneously (s.c.) with saline or 4 mg/kg Poly I:C (10 ml/kg) on GD 15. The weight of the dam was recorded before and 24 h after Poly I:C/vehicle injection to calculate the maternal weight response.

2.4. Magnetic resonance imaging (MRI)

Resting-state functional MRI was performed to assess spontaneous low-frequency (0.01-0.1 Hz) fluctuations in the blood-oxygen level dependent (BOLD) signal in brain regions, reflecting spontaneous neuronal activity patterns. A high correlation between the BOLD signal fluctuations of two anatomically separate brain regions suggests a high functional connectivity between these regions. This technique was used to assess functional connectivity in the DMN of MIA offspring.

Diffusion tensor imaging was performed to assess the random displacement of water molecules due to molecular diffusion or Brownian motion in each voxel and was used to probe tissue microstructure in the brain of MIA offspring.

Pharmacological functional MRI was used to assess the effect of the NMDAR antagonist MK-801 on BOLD signal, and thus indirectly neuronal activity, in the brain of MIA offspring.

2.4.1. Acquisition

All data were acquired on a 7T PharmaScan MR system (Bruker, Germany) with Paravision 5.1 software using a standard Bruker crosscoil set-up with a quadrature volume coil and a quadrature surface coil designed for rats (Bruker, Germany). The rats' head was immobilized in an MR-compatible stereotaxic device using blunt earplugs and a tooth bar.

RsfMRI, DTI and three-dimensional (3D) T_2-weighted anatomical MRI scans were all performed during one scanning session in PNW 12. PhMRI with the NMDAR antagonist MK-801 was performed during a second scanning session in PNW 13.

For the first scanning session, rats were anesthetized with isoflurane in a mixture of O_2 (30%) and N_2 (70%) (5% induction; Forene; Abbott, Belgium) after which a s.c. bolus injection of 0.05 mg/kg medetomidine hydrochloride (Domitor, Pfizer, Karlsruhe, Germany) was administered to sedate the animals. After 15 min, continuous s.c. infusion of 0.1 mg/kg/h medetomidine was started. Following bolus injection, isoflurane was gradually decreased to 0.4% during the rsfMRI scan. After the rsfMRI scan was finished, infusion of medetomidine was discontinued and isoflurane was increased to ± 1%. At the end of the scanning session, animals received a s.c. injection of 0.25 mg/kg atipamezole (Antisedan, Pfizer, Karlsruhe, Germany) to reverse the effects of medetomidine. For the second scanning session, only isoflurane was used as anesthetic (5% induction, 2% maintenance). Breathing rate and blood oxygenation were monitored constantly using a

pressure sensitive pad and a pulse oximeter (MR-compatible Small Animal Monitoring and Gating System, SA Instruments, Inc., USA) and maintained between normal physiological ranges. The temperature of the animals was monitored by means of a rectal probe and maintained at (37 ± 0.5) °C through a feedback-controlled warm air system (MR-compatible Small Animal Heating System, SA Instruments, Inc., USA).

Three orthogonal multi-slice Turbo Rapid Acquisition with Relaxation Enhancement (RARE) T_2-weighted images were acquired to ensure uniform slice positioning for rsfMRI, DTI and phMRI data of different animals. A field map was acquired in each scanning session to measure field homogeneity, followed by local shimming, which corrects for the measured inhomogeneity in a rectangular volume within the brain.

During the first scanning session, coronal rsfMR images were acquired between 40 and 50 min post-bolus medetomidine injection using a single-shot T_2*-weighted gradient-echo echo planar imaging (GE-EPI) sequence with the following parameters: repetition time (TR) 2000 ms, echo time (TE) 29 ms, 20 slices of 0.7 mm (limited to cerebrum), slice gap 0.1 mm, 300 volumes, scan duration 10 min. The field of view (FOV) was (30 x 30) mm^2 and the matrix size [128 x 128], resulting in voxel dimensions of (0.234 x 0.234 x 0.8) mm^3.

Coronal diffusion-weighted (DW) images were acquired with a two-shot spin-echo echo planar imaging (SE-EPI) sequence with 60 optimally spread diffusion gradient directions. In addition, 15 non-DW b_0 images (b-value 0 s/mm^2; 5 b_0 per 20 DW images) were acquired. The following imaging parameters were used: TR 7500 ms, TE 26 ms, diffusion gradient pulse duration δ 4 ms, diffusion gradient separation Δ 12 ms, b-value 800 s/mm^2, 20 slices of 0.7 mm (limited to cerebrum, same slices as rsfMRI), 0.1 mm slice gap,

scan duration approx. 20 min. The FOV was (30 x 30) mm^2 and the matrix size [128 × 128], resulting in voxel dimensions of (0.234 × 0.234 x 0.8) mm^3.

A 3D RARE T_2-weighted scan of the entire brain was acquired with the following parameters: TR 2250 ms, TE 11 ms (TE_{eff} 44 ms), RARE factor 8, scan duration 15 min. The FOV was (29 x 20 x 15) mm^3 and the acquisition matrix [256 × 64 × 50], resulting in a spatial resolution of (0.113 x 0.313 × 0.300) mm^3.

During the second scanning session, coronal phMR images were acquired using a single-shot T_2*-weighted GE-EPI sequence with the following parameters: TR 4000 ms, TE 25 ms, 13 slices of 1.0 mm (limited to cerebrum), slice gap 0.2 mm, 600 volumes, scan duration 40 min. The FOV was (25 x 25) mm^2 and the matrix size [128 x 64], resulting in voxel dimensions of (0.195 x 0.391 x 1.200) mm^3. Baseline scans were acquired for 10 min (150 volumes) after which a single bolus of 0.2 mg/kg MK-801 (0.8 ml/kg) was administered through an intravenous (i.v.) catheter and the measurements continued until 30 min (450 volumes) post-injection.

2.4.2. Data preprocessing

All image preprocessing was performed using SPM12 in MATLAB 2014a (MathWorks, USA).

Resting-state fMRI. First, images within each session were realigned to the first image using a least-squares approach and a 6-parameter (rigid body) spatial transformation. Secondly, EPI images were coregistered to the individual 3D RARE scan. The individual 3D RARE scans were normalised to a study-specific 3D T_2-weighted anatomical template using an affine transformation followed by the estimation of the nonlinear deformations. These transformation parameters were used to normalise all EPI datasets to the study-

specific 3D template. This template was made in Advanced Normalisation Tools (ANTs) using all individual 3D RARE scans. Next, in plane smoothing was done using a Gaussian kernel with full width at half maximum (FWHM) of twice the voxel size (FWHM (0.468 x 0.468 x 0.8) mm^3). Finally, datasets were filtered using the Resting State fMRI Data Analysis toolbox (REST1.8). The band-pass filter was set between 0.01 and 0.1 Hz to retain the low frequency fluctuations of the blood-oxygen level dependent (BOLD) signal time course.

Diffusion tensor imaging. Firstly, images were realigned by performing a rigid registration between the b_0 images, which was followed by an extended registration taking all the DW images into account. Secondly, DW images were coregistered to the individual 3D RARE scan. The individual 3D RARE scans were normalised to the study-specific 3D T_2-weighted anatomical template using an affine transformation followed by the estimation of the nonlinear deformations. These transformation parameters were used to normalise all DWI datasets to the study-specific 3D template.

Pharmacological fMRI with MK-801. All images within each session were realigned to the mean image using a rigid registration. Secondly, all datasets were normalised to the study-specific EPI template using an affine transformation followed by the estimation of the nonlinear deformations. The study-specific EPI template was made using the EPI data from 8 control rats. Finally, in plane smoothing was performed using a Gaussian kernel with FWHM of twice the voxel size (FWHM (0.390 x 0.782 x 1.200) mm^3).

2.4.3. Data analysis

Resting-state fMRI. A region of interest (ROI)-based analysis was performed for all three groups including the following ROIs: left and right anterior cingulate cortex (Cg),

retrosplenial cortex (RS), parietal association cortex (PtA), posterior parietal cortex (PtP), temporal association cortex (TeA), primary motor cortex (M1), primary somatosensory cortex (S1), primary auditory cortex (Au1) and primary visual cortex (V1). These ROIs were defined as square regions of four voxels using MRIcron software, based on the Paxinos and Watson rat brain atlas, and delineated on the study-specific 3D template. The filtered rsfMRI datasets and a mask for each ROI were used as an input to REST to extract for each subject the time courses for each ROI. Correlation coefficients between the time courses of each pair of ROIs were calculated and z-transformed using an in-house program developed in MATLAB. The average group z-transformed correlation values were presented in a functional connectivity (FC) strength matrix. The FC strength in the default mode-like network was computed by calculating the average of the pairwise correlation values between Cg, RS, PtA, PtP and TeA. The default mode-like network in rat was defined based on previous literature [30]. Orbitofrontal and prelimbic cortex could not be included in our analysis due to susceptibility artefacts in the images at the level of these regions. The FC strength in motor and sensory areas was computed by calculating the average of the pairwise correlation values between M1, S1, Au1 and V1.

Seed-based analyses were performed for each of the ROIs within the default mode-like network. BOLD signal time courses for each of these ROIs were extracted from all filtered datasets using the REST toolbox. These were used in SPM12 in a generalised linear model with the motion parameters resulting from the realignment as covariates to obtain individual statistical FC maps for all animals with the right Cg, RS, PtA, PtP and TeA as seed regions. A whole brain mask was implemented in this analysis. The total cluster sizes of these individual FC maps (family wise error (FWE) corrected, $p \leq 0.05$, minimal cluster size $k \geq 10$) were determined. In addition, the mean T-value was extracted from each

individual FC map. A mask containing all clusters that were significantly correlated with the seed region at group-level (one-sample t-test per group, FWE corrected, $p \leq 0.05$, $k \geq 10$) was used in this analysis. This mask was the sum of all clusters that were significantly correlated with the seed in each group. Finally, mean FC maps for each seed region were computed per group (one-sample t-test, FWE corrected, $p \leq 0.05$, $k \geq 10$). Further statistics to compare between groups are described in 2.7.

Diffusion tensor imaging. The diffusion tensor was estimated and the DTI parameter maps were computed (i.e. fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD)). Finally, the DTI parameter maps were smoothed in plane using a Gaussian kernel with FWHM of twice the voxel size (FWHM (0.468 x 0.468 x 0.8) mm^3).

Voxel-based analysis was performed for each of the obtained DTI metrics (i.e. FA, MD, AD and RD) in SPM12. First, we used a whole brain approach. Next, we explored differences within a ROI that contained all cortical DMN regions (bilateral Cg, RS, PtA, PtP and TeA), overlayed as a mask. Statistics are described in 2.7.

Pharmacological fMRI with MK-801. For each animal, the first 140 baseline scans ("pre") were compared with the last 140 scans (i.e. 20-30 min post-injection, "post") in SPM12. The motion parameters resulting from the realignment were added as covariates to account for movement. A whole brain mask was used in this analysis. Mean T-values were extracted from the individual T-maps (pre > post) in MATLAB 2014a for the following ROIs: anterior cingulate cortex (Cg), primary and secondary motor cortex (M1/2), striatum, hippocampus, thalamus, default mode-like network and entire cortex. Finally, mean difference maps were computed for each group (paired t-test, pre > post,

uncorrected, p≤0.01, k≥10). Further statistics to compare between groups are described in 2.7.

2.5. Behaviour

2.5.1. Prepulse inhibition of the acoustic startle reflex

Prepulse inhibition (PPI) of the acoustic startle reflex was assessed for prepulses of three different intensities (70 dB, 75 dB and 80 dB) using standard startle boxes (Kinder Scientific, USA) as previously described [27]. A startle pulse (120 dB) is preceded shortly by a prepulse and the animal's startle response to the startle pulse is measured. A strong prepulse normally results in pronounced PPI, i.e. a reduced startle response to the startle pulse. In schizophrenia patients, PPI is reduced, reflecting a sensorimotor gating deficit [31]. This test was performed to assess whether the MIA offspring displayed a sensorimotor gating deficit.

2.5.2. Spontaneous locomotor activity

Spontaneous ambulatory locomotion was measured for a period of 24 h (12 h light-dark) using a home-cage 4 x 8 photobeam activity system (San Diego Instruments, USA). The average number of photobeam crossings per hour (sum of beam crossings in X and Y direction) was calculated for both the light and the dark phase. This test was performed to assess changes in circadian rest-activity rhythms in MIA offspring, as has been observed in schizophrenia patients [32].

2.5.3. Open field test

Animals were placed in the periphery of a well-lit square arena (48 x 48 cm) and allowed to explore the novel environment for 10 min. During this trial, animals were video-

tracked with EthoVision XT software (version 10.0, Noldus, USA). For analysis, the arena was divided in a central zone (inner square, 24 x 24 cm) and a peripheral zone. The following parameters were calculated: latency to first entry in central zone, number of transitions from periphery to centre, % time spent in centre, % distance moved in centre, total distance moved and mean velocity. This test was performed to assess anxiety in the MIA offspring. Anxiety is a common symptom of schizophrenia [33].

2.5.4. Sucrose preference test

Sucrose preference and total liquid consumption were assessed as previously described during a test period of 24 h, following a habituation period of 48 h [27]. Briefly, two bottles were presented to the animals: one with water and one with sucrose solution. The animals' preference for the sucrose solution was calculated by weighing both bottles before and after the test period. This test was performed in order to assess anhedonia in the MIA offspring, which is commonly observed in schizophrenia patients [34].

2.5.5. MK-801 induced locomotor activity

MK-801 induced hyperlocomotion was measured using the home-cage 4 x 8 photobeam activity system as previously described [27] with one important modification: four hours of locomotor activity (i.e. beam crossings) were recorded following administration of 0.2 mg/kg MK-801 (s.c., 10 ml/kg). This test was performed to assess whether there was an altered behavioural response to an NMDAR antagonist in MIA offspring, as has been observed in schizophrenia patients [5].

2.6. Histology: GluN1

Animals were sacrificed by decapitation. Brains were resected and snap-frozen in isopentane on dry ice (3 min, -35°C) and stored at -80°C. Sagittal sections of 20 μm were

obtained using a CryoStar NX50 cryostat (Thermo Scientific, Belgium) at ca. 1.90 mm lateral to the midline (right hemisphere) and collected in triplicate.

Three consecutive sections per animal were incubated for 1 h with Dako wash buffer (Dako, Belgium) and for 30 min with 10% normal goat serum in PBS to block aspecific binding. Next, sections were incubated for 3 h with anti-GluN1 monoclonal mouse antibody (1:400 in Dako diluent) (Synaptic Systems, Germany). After washing, sections were incubated for 1 h with goat anti-mouse antibody conjugated with horseradish peroxidase (GAM IgG2b-HRP, 1:1000 in PBS), rinsed again, and incubated for 10 min with the colorimetric substrate 3,3'-diaminobenzidine (DAB) for visualization. The reaction was stopped with dH_2O and sections were gradually dehydrated and coverslipped.

Images were obtained with a NanoZoomer-XR slide scanner (Hamamatsu, Japan) equipped with a 20x objective and analysed with ImageJ software. The following ROIs were manually drawn on of the three replicate sections in ImageJ using the curation function of an in-house developed plugin SliceMap [35]: frontal cortex, cortex, corpus callosum, striatum, hippocampus and thalamus. To quantify the GluN1 staining, the image underwent a colour-to-grayscale conversion assuming DAB-like staining, after which the grayscale intensity was normalised to the average intensity in the corpus callosum (reference region). This effectively normalises the signal intensity in each ROI, in order to reduce inter-slice and inter-subject variability in staining. Pixels positive for GluN1 were depicted by a threshold of intensity equalling three times the standard deviation. Finally, for each region the area percentage of positive pixels was determined. The workflow of the image analysis is visualised in Suppl.Fig.1.

2.7. Statistics

Normal distribution of the data was tested using the D'Agostino-Pearson omnibus normality test. Outlier analyses were performed with the ROUT test. Linear mixed model analysis was performed on all data sets to investigate whether litter effects had to be accounted for. Linear mixed models with and without a random intercept for litter were made and a likelihood ratio test was performed to compare the goodness of fit of the two statistical models. Adding litter as a random intercept did not improve the statistical models, indicating that there were no observable litter effects in our study. This is in line with results from our previous study, where we observed that there was high variability in the cytokine response between littermates [27]. Since each of the offspring are enclosed in a separate amniotic sac with an individual foetal circulation, MIA can differentially affect the different offspring from one dam. Differences between the three groups (control, Poly I:C WG and Poly I:C WL offspring) in average FC strength in default mode-like network and motor/sensory areas, total cluster sizes of FC maps, acoustic startle reflex, prepulse inhibition and spontaneous locomotion were analysed using one-way ANOVA tests and Dunnett's multiple comparisons tests as post-hoc tests. When pooling all Poly I:C offspring, unpaired t-tests were used to compare the two groups (control and Poly I:C offspring). Differences in MK-801 induced hyperlocomotion between the groups were investigated using a repeated measures two-way ANOVA test with either Dunnett's (three groups) or Sidak's (two groups) multiple comparisons test as post-hoc tests. Differences between the three groups (control, Poly I:C WG and Poly I:C WL dams or offspring) in maternal weight change, T-values from phMRI T-maps, open field performance, sucrose preference, total liquid consumption, GluN1 levels were analysed using Kruskal-Wallis tests and Dunn's multiple comparisons tests as post-hoc tests. When pooling all Poly I:C offspring/dams, Mann-Whitney U tests were used to compare the two groups (control and Poly I:C offspring/dams). A chi-square test and chi-

square test for trend were used to investigate a difference in the proportion of the group that displayed anhedonia (defined as a sucrose preference of less than 90%) in the different groups. Pearson correlation tests were performed to investigate the relationship between *in vivo* imaging measurements and behavioural assessments. These analyses were performed using GraphPad Prism 6. Statistical significance was set at $p \leq 0.05$. Differences in seed-based FC maps and FA, MD, AD and RD maps between the three groups were investigated using one-way ANOVA tests with F-contrasts to compare between the three groups and t-contrasts to compare between two groups. When pooling all Poly I:C offspring, two-sample t-tests were used to investigate the difference in seed-based FC maps and FA, MD, AD and RD maps between the two groups. To investigate the difference in seed-based FC maps between the different groups, a mask was used that contained all clusters that were significantly correlated with the seed at group-level (FWE corrected, $p \leq 0.05$, $k \geq 10$). This mask was the sum of all clusters that were significantly correlated with the seed in each group. These analyses were performed in SPM12 and MATLAB 2014a. Finally, stepwise regression analyses were performed to investigate whether *in vivo* imaging measurements could explain the variability in the behavioural assessments. Forward stepwise regression analysis was performed in JMP Pro 13 with p-value threshold as stopping rule (probability to enter: 0.25, probability to leave: 0.1).

3. Results

3.1. Variable maternal response to MIA

Pregnant dams injected with Poly I:C exhibited a variable weight response as reported before [27], with some animals having a reduction in weight (N=4) or no weight change (N=1) (Poly I:C WL: N=5, mean ± SEM: (-3.2 ± 1.9) g, -1.00 ± 0.60 % decrease in body weight) and others showing weight gain (Poly I:C WG: N=4, mean ± SEM: (4.7 ± 2.2) g, 1.43 ± 0.56 % increase). There was a significant difference in weight change between Poly I:C WL and Poly I:C WG dams ($p \leq 0.05$) (Fig.2). Pregnant dams injected with saline showed a slight weight increase (N=4, mean ± SEM: (1.4 ± 0.8) g, 0.47 ± 0.26 % increase). There was no significant difference in weight change between controls on the one hand and Poly I:C-treated dams on the other hand (Fig.2).

3.2. Increased FC in DMN of adult Poly I:C WL offspring

Region-of-interest (ROI)-based analysis of the rsfMRI data revealed significantly increased average FC in the DMN of Poly I:C WL offspring vs. controls, but not in Poly I:C WG offspring ($p \leq 0.05$) (Fig.3). FC within the DMN was also significantly increased when comparing all Poly I:C offspring to controls ($p \leq 0.05$) (Fig.3). FC was not significantly altered in non-DMN cortical areas (motor and sensory cortical areas).

Seed-based analysis revealed i) significantly larger total cluster sizes of functionally connected clusters (FWE corrected, $p \leq 0.05$, $k \geq 10$) with posterior parietal cortex (PtP) ($p \leq 0.05$) (Fig.4A,B) and temporal association cortex (TeA) ($p \leq 0.01$) (Fig.4B), and ii) significantly higher mean T-values of the FC maps with parietal association cortex (PtA) ($p \leq 0.05$), PtP ($p \leq 0.05$) and TeA ($p \leq 0.001$) as seed regions in Poly I:C WL offspring vs. controls, but not in Poly I:C WG offspring. No significant group difference was observed

for i) total size of clusters significantly correlated with anterior cingulate cortex (Cg), retrosplenial cortex (RS) and PtA (Fig.4B), and ii) mean T-values of the FC maps with Cg and RS as seed regions. When pooling all Poly I:C offspring, we observed i) a significantly larger total cluster size of significantly correlated clusters with TeA ($p \leq 0.01$) (Fig.4B) and ii) significantly higher mean T-values of FC maps with PtP ($p \leq 0.05$) and TeA ($p \leq 0.01$) in Poly I:C offspring vs. controls. Trends towards significance were observed for i) increased total cluster size of clusters significantly correlated with PtP ($p=0.057$) (Fig.4B) and ii) higher mean T-value of the FC map with PtA as seed in all Poly I:C offspring vs. controls ($p=0.081$).

Statistical difference maps (uncorrected, $p \leq 0.001$, $k \geq 10$) of the FC maps with PtP and TeA as seed regions revealed a difference between Poly I:C WL offspring and controls (Poly I:C WL > control, data not shown). When pooling all Poly I:C offspring, a group difference with controls was only found for the FC maps with TeA as seed region. Statistical difference maps did not reveal any group difference between MIA and control offspring for the FC maps with Cg, RS or PtA as seed regions.

3.3. No microstructural changes in adult MIA offspring

Whole-brain voxel-based analysis (VBA) did not reveal significant differences in fractional anisotropy, mean, axial and radial diffusivity between MIA and control offspring (data not shown). VBA within the DMN did not reveal any significant group differences either (data not shown).

3.4. Attenuated BOLD response to NMDAR antagonist in adult MIA offspring

Administration of NMDAR antagonist MK-801 resulted in a decrease in blood-oxygen-level-dependent (BOLD) signal in all groups. However, in striatum and thalamus of Poly

I:C WG offspring the mean T-values of the pre>post contrast maps were significantly lower compared to controls ($p \leq 0.05$) (Fig.5). There were also trends for lower mean T-values in anterior cingulate ($p=0.088$) and motor cortices ($p=0.081$) of Poly I:C WG offspring vs. controls. The mean T-value in thalamus was also significantly lower in Poly I:C WL offspring compared to controls ($p \leq 0.05$). When taking all Poly I:C offspring together, mean T-values were significantly lower in striatum ($p \leq 0.05$) and thalamus ($p \leq 0.01$) vs. controls (Fig.5). In addition, a trend for a lower mean T-value in hippocampus was observed in all Poly I:C offspring vs. controls ($p=0.062$) (Fig.5). No differences were observed in mean T-values for hippocampus, DMN and entire cortex between any of the groups.

3.5. Subtle behavioural deficits in adult MIA offspring

As expected, the % prepulse inhibition (PPI) increased significantly with prepulse intensity. Surprisingly, a trend towards significance for increased PPI with the 75 dB prepulse was observed in all Poly I:C offspring vs. controls ($p=0.086$) (Fig.6A). No differences were observed in the magnitude of the startle reflex or % PPI with the other prepulse intensities between the different groups.

Poly I:C WG offspring showed a trend for increased spontaneous locomotion during light phase ($p=0.074$) (Fig.6B). A weak trend was observed for decreased activity during dark phase in all Poly I:C offspring vs. controls ($p=0.098$).

Poly I:C WL offspring showed trends for a decreased number of entries into the centre of the open field ($p=0.089$), decreased % time spent in centre ($p=0.072$) and decreased % distance travelled in centre ($p=0.063$) vs. controls (Fig.6C). When pooling all Poly I:C offspring, there was a significantly decreased number of entries into centre ($p \leq 0.05$),

decreased % time spent in centre ($p \leq 0.05$) and decreased % distance travelled in centre ($p \leq 0.05$), as well as a trend for increased latency to first entry into the centre of the open field vs. controls ($p=0.089$) (Fig.6C). There were no differences in total distance travelled or velocity between the groups.

There was no significant difference in sucrose preference or total liquid consumption between the groups (Fig.6D). There was a numerically higher proportion of animals with anhedonia (defined as sucrose preference <90%) in Poly I:C WL and WG groups (both 25%, respectively 3/12 and 4/16 rats) than in controls (9%, 1/11 rats), but this was not significant.

Overall, there was no significant difference in MK-801-induced hyperlocomotion between MIA offspring and control offspring. However, though Poly I:C WG offspring initially showed a similar hyperlocomotive response to MK-801 as controls, their responses started to deviate after approx. 85 min with the Poly I:C WG offspring exhibiting a reduced response to the drug compared to controls. This was significant at 95-100 min post-injection ($p \leq 0.05$) and a trend was observed at 125-130 min post-injection ($p=0.073$) (Fig.6E). No difference was observed between Poly I:C WL offspring and controls or when comparing all Poly I:C offspring vs. controls.

3.6. No altered GluN1 protein levels in MIA offspring

There were no significant differences in the % area of GluN1 staining between the groups in any of the investigated brain regions (data not shown).

3.7. FC in DMN correlates with behaviour in MIA offspring

Correlation analysis was performed between rsfMRI measurements in MIA offspring (FC in DMN, total cluster sizes and mean T-values of seed-based FC maps with Cg, RS, PtA, PtP

and TeA as seed regions) and phMRI measurements (mean T-values in anterior cingulate and motor cortices, striatum, hippocampus and thalamus) on the one hand and the following behavioural assessments in MIA offspring on the other hand: average locomotion during light phase, number of entries into centre, % time spent and % distance moved in centre, % sucrose preference and locomotion 95-100 min post-MK-801.

Correlation analysis revealed significant negative correlations between sucrose preference and i) average FC in DMN ($p \leq 0.01$, $r=-0.54$) (Fig.7), ii) total cluster size of the FC maps with Cg as seed region ($p \leq 0.01$, $r=-0.50$), iii) total cluster size ($p \leq 0.05$, $r=-0.46$) and mean T-value ($p \leq 0.05$, $r=-0.44$) of the FC maps with PtP as seed region, and iv) total cluster size of the FC maps with TeA as seed ($p \leq 0.05$, $r=-0.45$).

Significant negative correlations were observed between total cluster size and mean T-value of the FC maps with Cg as seed region and number of entries into centre open field (both $p \leq 0.05$, $r=-0.42$).

Positive correlations were observed between total cluster size and mean T-value of FC maps with TeA as seed region and MK-801-induced hyperlocomotion (both $p \leq 0.01$, $r=0.51$) (Fig.7).

No significant correlations were observed between phMRI measurements and behavioural assessments.

Forward stepwise regression analysis with all of the aforementioned FC measurements revealed that these parameters could predict some of the variability in i) number of entries into centre open field ($p \leq 0.05$, R^2 Adj=0.25), ii) sucrose preference ($p \leq 0.01$, R^2

535 Adj=0.43), and iii) MK-801-induced hyperlocomotion ($p \leq 0.05$, R^2 Adj=0.26) in MIA
536 offspring.

4. Discussion

To our knowledge, we showed for the first time altered functional connectivity (FC) in maternal immune activation (MIA) offspring. More precisely, we demonstrated increased FC in the default mode-like network (DMN) in adult offspring of dams that lost weight post-MIA. Furthermore, DMN FC correlated with behaviour in MIA offspring. Finally, we observed that MIA offspring have a different pathophysiology depending on the maternal response to the immune challenge. While Poly I:C Weight Loss (WL) offspring showed hypersynchronicity (i.e. increased functional connectivity) in the DMN, Poly I:C Weight Gain (WG) offspring exhibited a more pronounced deficit in NMDA receptor (NMDAR) antagonist response (i.e. smaller BOLD changes in response to MK-801).

The Poly I:C model is relevant for several neuropsychiatric disorders with a neurodevelopmental origin, including schizophrenia and autism spectrum disorders (ASD). MIA is considered a disease primer and is only one single risk factor for these disorders [1]. While MIA is sufficient to induce long-lasting alterations in brain and behaviour, it is not in itself capable to reproduce the entire symptomatology and pathology of schizophrenia or ASD, since these disorders have a multifactorial origin [3, 4]. MIA only leads to schizophrenia or ASD in combination with other environmental and/or genetic factors. It is important to bear this in mind when interpreting observations made in this animal model in the context of schizophrenia or ASD. While we set out to study brain functional connectivity, microstructure and NMDAR function and their relationship to behaviour in the MIA model in the context of schizophrenia (and thus chose behavioural tests relevant for this disorder), the results may also be relevant in the context of ASD.

4.1. Maternal response to MIA

In line with our previous work [27], pregnant dams showed a highly variable response to MIA in terms of weight change. Differential behavioural deficits in Poly I:C WL and Poly I:C WG offspring have been reported [21, 27-29].

4.2. Increased FC in DMN of MIA offspring in the absence of microstructural changes

We observed increased FC in the DMN of adult Poly I:C WL offspring, in agreement with most DMN studies in schizophrenia patients. This hypersynchronicity was most pronounced with posterior parietal and temporal association cortices of the DMN. Interestingly, these regions have been shown to comprise different modules of the rat DMN [36]. However, little is known about the functional relevance of these different modules.

While there are some differences with regard to the exact brain regions that constitute the DMN in rats vs. humans, it has been established using resting-state functional MRI that the overall DMNs of rats and humans are broadly similar [30, 37]. Therefore, we limit the interpretation of our results to the overall DMN instead of focusing on specific regions of the DMN. While the function of the DMN in rats has not yet been extensively investigated, one study has shown deactivation of DMN in rats during a prepulse inhibition (PPI) session using PET imaging [38]. Electrophysiological data have also provided evidence that the rat DMN is very similar to its human counterpart [39].

While FC was increased in the DMN, no microstructural changes could be observed in MIA offspring vs. controls. This is in contrast with previous studies of MIA offspring. However, these studies differ in many aspects from the current study. Beloosesky, Ginsberg and colleagues evaluated apparent diffusion coefficient in very young

584 (postnatal day 25) female offspring of rats injected intraperitoneally with
585 lipopolysaccharide, in which they found evidence for diffuse cerebral injury [13, 16].
586 Fatemi and colleagues investigated fractional anisotropy (FA) in selected white matter
587 structures of male mice prenatally exposed to human influenza virus and observed some
588 alterations at different ages (ranging from birth to young adulthood), which were
589 different depending on the exact timing of the prenatal immune challenge [14, 15]. Only
590 one study investigated FA throughout the entire brain of male adult mice exposed to Poly
591 I:C during gestation and reported changes throughout fronto-striatal-limbic circuits,
592 which were more pronounced when mice were exposed during early gestation [17].
593 Differences in nature and timing of the immune challenge, species and age of the offspring
594 may explain the difference between our results and the results obtained in these studies.

595 **4.3. Attenuated response to NMDAR antagonist in MIA offspring, but no change**
596 **in NMDAR levels**

597 Pharmacological functional MRI (fMRI) with an NMDAR antagonist is ideally suited to
598 investigate whole-brain NMDAR antagonist responses, which may reveal underlying
599 NMDAR dysfunction. As expected, we observed an altered response to MK-801 in MIA
600 offspring, which was most pronounced in Poly I:C WG offspring. The altered response
601 was mostly detectable in subcortical structures, i.e. striatum, thalamus and hippocampus,
602 which have all been implicated in schizophrenia [40, 41]. However, a trend for an altered
603 response could also be observed in frontal cortical regions in Poly I:C WG offspring.
604 Frontal cortex abnormalities have also been widely reported in schizophrenia [42]. We
605 observed negative BOLD responses in the different investigated brain regions following
606 acute intravenous administration of 0.2 mg/kg MK-801 in our Wistar Han IGS rats using
607 2% isoflurane. On the contrary, previous work has shown positive BOLD responses in

608 several brain regions following acute intraperitoneal administration of 0.3 mg/kg MK-
609 801 in Lewis rats using 1.2% isoflurane [43]. Another study showed that the used
610 anaesthesia regimen has a major effect on the BOLD response to ketamine, another non-
611 competitive NMDAR antagonist. When using α-chloralose, positive BOLD responses were
612 observed following the acute administration of ketamine in Sprague-Dawley rats.
613 However, when using 1.5% isoflurane, negative BOLD responses were observed [44].
614 Another study showed positive BOLD responses in awake Sprague-Dawley rats following
615 acute administration of ketamine [45]. In another study from our lab, acute
616 administration of memantine, another non-competitive NMDAR antagonist, resulted in
617 both BOLD increases and decreases in Lister Hooded rats using a range of 0.9-2%
618 isoflurane [46]. Possibly, a different concentration of isoflurane may lead to different
619 BOLD responses after acute administration of NMDAR antagonists. Higher
620 concentrations of isoflurane may lead to negative BOLD responses, while lower
621 concentrations may lead to positive BOLD responses. However, it is difficult to draw any
622 clear conclusions from these studies since they used different NMDAR antagonists (as
623 well as different doses and administration routes) and different rat strains, in addition to
624 different anaesthesia regimens.

625 A slightly attenuated hyperlocomotive response to MK-801 was observed in Poly I:C WG
626 offspring, but not Poly I:C WL offspring. The pharmacological fMRI response to MK-801
627 also showed the most pronounced difference in Poly I:C WG offspring compared to
628 controls. The fact that a smaller difference between the groups was seen in the
629 behavioural test than in the phMRI assessment may be due to behavioural sensitisation
630 towards MK-801, which has previously been reported [47]. However, we only
631 administered the drug two times to each animal, and not on consecutive days. Therefore,

632 we cannot be sure that behavioural sensitisation was an issue in our experimental design.
633 Both assessments suggest that NMDAR antagonist response is most altered in Poly I:C
634 WG offspring. A deficit in NMDAR antagonist response may suggest NMDAR
635 hypofunction. In literature, both exaggerated [19, 20, 48] and attenuated [49] responses
636 to MK-801 in MIA offspring have been reported as well as no difference vs. controls [50].
637 Some authors have also described a difference in MK-801-induced hyperlocomotion
638 depending on the maternal weight response to the immune challenge. A decreased
639 response to MK-801 has been demonstrated in Poly I:C WL offspring, with either a similar
640 but less pronounced response in Poly I:C WG offspring [29], a slightly increased response
641 in Poly I:C WG offspring [21, 27] or a response comparable to controls [28]. It is clear that
642 most studies have observed a disturbed NMDAR antagonist response using MK-801, but
643 the responses are not consistent across studies and are likely dependent on the precise
644 perturbation of the neurodevelopment.

645 Altered NMDAR antagonist response may potentially be explained by a change in total
646 number of NMDARs. However, no difference was observed in GluN1 levels, the obligatory
647 NMDAR subunit. While some studies have shown decreased GluN1 in MIA offspring [20,
648 22, 23], others have demonstrated increased GluN1 levels [51] or no change [52]. Altered
649 NMDAR antagonist response may also be explained by a differential subunit composition,
650 i.e. a different contribution of GluN2A- or GluN2B-subunits, or a different
651 phosphorylation level of the subunits. While some studies have shown no difference in
652 GluN2A/B subunits [22, 23], others have reported increased GluN2B [53], decreased
653 GluN2B levels [54] or no difference in GluN2B, but increased GluN2A levels [51]. Clearly,
654 the changes are not consistent across studies.

An interesting future direction in determining the relationship between altered FC and altered glutamate signalling in schizophrenia/ASD may lie in MR spectroscopy studies targeting glutamate in the MIA model or other animal models for these disorders. For example, a recent study that made use of resting-state fMRI and MR spectroscopy observed that cerebro-cerebellar FC was positively associated with the cerebellar excitation/inhibition balance (glutamate+glutamine/GABA) in adolescents/adults with ASD [55].

4.4. Subtle behavioural deficits in MIA offspring

The most prominent behavioural deficit was increased anxiety in the open field, which was most pronounced in Poly I:C WL offspring. Anxiety symptoms and comorbid anxiety disorders have often been described in schizophrenia patients [33]. Several other groups have also reported increased anxiety in open field in MIA offspring [56-59], while others reported no difference [28, 29, 60] or decreased thigmotaxis [61].

A trend for increased prepulse inhibition (PPI) was observed in the MIA offspring with the 75 dB prepulse, but not with the other prepulse intensities. Interestingly, increased PPI has been observed in autistic children with a 76 dB prepulse and not with a stronger prepulse [62]. While many studies have shown decreased PPI in MIA offspring [63, 64], some failed to observe a difference [65] or showed increased PPI [29].

A trend for increased spontaneous locomotion during light phase and decreased activity during dark phase were observed in the MIA offspring, which suggests a disturbed rest-activity/sleep-wake rhythm. Circadian rest-activity/sleep-wake disruptions are a common problem in schizophrenia [32]. Recently, a study has shown persistent sleep alterations in MIA offspring with increased locomotion during the light phase [66].

Increased locomotion during the light phase has also been shown by others [49, 57, 67], but decreased locomotion has been observed as well [68].

Though there was a numerically higher proportion of subjects with anhedonia (defined as sucrose preference <90%) in the MIA offspring groups vs. controls, this difference was not significant. Again, anhedonia has not been consistently reported in MIA offspring [25, 27, 57, 69, 70].

4.5. Hypersynchronicity in the DMN is related to behaviour in MIA offspring

Interestingly, FC in the DMN of MIA offspring correlated with their behavioural outcome. Correlation and stepwise regression analyses showed that FC in the DMN could predict the variability in several behavioural parameters: sucrose preference, thigmotaxis in open field and MK-801-induced hyperlocomotion. Higher FC in DMN was related to lower sucrose preference and increased anxiety. Similarly, hypersynchronicity in the DMN of schizophrenia patients has been related to psychopathological symptom severity [71, 72]. Hypersynchronicity and hyperactivity of the DMN are thought to blur the line between internal thoughts/feelings and external perceptions in schizophrenia patients, resulting in exaggerated self-relevance of neutral events and aberrant integration of internal and external stimuli, ultimately leading to symptoms such as paranoia and hallucinations [72]. On the other hand, higher FC in DMN was related to a higher (more normal) MK-801-induced hyperlocomotive response. Indeed, Poly I:C WG offspring with normal FC in DMN had a decreased MK-801-induced hyperlocomotive response while Poly I:C WL offspring with increased FC had a normal MK-801-induced hyperlocomotive response. The pharmacological fMRI response to MK-801 was not related to behaviour in the MIA offspring.

4.6. Relevance for autism spectrum disorders

As mentioned earlier, the MIA model has also been used as an animal model with relevance for autism, since maternal infection is also an important risk factor for this neurodevelopmental disorder [73]. Patients with ASD also display aberrant DMN function and FC. Most studies have reported increased within-network FC between core DMN nodes in children with ASD, but decreased FC in adolescents and adults with ASD (reviewed in [74]). Moreover, increased FC in DMN was related to social impairment severity in children with ASD [75]. NMDAR dysfunction has also been observed in ASD (reviewed in [76]). Unfortunately, we did not perform any behavioural tests in this study to assess whether our MIA offspring displayed a behavioural phenotype relevant to the core features of ASD, namely deficits in social interaction and communication, and repetitive behaviours/restricted interests [4]. However, we did observe some subtle behavioural abnormalities that are relevant for ASD. Anxiety symptoms and comorbid anxiety disorders are common in ASD patients [77]. An increased PPI with a 76 dB prepulse has been observed in children with ASD [62] and disturbed sleep-wake patterns are also common [78]. The link between increased FC in DMN and some of the behaviour in our MIA offspring is similar to the association that has been observed between increased FC in DMN and social impairment in children with ASD [75], though different aspects of behaviour were studied. Hence, we can conclude that our results may also be relevant for autism.

4.7. Limitations

We chose to investigate only male rats, since the hormonal cycle in female rats complicates the behavioural read-out in females. In future studies, however, female rats should also be investigated.

Every kind of anaesthesia affects *in vivo* functional imaging read-outs. However, a recent study that investigated FC patterns under six different anaesthesia protocols and the awake condition in rats concluded that FC patterns measured using a combination of isoflurane and medetomidine (as used in this study) had a good correspondence to those measured in awake rats [79]. Therefore, we are confident that our results are trustworthy. In future studies, however, it may be better to use propofol instead, since FC patterns obtained under propofol anaesthesia have been shown to be most similar to those measured in awake rats, or to use awake rats [79]. The latter, however, is a time-consuming process since animals have to be trained for a long period [80]. As described before (see 4.3), the choice of anaesthesia also greatly affects the outcome in pharmacological MRI combined with NMDAR antagonists. There is no consensus on which anaesthesia regimen should be used when testing responses to NMDAR antagonists and one should always keep in mind that the outcome of a study could be different based on the anaesthesia that is used.

5. Conclusions and future perspectives

We observed increased functional connectivity (FC) in the default mode-like network (DMN) of maternal immune activation (MIA) offspring, a neurodevelopmental model with relevance for several neuropsychiatric disorders. Moreover, increased FC in DMN was associated with a worse outcome on several behavioural tests (especially affective measures: anxiety, anhedonia) in our study, similar to the association between increased FC in DMN of schizophrenia patients and children with autism spectrum disorders (ASD) and their respective symptomatology. Since it has been shown that hypersynchronicity in the DMN of schizophrenia patients can be normalised by antipsychotic treatment, hypersynchronicity in the DMN of MIA models could be a potential biomarker to test

749 novel therapies for schizophrenia, but also for ASD. While behavioural deficits were
750 subtle in our study, the altered FC was clear and may be a more reliable read-out of the
751 animal model than the behaviour. Finally, it remains to be seen if altered FC in the DMN
752 of MIA offspring precedes the behavioural deficits and may be useful as a novel
753 prognostic biomarker. All of these issues need to be addressed in future studies. Finally,
754 we showed that Poly I:C WL offspring exhibited DMN hypersynchronicity, while Poly I:C
755 WG offspring displayed a more pronounced deficit in NMDAR antagonist response, which
756 could suggest un underlying NMDAR dysfunction. This underlines the importance of
757 taking the individual maternal response into account in studying the long-lasting effects
758 of disturbed neurodevelopment following prenatal immune activation.

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Figure legends

Fig.1. Study design. Pregnant dams were administered 4 mg/kg Poly I:C (maternal immune activation: MIA) or saline on gestational day (GD) 15. Twenty-four hours post-injection, the maternal weight response was recorded. Male offspring were tested during adulthood in postnatal weeks (PNW) 12 and 13. Behavioural testing included assessment of the prepulse inhibition (PPI) of the acoustic startle reflex, spontaneous locomotor activity (LMA), open field (OFT) and sucrose preference tests (SPT), and finally assessment of the locomotor response to the NMDA receptor (NMDAR) antagonist MK-801. Animals underwent resting-state functional MRI (fMRI) and diffusion tensor imaging (DTI) during PNW 12 and pharmacological fMRI with MK-801 during PNW 13. Rats were sacrificed in the beginning of PNW 14 for histological assessments.

Fig.2. Maternal response to the viral mimetic Poly I:C. Maternal weight response at 24 hours following Poly I:C or vehicle injection. Poly I:C-treated dams exhibited a wide range of weight change responses to the immune challenge in comparison with controls and were divided into dams that lost weight (Poly I:C WL) and gained weight (Poly I:C WG). Data are presented as boxplots. Control dams: N=4, Poly I:C WG dams: N=4, Poly I:C WL dams: N=5. Kruskal-Wallis test with Dunn's multiple comparisons test. *$p \leq 0.05$

Fig.3. Region of interest-based analysis of resting-state functional MRI data reveals increased functional connectivity (FC) in the default mode-like network (DMN) of MIA offspring. A. Average group z-transformed FC matrices of DMN-like, motor and sensory cortical regions. From top to bottom: left (L) and right (R) anterior cingulate cortex (Cg), left and right retrosplenial cortex (RS), left and right parietal association cortex (PtA), left and right posterior parietal cortex (PtP), left and right temporal association cortex (TeA), left and right primary motor cortex (M1), left and right primary

somatosensory cortex (S1), left and right primary auditory cortex (Au1), left and right primary visual cortex (V1). The black triangle indicates the correlations between DMN-like regions. The colour scale indicates the z-transformed correlation values. The values within the DMN-like network are higher (more red) in Poly I:C WL offspring compared to controls. **B.** The average zFC correlation values within the DMN-like network (left) and primary motor and sensory cortices (right). The average FC within the DMN-like network is significantly higher in Poly I:C WL offspring vs. controls. Mean ± SEM is shown. Control offspring: n=11, Poly I:C WG offspring: n=10, Poly I:C WL offspring: n=15. One-way ANOVA with Dunnett's multiple comparisons test. *p≤0.05

Fig.4. Seed-based analysis of resting-state functional MRI data reveals increased functional connectivity (FC) in the default mode-like network (DMN) of MIA offspring. A. Group statistical seed-based FC maps with posterior parietal cortex as seed region (one-sample t-test, FWE corrected, p<0.05, minimal cluster size k≥10). The colour scale indicates T-values. **B.** Total cluster size of all significantly correlated clusters (FWE corrected, p<0.05, k≥10) with different seed regions of the DMN-like network: right cingulate cortex (Cg R), right retrosplenial cortex (RS R), right parietal association cortex (PtA R), right posterior parietal cortex (PtP R), and right temporal association cortex (TeA R). The total cluster sizes of the FC maps with PtP R and TeA R as seed regions are significantly higher in Poly I:C WL offspring vs. controls. Mean ± SEM is shown. Control offspring: n=11, Poly I:C WG offspring: n=10, Poly I:C WL offspring: n=15. One-way ANOVA with Dunnett's multiple comparisons test. #p<0.1, *p≤0.05, **p≤0.01

Fig.5. Pharmacological fMRI with the NMDA receptor antagonist MK-801 reveals altered NMDAR function in MIA offspring. A. Group statistical difference maps of BOLD signal before > after (20-30 min post) MK-801 administration (uncorrected, p<0.01,

minimal cluster size k≥10). MIA offspring showed a significantly different response to the NMDAR antagonist in comparison with controls, which was most pronounced in Poly I:C WG offspring. The colour scale indicates T-values. 1: anterior cingulate cortex, 2: motor cortex, 3: striatum, 4: thalamus, 5: hippocampus. **B.** Mean T-values in anterior cingulate cortex, motor cortex, striatum, thalamus and hippocampus. Mean T-values were significantly lower in striatum of Poly I:C WG offspring vs. controls, and in thalamus of Poly I:C WG and Poly I:C WL offspring vs. controls. A trend was observed for lower mean T-values in frontal cortical regions (i.e., anterior cingulate cortex and motor cortex) of Poly I:C WG offspring vs. controls. There was no significant difference in mean T-values in hippocampus between the three groups. Data are presented as boxplots. Control offspring: n=7, Poly I:C WG offspring: n=8, Poly I:C WL offspring: n=14. Kruskal-Wallis test with Dunn's multiple comparisons test. #$p<0.1$, *$p\leq0.05$, **$p\leq0.01$

Fig.6. Subtle behavioural changes in MIA offspring. A. There were no significant differences in % prepulse inhibition with any of the tested prepulse intensities between the three offspring groups. **B.** Poly I:C WG offspring showed a trend for increased baseline locomotor activity (average beam crossings per hour) during the light phase compared to controls. **C.** Poly I:C WL offspring showed trends for a decreased number of entries into the centre of the open field and a lower % distance moved in the centre vs. controls. **D.** There was no significant difference in % sucrose preference between the three groups. **E.** Poly I:C WG offspring showed a slightly attenuated hyperlocomotion response to MK-801 compared to controls. The arrow indicates the time-point of MK-801 administration. Mean ± SEM is shown in panels A, B and E. Data in C and D are presented as boxplots. All behavioural tests: Control offspring: n=11, Poly I:C WG offspring: n=12, Poly I:C WL offspring: n=16. A, B: one-way ANOVA with Dunnett's multiple comparisons test; C, D:

Kruskal-Wallis test with Dunn's multiple comparisons test, E: two-way repeated measures ANOVA with Dunnett's multiple comparisons test. #p<0.1, *p≤0.05

Fig.7. Correlation between functional connectivity (FC) in the default mode-like network (DMN) and behavioural outcome in MIA offspring. A. A negative correlation was observed between average FC in the DMN and sucrose preference in MIA offspring. **B.** A positive correlation was seen between the total cluster size of the FC maps with right temporal association cortex as seed region and MK-801 induced hyperlocomotion in MIA offspring. Pearson correlation.

Suppl.Fig.1. Illustration of the workflow of the image analysis procedure for the immunohistochemistry (IHC) images of GluN1-stained sections. From three technical replicates of each biological sample a single slice was randomly chosen and annotated. To quantify the GluN1 staining, the image underwent a colour-to-grayscale conversion assuming DAB-like staining, after which the grayscale intensity was normalised to the average intensity in the corpus callosum (reference region). Pixels positive for GluN1 were depicted by a threshold of intensity equalling 3 times the standard deviation. Finally, for each region the area percentage of positive pixels was determined. Fuchsia: frontal cortex, light green: cortex, dark green: corpus callosum, yellow: hippocampus, light red/pink: striatum, dark red: thalamus.