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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 prepulse 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

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FW and RS: Conducted biochemical analysis.
CH and IW: Contributed to design of experiments and writing ms.
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Conflict of Interest
The authors declare no conflict of interest.

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.

Keywords

Poly I:C; Schizophrenia; Sensorimotor deficits; FDG-PET; Neurotransmission; Neurobiological trajectories

1. Introduction

Converging evidence from epidemiology, neuroimaging and postmortem 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 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 interstimulus 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 × 0.3875 × 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 when compared to PND60 and 35. Furthermore, a significant difference was found between poly I:C and saline offspring at PND100 ($t(3.84)$, $p = 0.001$) but not at PND35 ($t(-1.76)$, $p = 0.09$) or PND60 ($t(0.325)$, $p = 0.74$). At PND100, poly I:C offspring exhibited lower levels of PPI when compared to saline offspring (Fig. 1).

3.2. Neurochemistry

3.2.1. Age-related effects (Table 1 and Fig. 2)—In the ***mPFC,*** contents of DA and GABA increased while 5-HIAA and DA-turnover decreased with age. In the ***Hipp,*** age-related effects were found for glutamate and GABA with higher levels being detected at PND100 as compared to PND35. In the ***Thal***, contents of DA increased, whereas those of 5-HIAA and DA-turnover decreased with age. In the ***Nacc*** PND100 rats displayed increased levels of DA, DOPAC, and glutamate as well as decreased levels of 5-HIAA and GABA when compared to PND35 rats. In the ***CPU,*** contents of GABA increased whereas those of glutamate, DA and 5-HT-turnover decreased with age. In the ***GP,*** levels of GABA increased whereas 5-HT, 5-HIAA, glutamate and DA-turnover decreased over time. Finally, in the ***VTA***, 5-HIAA and DOPAC decreased whereas glutamate was increased when PND100 were compared to PND35 rats.

3.2.2. MIS-related effects (Table 1 and Fig. 2)—In the ***mPFC*** MIS led to significantly lower contents in DOPAC and 5-HIAA. In the ***Hipp***, it led to significantly lower 5-HIAA-content and a significant MIS × age interaction for this metabolite. Post-hoc pairwise comparisons confirmed a significant increase of 5-HIAA with age in saline offspring ($p < 0.05$) and significant higher 5-HIAA-levels in PND100 saline offspring when compared to poly I:C ($p < 0.05$). A trend towards a significant interaction between MIS and age was found for DA. In the ***Thal*** no MIS-related effects were seen. In the ***Nacc***, maternal exposure to poly I:C increased DA-contents, whereas in the ***CPU*** it reduced 5-HT-contents. In the ***GP***, we found a significant MIS × age interaction for 5-HT with post-hoc pairwise comparisons confirming an effect of age in both poly I:C ($p < 0.05$) and saline offspring ($p < 0.05$) as well as significantly reduced levels of 5-HT in adult poly I:C as compared to adult saline offspring ($p < 0.05$). Further, a significant MIS × age interaction was found for GABA with post hoc pairwise comparisons confirming significantly lower levels of GABA in adults as compared to adolescent poly I:C offspring ($p < 0.05$) as well as in adult poly I:C as compared to adult saline offspring ($p < 0.05$). A trend to significant MIS × age interaction was found for DA. In the ***VTA***, a significant MIS × age interaction was observed for DA and 5-HT. For DA, post-hoc pairwise comparisons confirmed a significant reduction of DA-content with ageing in saline offspring ($p < 0.05$). For 5-HT, it revealed a significant increment of 5-HT with ageing in poly I:C offspring ($p < 0.05$) and a significant higher 5-HT-content in poly I:C than in saline offspring at adulthood ($p < 0.05$).

3.3. Imaging

3.3.1. Age-related effects (Fig. 3)—PND100 ***saline offspring*** as well as PND100 ***poly I:C offspring*** showed lower glucose metabolism in the cortex in both hemispheres and prefrontal cortex as well as higher glucose metabolism in the ventral Hipp, cerebellum and periaqueductal gray matter (PAG), hypothalamus and septum when compared to respective PND35 animals.

3.3.2. MIS-related effects (Fig. 4)—At ***PND35*** poly I:C offspring showed lower glucose metabolism in the ventral Hipp in both hemispheres, in the cortex/PFC and in the cerebellum as well as higher glucose metabolism in the Thal and GP when compared to saline offspring. Most of these MIS-dependent differences seen in adolescence were maintained in adulthood. At ***PND100,*** poly I:C offspring showed lower glucose metabolism in the ventral Hipp in both hemispheres and cortex/PFC as well as higher glucose metabolism in Thal when compared to controls. In contrast to adolescent, adult poly I:C offspring additionally showed higher glucose metabolism in the amygdala and Nacc in both hemispheres when compared to saline offspring but no difference in glucose metabolism in the cerebellum anymore.

4. Discussion

In recent years there has been a growing body of evidence pointing to neurotransmission and network abnormalities in schizophrenia. However, most of the data derived from patients is confounded by heterogeneous symptom profile, medication status and disease history. Animal models may overcome this limitation. In line with previous demonstrations of abnormal brain volumetric trajectories underlying delayed symptom manifestation in poly I:C offspring, we were now able to demonstrate that MIS affects metabolic brain activity, neurotransmission and sensorimotor gating, in a maturation-dependent manner.

4.1. Behavioral trajectories

Sensory gating deficits as reflected in impaired PPI constitute one of the core features of schizophrenia (Braff et al., 2001). Being a cross species phenomenon (Swerdlow et al., 2008), PPI has been previously shown to be disrupted in adult poly-I:C offspring (Ozawa et al., 2006; Meyer and Feldon, 2010; Mattei et al., 2014) and here we replicated this finding. Furthermore, our longitudinal assessment revealed that the PPI deficit emerged post-pubertally so that there were no differences in PPI levels between poly I:C and controls on PND35 and PND60 but poly-I:C offspring had lower PPI levels than controls on PND100. It could be argued that time-dependent emergence of PPI disruption in poly-I:C exposed offspring is due to low levels of PPI in controls, which make any decrease in PPI difficult to demonstrate due to a potential “floor” effect. However, a different interpretation is possible when comparing the developmental trajectories of PPI in controls and poly-I:C offspring: While in control animals PPI increased in magnitude from 35 to 100 days of age, such maturation-dependent increase in sensorimotor gating was prevented/attenuated in the offspring of dams exposed to poly-IC, leading to an adult deficit in this behavioral phenomenon. Very similar results and interpretation have been presented by Romero et al. (2010) on the longitudinal effects of prenatal administration of the MIS-inducing agent bacterial endotoxin lipopolysaccharide (LPS) on PPI. Prenatal LPS treatment also led to the

induction of a behavioral deficit, which emerged first at puberty and persisted throughout adulthood (Romero et al., 2010). The present results are in agreement with the impaired developmental trajectory of PPI observed after prenatal administration of the MIS-inducing agent bacterial endotoxin lipopolysaccharide (Romero et al., 2010). Altogether, our data are consistent with the well-documented post-pubertal onset of psychotic behavior in the majority schizophrenic patients.

4.2. Neurobiological trajectories

We found age-dependent changes in all neurotransmission systems as well as brain structures examined regardless of the MIS status and a high overlap between age-related brain activity changes in MIS and control offspring. Neurobiological changes that develop over time regardless of the maternal immune status most likely reflect normal neurobiological maturation processes whereas those that develop against the maternal immune status indicate abnormal maturation processes and are likely to contribute to the delayed manifestation of behavioral deficits seen in poly I:C offspring (Piontkewitz et al., 2012).

Adding on to previous data (Winter et al., 2009; Giovanoli et al., 2013) on a neurochemical level, probably our most exciting MIS-related effects refer to the DA-system such that poly I:C offspring show higher levels of DA in the Nacc and lower levels of DOPAC in the mPFC when compared to saline offspring. Compelling evidence points to the participation of abnormally enhanced DA-activity in the mesolimbic system, especially the Nacc, in the disruption of sensorimotor gating (Geyer et al., 2001). Moreover, alterations in the DA-system in the form of imbalances between subcortical and cortical DA are well documented in schizophrenia. Specifically, it has been suggested that in line with the data of the present study enhanced activity in the mesolimbic DA-system together with hypoactive mesocortical DA-projections to the PFC contribute to the pathophysiology of schizophrenia (Abi-Dargham and Moore, 2003; Winterer and Weinberger, 2004; Giulivi et al., 2013). As abnormal glucose metabolism is thought to be an indicator of an underlying pathology, our FDG-PET data further strengthen this notion demonstrating that in comparison to controls poly I:C offspring exhibit lower glucose metabolism in the ventral Hipp, cortex and PFC and higher glucose metabolism in the amygdala and Nacc.

Interestingly, DAergic dysregulations as well as alterations in metabolic activity pattern differed across mPFC and Nacc with respect to first appearance and further development. In the mPFC, poly I:C offspring showed reduced levels of DOPAC as well as metabolic activity prior to and with manifestation of behavioral deficits in adolescent and adult states as compared to age-matched controls. Together with an early reduction in 5-HIAA, this finding reflects an early impairment of the mPFC in the poly I:C model of schizophrenia. This is of special interest as cognitive processes that are supported by the integrity of the PFC are among the first to occur in schizophrenia patients, presenting much before the onset of psychotic symptoms (Volk and Lewis, 2014). In contrast, DA-contents in the Nacc increased with age in both, saline and poly I:C offspring with the latter presenting further increments as compared to controls only during adulthood. Likewise, adult but not adolescent poly I:C offspring displayed increased Nacc metabolic activity as compared to saline offspring. A

recent study by Alam et al. (2015) demonstrated reduced firing rates and burst behavior of mPFC neurons along with increased firing rates of Nacc neurons to subserve impaired sensorimotor gating in rats. This data suggests that only the combined deficit in mPFC and Nacc functioning as found here in adult but not adolescent poly I:C offspring is capable of inducing PPI deficits in rats.

Schizophrenia has further been associated with hippocampal abnormalities. Structural imaging and post mortem studies consistently report decreased hippocampal volumes in schizophrenia patients and functional imaging studies demonstrate elevated basal cerebral perfusion and point to a failure in recruiting the Hipp during memory tasks (Heckers et al., 1998; Medoff et al., 2001; Weiss et al., 2003; Schobel et al., 2009; Tamminga et al., 2010). Not much is known about the onset of hippocampal abnormalities in relation to symptom manifestation. Piontkewitz and colleagues (Piontkewitz et al., 2011) found in the poly I:C model of schizophrenia reduced hippocampal volumes at PND46, still prior to behavioral deficit manifestation. Poly I:C offspring has further been shown to exhibit down-regulated hippocampal neurogenesis (Meyer et al., 2010; Wolf et al., 2011; Mattei et al., 2014) that likewise precedes behavioral deficits manifestation (Piontkewitz et al., 2012) and probably associates to hippocampal volume reductions since normal adult hippocampal neurogenesis results in a pool of new neurons and volume gain (Ming and Song, 2005). Here, we report that hippocampus-related functional alteration may also occur prior to symptom manifestation. As such we found previously reported abnormalities in the serotonergic system (Winter et al., 2009) and decreased metabolic activity in the hippocampus at both adolescent and adult stages.

4.3. Conclusion

Taken together, to the best of our knowledge this is the first report of FDG-PET in parallel with neurochemical investigations to study and identify brain networks associated to the delayed emergence of schizophrenia-related behavioral deficits in an animal model of this affliction. Our finding suggest that MIS-induced metabolic and neurochemical changes are neurodevelopmental in nature and either progressive or non-progressive and that the behavioral deficits manifest as these abnormalities increase.

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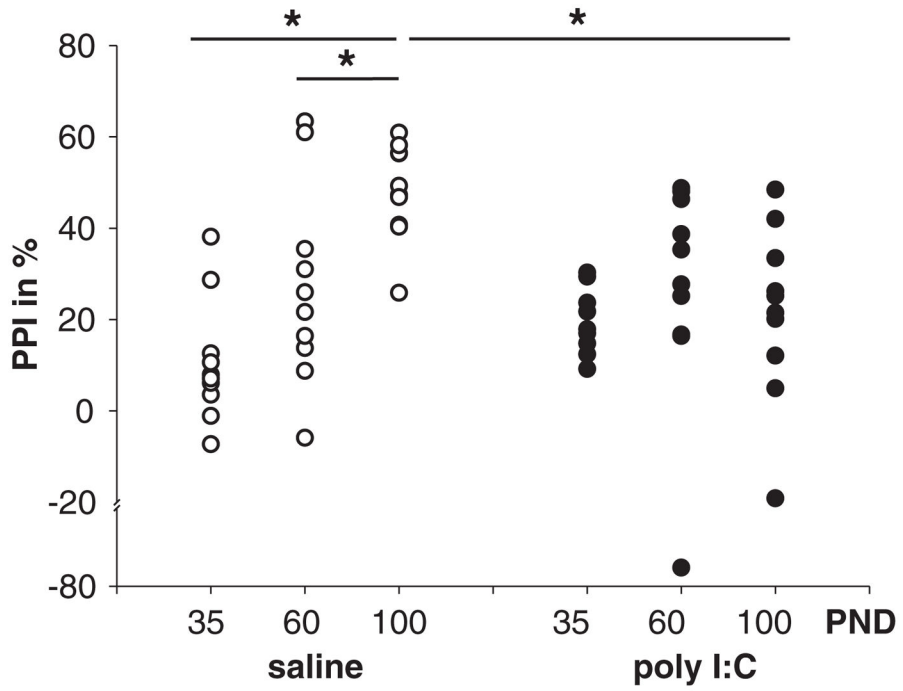


Fig. 1.

The developmental course of PPI in saline and poly I:C offspring. Offspring of saline and poly I:C treated dams were tested for PPI at PND35, PND60 and PND100. Vertical point plots depict the percentage of PPI inhibition (%PPI) in each animal for each condition. In saline rats (left) PPI develops over time: a significant difference between PND35 and PND100 as well as between PND60 and PND100 was found. In poly I:C rats (right) no significant differences were found over all time-course comparisons. Comparisons between saline and poly I:C offspring revealed a significant difference at PND100 but not between PND30 or PND60. Significant differences indicated by asterisks (*); $p < 0.05$.

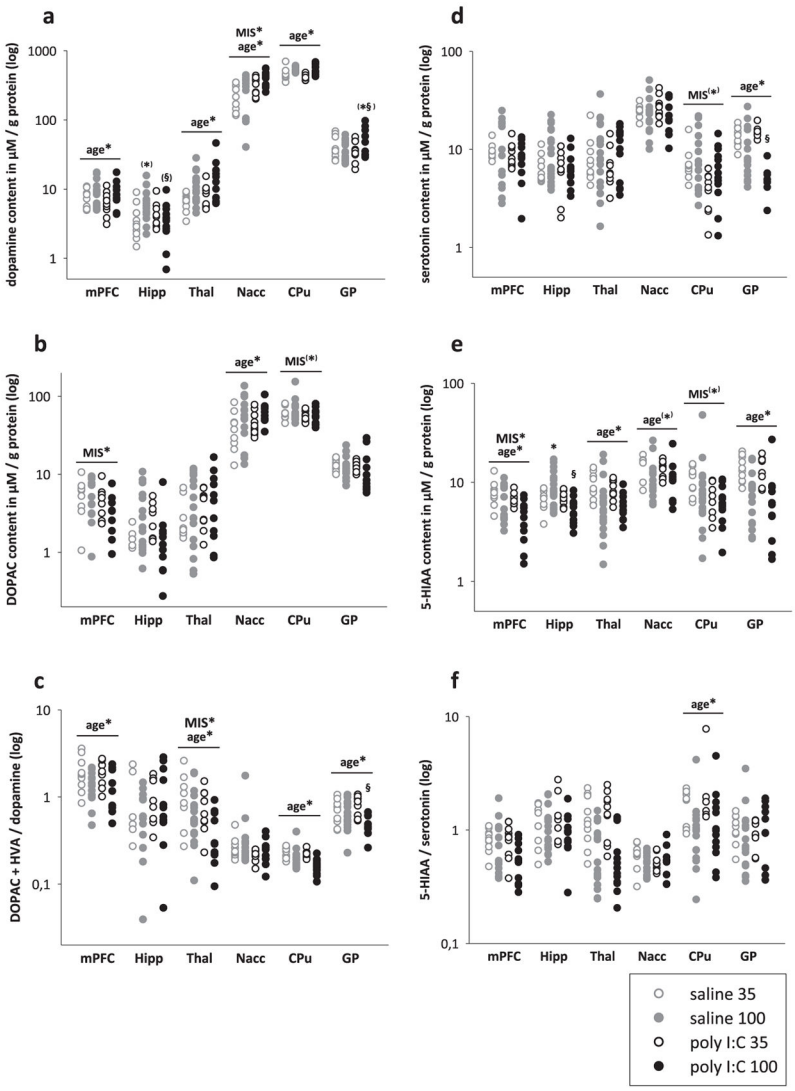
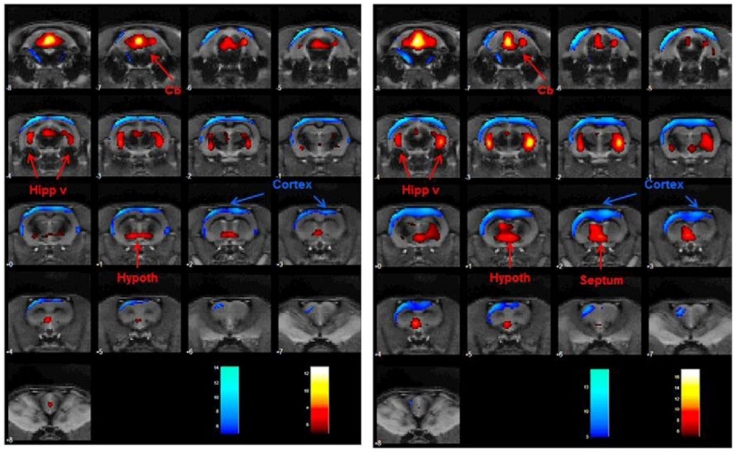


Fig. 2.

Effects of maternal immune stimulation and age on monoamine levels (dopamine, serotonin) and their metabolites (DOPAC, HVA, 5-HIAA). Neurochemical contents were examined in adolescent (35) and adult (100), control (saline) or MIS (poly-I:C) offspring. Vertical point plots depict the contents of monoamines and their metabolites measured in the mPFC, Hipp, Thal, Nacc, CPU and GP and are expressed in µM per g protein. The presence of age- and MIS-dependent effects are depicted by age* and MIS*, respectively (*$p < 0.05$), based on two-way ANOVA of the neurochemical content in the corresponding brain area. Significant differences of post hoc comparisons are indicated by asterisks (*, versus respective juvenile group) and paragraph (§, versus age-matched control group). a) Results for dopamine. b) Results for DOPAC. c). Results for dopamine turnover (DOPAC + HVA)/dopamine. d) Results for serotonin. e) Results for 5-HIAA. f) Results for serotonin turnover (5-HIAA/serotonin).

a



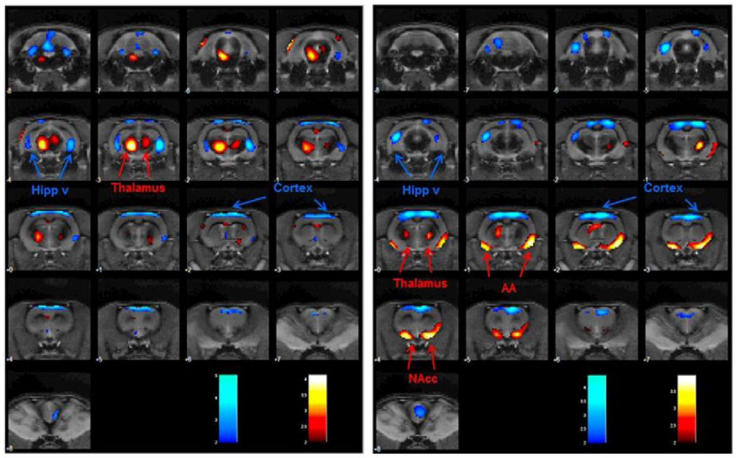
b

MIS	ROI	Side	PND 100 vs PND 35	T-Value	P-Value
saline	C	R	↓	14.17	0.000
	C	L	↓	11.17	0.000
	PFC	R, L	↓	10.47	0.000
	Cb, PAG, vHipp L	R, L	↑	12.8	0.000
	vHipp	R	↑	8.67	0.000
	Sept, Hypoth	R, L	↑	7.11	0.000
poly I:C	C	R	↓	18.57	0.000
	C	L	↓	15.16	0.000
	PFC	R, L	↓	13.39	0.000
	Cb, PAG, vHipp	R, L	↑	17.38	0.000
	Sept, Hypoth	R, L	↑	10.72	0.000

Fig. 3.

Age-related effects on glucose metabolism measured via FDG-PET. a) Colored PET overlays on the MR reference indicate increased [^{18}F]FDG uptake (hot colors) or decreased [^{18}F]FDG uptake (cold colors) following maturation in saline offspring (left panel) and MIS offspring (right panel). b) saline and poly I:C offspring (MIS), region of interest (ROI), side left and right, T-value, glucose metabolism (increase ↑ or decrease ↓), statistical p-value for cortex (C), prefrontal cortex (PFC), cerebellum (Cb), periaqueductal gray matter (PAG), ventral Hipp (vHipp), septum (Sep) and hypothalamus (hypoth).

a



b

PND	ROI	Side	poly I:C vs saline	T-Value	P-Value	FDR
	C, PFC	R, L	↓	4.58	0.000	0.08
	vHipp	L	↓	5.04	0.000	0.08
35	vHipp	R	↓	3.39	0.000	0.094
	Thal, GP	R, L	↑	4.15	0.000	0.266
	Cb	R, L	↓	3.66	0.000	0.08
	C, PFC, Hipp	R	↓	18.57	0.000	0.092
	vHipp	L	↓	15.16	0.000	0.092
100	Nacc, AA	R, L	↑	13.39	0.000	0.132
	Thal, GP	L	↑	17.38	0.000	0.132
	Thal	R	↑	10.72	0.003	0.169

Fig. 4.

Age-related effects on glucose metabolism measured via FDG-PET. a) Colored PET overlays on the MR reference indicate increased [^{18}F]FDG uptake (hot colors) or decreased [^{18}F]FDG uptake (cold colors) following maturation in saline offspring (left panel) and MIS offspring (right panel). b) post natal day (PND) 35 and 100, region of interest (ROI), side left and right, T-value, glucose metabolism (increase ↑ or decrease ↓), statistical p value for cortex (C), prefrontal cortex (PFC), cerebellum (Cb), periaqueductal gray matter (PAG), ventral Hipp (vHipp), septum (Sep) and hypothalamus (hypoth).

Table 1

Effects of maternal immune stimulation and age on neurotransmitter levels. Neurochemical contents were examined in adolescent (35) and adult (100), control and MIS (poly-I:C) offspring. Neurotransmitters (DA, DOPAC, HVA, dopamine turnover (DOPAC + HVA/dopamine) were measured in the mPFC, Hipp, Thal, Nacc, CPU, GP, and VTA and are expressed as mean values ± S.E.M. of μM per g protein. The presence of age- and MIS-dependent effects are depicted by age, MIS, and interaction, degrees of freedom (DF), F-values and statistical p-values based on two-way ANOVA of the neurochemical content in the corresponding brain area.

Transmitter	Region	MIS	PND	Content in μM/g protein	Two-way ANOVA effects	DF	F-value	p-Value
DA	mPFC	control	35	7.21 ± 0.73	MIS	1,41	0.266	0.609
			100	9.28 ± 1.13	age	1,41	4.403	0.042
		poly I:C	35	6.58 ± 0.77	interaction	1,41	0.008	0.093
			100	8.84 ± 1.15				
	Hipp	control	35	3.78 ± 0.74	MIS	1,48	1.666	0.203
			100	6.58 ± 0.78	age	1,48	2.569	0.116
		poly I:C	35	4.28 ± 0.66	interaction	1,48	3.670	0.061
			100	4.03 ± 0.66				
	Thal	control	35	6.68 ± 0.62	MIS	1,47	1.603	0.211
			100	11.36 ± 1.40	age	1,47	8.154	0.006
		poly I:C	35	8.20 ± 1.03	interaction	1.47	0.256	0.616
			100	14.88 ± 2.83				
	Nacc	control	35	224.15 ± 30.02	MIS	1,40	7.529	0.009
			100	291.67 ± 36.02	age	1,40	4.971	0.032
		poly I:C	35	309.24 ± 28.36	interaction	1,40	0.064	0.802
			100	394.00 ± 30.58				
	CPu	control	35	458.38 ± 29.87	MIS	1,47	0.015	0.903
			100	548.46 ± 9.86	age	1,47	36.817	0.000
		poly I:C	35	426.49 ± 12.55	interaction	1,47	2.237	0.141
			100	575.52 ± 24.70				
	GP	control	35	42.10 ± 5.45	MIS	1,41	1.101	0.300
			100	38.58 ± 3.10	age	1,41	1.687	0.201
		poly I:C	35	37.32 ± 4.20	interaction	1,41	3.973	0.053
			100	54.00 ± 7.46				

Transmitter	Region	MIS	PND	Content in µM/g protein	Two-way ANOVA effects	DF	F-value	p-Value
	VTA	control	35	135.70 ± 5.59	MIS	1,48	0.423	0.519
			100	98.77 ± 9.84	age	1,48	0.964	0.331
		poly I:C	35	102.49 ± 16.47	interaction	1,48	5.334	0.025
			100	117.38 ± 7.79				
DOPAC	mPFC	control	35	5.75 ± 0.81	MIS	1,40	6.209	0.017
			100	5.83 ± 0.77	age	1,40	0.851	0.362
		poly I:C	35	4.63 ± 0.70	interaction	1,40	1.040	0.314
			100	3.14 ± 0.66				
	Hipp	control	35	1.58 ± 0.19	MIS	1,38	0.003	0.858
			100	3.37 ± 0.74	age	1,38	0.230	0.634
		poly I:C	35	2.86 ± 0.51	interaction	1,38	3.284	0.078
			100	1.81 ± 0.63				
	Thal	control	35	3.05 ± 0.73	MIS	1,44	0.048	0.828
			100	5.17 ± 0.98	age	1,44	1.676	0.202
		poly I:C	35	3.94 ± 0.63	interaction	1,44	0.326	0.571
			100	4.77 ± 1.38				
	Nacc	control	35	40.61 ± 7.46	MIS	1,40	0.550	0.463
			100	58.97 ± 8.99	age	1,40	5.357	0.026
		poly I:C	35	46.48 ± 5.18	interaction	1,40	0.000	1,000
			100	64.84 ± 5.99				
	CPu	control	35	60.77 ± 3.45	MIS	1,47	3.477	0.069
			100	67.27 ± 5.83	age	1,47	0.504	0.481
		poly I:C	35	54.52 ± 2.45	interaction	1,47	0.362	0.550
			100	55.05 ± 3.42				
	GP	control	35	12.95 ± 0.70	MIS	1,42	0.000	0.994
			100	12.27 ± 1.03	age	1,42	0.229	0.635
		poly I:C	35	13.01 ± 0.63	interaction	1,42	0.001	0.973
			100	12.24 ± 2.47				
	VTA	control	35	30.32 ± 1.67	MIS	1,47	0.000	0.989
			100	18.04 ± 1.83	age	1,47	8.339	0.006
		poly I:C	35	25.72 ± 4.28	interaction	1,47	3.077	0.086

Transmitter	Region	MIS	PND	Content in µM/g protein	Two-way ANOVA effects	DF	F-value	p-Value
			100	22.72 ± 2.12				
HVA	mPFC	control	35	7.64 ± 1.14	MIS	1,41	0.390	0.536
			100	5.18 ± 0.95	age	1,41	1.418	0.241
		poly I:C	35	7.34 ± 0.89	interaction	1,41	0.765	0.387
			100	6.96 ± 1.61				
	Hipp	control	35	1.93 ± 1.29	MIS	1,47	0.760	0.388
			100	1.07 ± 0.26	age	1,47	0.279	0.560
		poly I:C	35	2.28 ± 1.04	interaction	1,47	0.216	0.644
			100	2.23 ± 0.98				
	Thal	control	35	2.83 ± 1.07	MIS	1,48	1.195	0.280
			100	2.24 ± 0.66	age	1,48	0.552	0.461
		poly I:C	35	1.99 ± 0.56	interaction	1,48	0.006	0.940
			100	1.51 ± 0.33				
	Nacc	control	35	20.70 ± 4.49	MIS	1,40	0.181	0.673
			100	22.63 ± 3.46	age	1,40	1.585	0.215
		poly I:C	35	19.69 ± 2.32	interaction	1,40	0.506	0.481
			100	26.62 ± 2.94				
	CPu	control	35	46.33 ± 3.51	MIS	1,47	1.304	0.259
			100	36.74 ± 1.86	age	1,47	5.990	0.018
		poly I:C	35	40.24 ± 2.03	interaction	1,47	1.282	0.263
			100	36.72 ± 3.11				
	GP	control	35	13.71 ± 1.00	MIS	1,43	0.586	0.448
			100	11.42 ± 1.53	age	1,43	2.879	0.097
		poly I:C	35	15.39 ± 1.24	interaction	1,43	0.076	0.784
			100	12.21 ± 1.90				
	VTA	control	35	15.00 ± 2.38	MIS	1,47	0.725	0.399
			100	10.96 ± 1.71	age	1,47	7.511	0.009
		poly I:C	35	17.54 ± 2.25	interaction	1,47	0.285	0.595
			100	11.54 ± 0.78				
5-HT	mPFC	control	35	9.66 ± 0.54	MIS	1,41	1.021	0.318
			100	10.54 ± 2.07	age	1,41	0.029	0.867

Transmitter	Region	MIS	PND	Content in µM/g protein	Two-way ANOVA effects	DF	F-value	p-Value
		poly I:C	35	8.79 ± 0.80	interaction	1,41	0.181	0.673
			100	8.41 ± 1.05				
	Hipp	control	35	6.49 ± 0.70	MIS	1,47	1.997	0.164
			100	9.89 ± 1.37	age	1,47	1.860	0.179
		poly I:C	35	6.46 ± 0.96	interaction	1,47	1.927	0.172
			100	6.43 ± 0.74				
	Thal	control	35	8.48 ± 1.68	MIS	1,49	0.111	0.741
			100	11.01 ± 2.00	age	1,49	3.393	0.072
		poly I:C	35	6.94 ± 1.05	interaction	1,49	0.241	0.626
			100	11.31 ± 1.45				
	Nacc	control	35	24.96 ± 1.23	MIS	1,40	0.005	0.946
			100	23.71 ± 2.85	age	1,40	1.623	0.210
		poly I:C	35	27.24 ± 2.36	interaction	1,40	0.637	0.430
			100	21.79 ± 2.62				
	CPu	control	35	7.19 ± 1.04	MIS	1,46	4.884	0.032
			100	9.44 ± 1.56	age	1,46	3.049	0.088
		poly I:C	35	4.20 ± 0.54	interaction	1,46	0.002	0.960
			100	6.58 ± 1.03				
	GP	control	35	13.86 ± 0.96	MIS	1,42	1.987	0.166
			100	10.06 ± 1.58	age	1,42	26.424	0.000
		poly I:C	35	15.08 ± 0.67	interaction	1.42	5.376	0.025
			100	5.04 ± 0.49				
	VTA	control	35	27.66 ± 2.51	MIS	1,48	0.407	0.527
			100	20.31 ± 2.42	age	1,48	0.443	0.509
		poly I:C	35	20.21 ± 2.27	interaction	1,48	7.335	0.009
			100	32.35 ± 4.39				
5-HIAA	mPFC	control	35	7.97 ± 0.71	MIS	1,41	4.653	0.037
			100	6.00 ± 0.70	age	1,41	12.320	0.001
		poly I:C	35	6.86 ± 0.32	interaction	1,41	0.180	0.674
			100	4.35 ± 0.59				
	Hipp	control	35	6.95 ± 0.53	MIS	1,47	6.323	0.015

Transmitter	Region	MIS	PND	Content in μM/g protein	Two-way ANOVA effects	DF	F-value	p-Value
			100	9.26 ± 0.84	age	1,47	0.183	0.671
		poly I:C	35	7.07 ± 0.36	interaction	1,47	7.171	0.010
			100	5.39 ± 0.42				
	Thal	control	35	9.29 ± 0.93	MIS	1,49	0.836	0.365
			100	6.67 ± 1.04	age	1,49	9.405	0.003
		poly I:C	35	8.67 ± 0.60	interaction	1,49	0.066	0.798
			100	5.58 ± 0.45				
	Nacc	control	35	15.04 ± 1.16	MIS	1,40	0.461	0.501
			100	11.93 ± 1.44	age	1,40	3.781	0.059
		poly I:C	35	13.74 ± 0.77	interaction	1,40	0.054	0.817
			100	11.29 ± 1.73				
	CPu	control	35	10.96 ± 0.99	MIS	1,47	3.294	0.076
			100	9.64 ± 2.41	age	1,47	0.422	0.519
		poly I:C	35	7.44 ± 0.82	interaction	1,47	0.002	0.959
			100	6.31 ± 0.71				
	GP	control	35	14.16 ± 1.29	MIS	1,43	0.052	0.821
			100	8.07 ± 1.10	age	1,43	17.963	0.000
		poly I:C	35	14.20 ± 1.35	interaction	1,43	0.067	0.798
			100	7.33 ± 2.12				
	VTA	control	35	18.08 ± 1.32	MIS	1,48	0.274	0.603
			100	10.55 ± 1.15	age	1,48	4.552	0.038
		poly I:C	35	15.46 ± 2.24	interaction	1,48	3.772	0.058
			100	15.10 ± 1.99				
Gutamate	mPFC	control	35	100.73 ± 1.82	MIS	1,45	0.591	0.446
			100	105.43 ± 3.11	age	1,45	2.136	0.151
		poly I:C	35	98.14 ± 2.43	interaction	1,45	1.030	0.316
			100	124.19 ± 20.35				
	Hipp	control	35	73.85 ± 1.59	MIS	1.50	0.226	0.637
			100	86.40 ± 3.83	age	1,50	13.631	0.001
		poly I:C	35	74.22 ± 2.62	interaction	1,50	0.142	0.708
			100	89.62 ± 3.40				

Transmitter	Region	MIS	PND	Content in μM/g protein	Two-way ANOVA effects	DF	F-value	p-Value
	Thal	control	35	88.19 ± 3.17	MIS	1,50	0.506	0.480
			100	88.6 ± 4.90	age	1,50	0.431	0.515
		poly I:C	35	88.33 ± 2.72	interaction	1,50	0.546	0.464
			100	81.00 ± 5.86				
	Nacc	control	35	65.12 ± 2.60	MIS	1,47	0.644	0.426
			100	71.38 ± 3.52	age	1,47	9.387	0.003
		poly I:C	35	64.31 ± 1.95	interaction	1,47	1.123	0.294
			100	77.21 ± 2.61				
	CPu	control	35	74.17 ± 2.45	MIS	1,48	0.137	0.712
			100	66.71 ± 2.92	age	1,48	8.458	0.006
		poly I:C	35	76.43 ± 1.90	interaction	1,48	0.924	0.341
			100	61.60 ± 5.31				
	GP	control	35	37.73 ± 1.78	MIS	1,49	0.248	0.621
			100	47.62 ± 4.16	age	1,49	7.735	0.008
		poly I:C	35	37.90 ± 0.96	interaction	1,49	0.210	0.649
			100	51.69 ± 4.61				
	VTA	control	35	49.99 ± 1.69	MIS	1,48	0.027	0.870
			100	69.76 ± 10.88	age	1,48	4.688	0.035
		poly I:C	35	45.73 ± 2.69	interaction	1,48	0.062	0.804
			100	70.63 ± 9.68				
GABA	mPFC	control	35	17.34 ± 0.41	MIS	1,45	0.838	0.365
			100	21.31 ± 0.81	age	1,45	5.664	0.022
		poly I:C	35	17.82 ± 0.57	interaction	1,45	0.507	0.480
			100	25.19 ± 4.57				
	Hipp	control	35	22.94 ± 1.18	MIS	1,50	0.024	0.877
			100	26.93 ± 1.06	age	1,50	15.156	0.000
		poly I:C	35	22.15 ± 0.90	interaction	1,50	0.260	0.613
			100	27.35 ± 1.09				
	Thal	control	35	22.30 ± 1.66	MIS	1,51	0.000	0.977
			100	27.28 ± 1.47	age	1,51	11.343	0.001
		poly I:C	35	20.97 ± 0.65	interaction	1,51	0.468	0.497

Transmitter	Region	MIS	PND	Content in µM/g protein	Two-way ANOVA effects	DF	F-value	p-Value
			100	28.50 ± 2.33				
	Nacc	control	35	58.68 ± 3.60	MIS	1,47	0.494	0.485
			100	45.59 ± 4.31	age	1,47	5.353	0.025
		poly I:C	35	53.39 ± 4.64	interaction	1,47	0.152	0.698
			100	44.08 ± 5.33				
	CPu	control	35	16.75 ± 0.88	MIS	1,48	0.026	0.871
			100	32.43 ± 6.00	age	1,48	7.95	0.007
		poly I:C	35	18.41 ± 0.53	interaction		0.023	0.879
			100	32.48 ± 5.35				
	GP	control	35	55.16 ± 4.17	MIS	1,49	0.292	0.592
			100	51.02 ± 4.27	age	1,49	9.930	0.003
		poly I:C	35	62.36 ± 3.27	interaction	1,49	4.831	0.033
			100	39.13 ± 3.51				
	VTA	control	35	51.86 ± 3.57	MIS	1,48	0.006	0.940
			100	46.77 ± 4.04	age	1,48	0.652	0.423
		poly I:C	35	50.28 ± 4.80	interaction	1,48	0.064	0.801
			100	47.62 ± 4.61				