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Research report

Maternal immune activation leads to atypical turning asymmetry and reduced DRD2 mRNA expression in a rat model of schizophrenia

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ABSTRACT

Atypical asymmetries have been reported in individuals diagnosed with schizophrenia, linking higher symptom severity to weaker lateralization. Furthermore, both lateralization and schizophrenia are influenced by the dopaminergic system. However, whether a direct link between the etiology of schizophrenia and atypical asymmetries exists is yet to be investigated. In this study, we examined whether maternal immune activation (MIA), a developmental animal model for schizophrenia and known to alter the dopaminergic system, induces atypical lateralization in adolescent and adult offspring. As the dopaminergic system is a key player in both, we analyzed neuronal dopamine D2 receptor (DRD2) mRNA expression. MIA was induced by injecting pregnant rats with 10 mg/kg polyinosinic:polycytidylic (PolyI:C) at gestational day 15. Controls were injected with 0.9 % NaCl. Offspring were tested at adolescence or early adulthood for asymmetry of turning behavior in the open field test. The total number of left and right turns per animal was assessed using DeepLabCut. Strength and preferred side of asymmetry were analyzed by calculating lateralization quotients. Additionally, DRD2 mRNA expression in the prefrontal cortex of offspring at both ages was analyzed using real-time PCR. MIA was associated with a rightward turning behavior in adolescents. In adults, MIA was associated with an absence of turning bias, indicating reduced asymmetry after MIA. The analysis of DRD2 mRNA expression revealed significantly lower mRNA levels after MIA compared to controls in adolescent, but not adult animals. Our results reinforce the association between atypical asymmetries, reduced DRD2 mRNA expression, and schizophrenia. However, more preclinical research is needed.

1. Introduction

Schizophrenia is one of the top 15 leading causes of disability worldwide, with an estimate of 20 million people affected around the globe [1]. Although the symptoms and etiology of schizophrenia have been researched for more than a century [2], there are still missing pieces to the full picture. Different perspectives combining clinical and basic research might help advance our understanding of the disorder. Besides the diagnostic criteria symptoms, one interesting finding that deserves further investigation is atypical asymmetries in individuals diagnosed with schizophrenia [3,4]. Generally, atypical asymmetries have been reported in several neurodevelopmental, psychiatric, and neurological disorders [4]. Several meta-analyses revealed a more than 1.5 fold increased odds ratio for non-right-handedness (meaning left-handedness and mixed-handedness) in people diagnosed with schizophrenia when comparing to healthy controls [5,6]. Likewise, the chance of being prescribed antipsychotics was increased by 53 % in left-handed compared to right-handed hospitalized children [7], underlining the association of atypical functional and behavioral lateralization and schizophrenia. Besides handedness, language

Abbreviations: CG, control group; Ct, circle threshold; DRD2, dopamine D2 receptor; LQ, lateralization quotient; MIA, maternal immune activation; NaCl, natrium chloride; PFC, prefrontal cortex; PND, postnatal day; PolyI:C, polyinosinic:polycytidylic; rtPCR, real-time PCR.

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lateralization is another form of motor asymmetry that has been investigated in patients diagnosed with schizophrenia [8]. Especially, patients who experience symptoms affecting the language system show a greater reduction of leftward language lateralization in the divided visual field paradigm [8,9] and the dichotic listening test [8].

Atypical asymmetries in schizophrenia have also been reported in brain structure [10]. Interestingly, for some brain regions, e.g., the planum temporale, the amount of decrease of leftward asymmetry also correlated with symptom severity in patients suffering from schizophrenia [11]. Others found an association between altered leftward lateralization in white matter integrity and severity of positive psychotic symptoms like delusion and hallucination [12]. Therewith, studies on atypical asymmetries in individuals affected with schizophrenia support a link between symptom severity and altered hemispheric asymmetries particularly for symptoms such as auditory verbal hallucinations and changes in language lateralization [8,13]. Even though several studies confirm altered asymmetries in schizophrenia, the cause for this relationship remains unclear. Here, animal models could provide useful insights, but suitable studies are rare so far.

It is known that rodents show an intrinsic side preference that is modulated by dopamine release [14]. Thus, rodents tend to turn contralateral to the striatum side containing more dopamine than the other and this turning behavior can be modulated by e.g., amphetamine, potentially leading to circling behavior [14,15]. Interestingly, stress exposure can result in increased dopamine release in the contralateral striatum side leading to atypical turning behavior [15]. Similarly, researchers have reported hemispheric dopaminergic asymmetries in the medial prefrontal cortex (PFC) consistent with the turning preferences of rats after ethanol injection. So right-turning rats show activation in the right medial PFC [16] as well as dominant right PFC cortex activation during stress regulation [17]. Laterality research in rodents might thus indicate that alterations in the dopaminergic system may be relevant for atypical asymmetries reported in patients diagnosed with schizophrenia.

Given that alterations in dopamine in the mesolimbic and prefrontal brain regions have repeatedly been reported in patients diagnosed with schizophrenia [18], a modulating role of dopamine in the expression of both schizophrenia and atypical asymmetries is highly likely. Findings from preclinical animal studies suggest that impairments in dopamine signaling in early brain development lead to schizophrenic-like phenotypes and long-lasting alterations in neuronal dopamine signaling [19]. Thus, alterations in dopamine signaling in the mesolimbic and prefrontal brain regions have repeatedly been reported in patients diagnosed with schizophrenia [18]. It is further hypothesized that in patients suffering from schizophrenia the mesolimbic areas are affected in terms of a hyperactive dopamine transmission meanwhile the prefrontal cortex is marked by a hypoactive dopamine transmission [18]. It has also been postulated that increased dopaminergic release in the subcortical regions, leading to increased dopamine D2 receptor (DRD2) activation in the frontal cortex, is responsible for positive symptoms of schizophrenia. In contrast, reduced dopamine D1 receptor (DRD1) activation in the PFC may be responsible for negative symptoms in humans [20].

The relevant role of dopamine in schizophrenia is further reinforced by the fact that frequently applied antipsychotic drugs for treating schizophrenia work by blocking the DRD2 [21–24]. In a postmortem study investigating the expression pattern of DRD1 and DRD2 expression in the dorsolateral PFC, hippocampus, and nucleus caudate, patients previously diagnosed with schizophrenia showed alterations only in the dorsolateral PFC with an increased expression of presynaptic DRD2 and decreased expression of post-synaptic DRD2 variants in the dorsolateral PFC compared to controls [25]. Furthermore, expression of DRD2 and DRD1 was again lower in patients diagnosed with schizophrenia compared to patients diagnosed with bipolar disorder or major depressive disorder indicating that these changes were specific to schizophrenia [25]. However, the excepted direction of change (increased or decreased) in DRD2 expression or activation is for one, dependent on the region being investigated and, dependent on the molecular level of investigation e.g., mRNA, protein, receptor-binding activity [18].

The implication of the dorsolateral PFC as a key region being altered in schizophrenia has been reinforced by further postmortem studies revealing altered expression of multiple genes and micro RNAs in the dorsolateral PFC in patients previously diagnosed with schizophrenia [26–28]. On the preclinical side, applying the maternal immune activation (MIA) animal model for schizophrenia also lead to alterations in the dopaminergic system (for review see [29]) such as decreased DRD2 binding in the striatum [30] reduced density of DRD1 and DRD2 in the PFC and enhanced tyrosine hydroxylase in striatal structures [31].

In rodents, the polyinosinic:polycytidylic (PolyI:C) MIA model is a well-established model to induce a schizophrenia-like phenotype [29,32], especially as this model enables to investigate induced neurodevelopmental alterations throughout development [33]. PolyI:C is an immunostimulant that structurally mimics double-stranded RNA and thus, can simulate viral infections [34]. Given the widely accepted implementation of the PolyI:C MIA model for studying schizophrenic-like alterations, it is of great interest to investigate whether this model can render valuable insights into the association between schizophrenia and atypical lateralization as well.

Comparable to humans, motor lateralization can be assessed in rodents and other species by investigating different behaviors [35]. In rodents, motor lateralization has been investigated in the form of e.g. paw preference [36], head-turning [37], or general body turning behavior [15,16,38,39]. One advantage of analyzing general turning behavior in an open setup or maze is that turning behavior occurs more naturally, especially, when animals have to navigate through a maze as in the open field, the T-maze, and the elevated plus-maze test [39]. Studies analyzing the turning behavior of rodents in mazes report a favor of the right side [39]. However, when assessing other asymmetry behaviors in rodents, the individual shows strong lateralization but at the population level, no side bias has been found in e.g. paw preference (54 % right-sided) [36] and head-turning [37]. Of note, a study examining head-turning asymmetry in the context of mood disorders found that left-turning rats showed increased behavioral despair in the forced swim test compared to right-turning rats [37]. Even though laterality research has made tremendous progress over the last decades [40], it still faces some problems concerning the methodological assessment of lateral behavior [41]. Here, new advances in deep learning can improve the so-called 'computational ethology' [42]. Especially the use of toolboxes like DeepLabCut allows for precise and fast tracking of complex behaviors [43,44]. DeepLabCut has therefore already proven to be a reliable and valuable method for analyzing e.g., turning behavior [45] or more laborious assessments like eye use of birds [46] and thus should be implemented when possible in the study of behavioral asymmetries. The use of these toolboxes will also enable more comparable and replicable results thus promoting the validity of study results.

Recently we showed that postnatal early life stress, a risk factor for several psychiatric disorders, leads to atypical leftward turning behavior in rats [45] underlining the potential of psychiatric animal models to help advance laterality research. Given the important modulating role of dopamine in schizophrenia and asymmetries, the question arises whether MIA also induces atypical asymmetries in rats similar to the atypical asymmetries reported in humans diagnosed with schizophrenia. To our knowledge, this is the first study to investigate whether MIA as a model for schizophrenia leads to atypical turning asymmetries in adolescent and adult rats in the open field test.

2. Methods

2.1. Animals

All animals were housed under standard conditions (22 ± 2 °C room temperature, 55 ± 25 % humidity) and standard lighting (12 h/12 h) with free access to water and food. 9 timed-pregnant Sprague-Dawley

rats (Charles River Laboratories, Sulzfeld, Germany) were single housed upon mating and divided into the MIA (N = 5) or control group (CG, N = 4). Day of birth was considered postnatal day (PND) 0 and at PND2 pups were sexed and when necessary culled to 10, if possible five male and five female pups. At PND21, pups were weaned and group-housed with same-sex siblings until testing. Experiments were conducted under the principles of Germany's Animal Welfare Act after approval by the LANUV (Landesamt für Natur, Umwelt und Verbraucherschutz North Rhine-Westphalia).

2.2. Maternal immune activation

In rats, an injection of 10 mg/kg PolyI:C on gestation day (GD) 15 is a long-established used experimental approach for inducing MIA to investigate early neurodevelopmental changes [47–49]. Thus, in this study, on GD15 pregnant rats were intraperitoneally injected with either:

I) 10 mg / kg PolyI:C (10 mg PolyI:C dissolved in 1 ml 0.9 % NaCl) (MIA) or
II) 0.9 % NaCl (1 ml / kg) (CG).

Offspring was tested at either PND39 (adolescence) or PND59 (early adulthood). At PND39, a total of 13 CG (5 ♀ + 8 ♂) and 18 MIA (7 ♀ + 11 ♂) animals were tested. In adulthood, 9 CG (4 ♀ + 5 ♂) and 12 MIA (4 ♀ + 8 ♂) rats are included in this study. Not more than 2 animals per sex and age group from the same litter were included to avoid litter effects. Animals were sacrificed three days after testing.

2.3. Analysis of turning behavior asymmetries

All behavioral testing was performed during the dark (= active) phase under red light. Offspring were tested for turning behavior in the open field test consisting of an area of 100cm × 100cm with a 30 cm high border. Therefore, animals were placed into the center of the open field and were allowed to run freely for 5 min while being filmed using an HD Webcam (C920 Pro, Logitech) connected to a laptop. Then, movement trajectories were obtained using video-based offline tracking via python software 'DeepLabCut' [44,45]. Extracted x–y coordinates are smoothed before analysis using the smooth spline function to remove noise. To detect turning behavior, angular movements were measured as the angular difference of body-head orientation between two consecutive frames. The turning behavior was defined as cumulative angular movements of more than 45°. To prevent the double count (or, potentially more duplicated counts) of turning more than 90°, an occurrence of a turning behavior was defined, once the angular difference was smaller than 2°. The rationale of this criteria is that a small angular difference between frames indicates animals stopped their turning movement or started to move forward. Both left and right turning behaviors were separately counted and used for subsequent analysis.

2.4. Dopamine D2 receptor mRNA expression analysis in the PFC

DRD2 mRNA expression in the PFC was examined using realtime (rt) PCR analysis. First, the PFC (including cingulate and prelimbic cortices) was dissected according to Watson's The Rat Brain in Stereotaxic Coordinates at bregma 5.64 – 4.20 mm [50]. RNA was isolated using the NucleoSpin TriPrep (Macherey–Nagel, Düren, Germany) with the only modification that for each sample, 40 µl of RNase-free water was added to obtain RNA. The quality and concentration of obtained RNA samples were then checked measuring 1 µl of the sample in NanoDrop ND-1000 Spectrophotometer (PEQLAB Biotechnologie, Erlangen, Germany). Gained mRNA was then transcribed into cDNA using the High-Capacity RNA-to-cDNA™ Kit (Thermofisher Scientific, Darmstadt, Germany) according to the manufacturer's protocol. Then, 30 ng cDNA to detect DRD2 mRNA in the PFC was quantified. The TaqMan hybridization with the TaqMan™ Gene Expression Master Mix and TaqMan gene expression assay for DRD2 (Rn00561126_m1), glyceraldehyde-3-phosphate dehydrogenase (Rn01775763_g1), and beta-actin (Rn00667869_m1) was used. The rtPCR reaction (Applied Biosystems 7500 Fast Real-Time PCR System) was run with 40 cycles and quantified by the number of cycle thresholds (ΔCt method). After rtPCR, the target gene DRD2 Ct was normalized to the Ct of the housekeeping genes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and beta-actin (bACT). All samples were tested in duplicates. The ΔCt value was calculated as previously described [51] and as follows: ΔCt = Mean Ct (DRD2) – Mean Ct (GAPDH + bACT).

2.5. Statistical analysis

For analysis of behavioral data, a 2 × 2 × 2 repeated-measures ANOVA with the within-subjects factors side (left turning, right turning) and the between-subjects factors experimental group (MIA, CG) and age group (PND39, PND59) was conducted. DRD2 ΔCt was analyzed using univariate ANOVA with the between-subjects factor treatment group (MIA, CG) and age group (PND42, PND62) and the dependent variable ΔCt DRD2. Post-hoc tests were corrected for multiple comparisons using Bonferroni correction.

Lateralization quotient (LQ) was determined for each animal following the formula LQ = ((R-L) / (R + L)) ×100, with R indicating the number of right turns and L indicating the number of left turns. The LQ ranges between -100 and 100, with positive values representing a right-sided turning bias and negative values indicating a left-sided turning bias.

3. Results

3.1. Relative DRD2 mRNA expression given as ΔCt

To investigate the effect of MIA on relative DRD2 mRNA expression, we used a univariate ANOVA with the between-subjects factors treatment group (MIA, CG) and age group (PND42, PND62) and the dependent variable ΔCt DRD2. There was a trend towards significance for the main effect of treatment group ($F_{(1,44)} = 3.54$; $p = 0.067$; partial $\eta^2 = 0.08$). Absolute ΔCt values showed a trend to be higher in the MIA group (9.74 +/- 0.13) than in the CG (9.37 +/- 0.15), indicating a trend towards lower DRD2 mRNA levels in the MIA group compared to CG. The main effect of age group ($p = 0.92$) and the interaction experimental group × age group ($p = 0.34$) failed to reach significance.

To further explore this trend, we ran this analysis separately for the PND42 and the PND62 group. For the PND42 group, the effect reached significance ($F_{(1,25)} = 4.39$; $p = 0.046$; partial $\eta^2 = 0.15$), indicating higher ΔCt values (lower mRNA levels) in the MIA group than in the CG. For the PND62 group this effect failed to reach significance ($F_{(1,17)} = 0.44$; $p = 0.52$).

3.2. Number of turns

We first analyzed whether there was a difference in the overall number of turns between age and treatment groups to assess whether there were any general differences in turning behavior frequency. To this end, we analyzed the overall number of turns for each individual using a 2 × 2 univariate ANOVA with the between-subjects factors experimental group (MIA, CG) and age group (PND39, PND59). In the age group PND39, the overall number of turns was 68.00 (+/- 10.77) in the CG and 63.17 (+/- 8.85) in the MIA group. In the age group PND59, the overall number of turns was (59.33 +/- 10.62) in the CG and 62.25 (+/- 8.13) in the MIA group. The main effects of the experimental group ($p = 0.73$) and age group ($p = 0.09$) failed to reach significance, as did the interaction experimental group × age group ($p = 0.16$). Therefore, there were no significant group differences in the overall number of turns.

3.3. Turning bias

The distribution of left turns and right turns in the two experimental and the two control groups are shown in Fig. 1. We conducted a 2 × 2 × 2 repeated-measures ANOVA with the within-subjects factors side (left turning, right turning) and the between-subjects factors experimental group (MIA, CG) and age group (PND39, PND59). The main effect of side ($F_{(1,48)} = 5.97$; $p = 0.018$; partial $\eta^2 = 0.11$) reached significance, indicating that on average, animals made more right (32.18 +/- 0.75) than left turns (31.01 +/- 0.70). The main effects of the experimental group ($p = 0.73$) and age group ($p = 0.09$) failed to reach significance, as did the interactions experimental group × age group ($p = 0.16$), experimental group × side ($p = 0.71$), and age group × side ($p = 0.59$). However, the interaction side × experimental group × age group reached significance ($F_{(1,48)} = 6.20$; $p = 0.016$; partial $\eta^2 = 0.11$). To further investigate this interaction, we conducted Bonferroni-corrected post-hoc tests. In age group PND39, the comparison between left and right turns reached significance in the MIA ($p = 0.006$), but not in the CG group ($p = 0.62$). Here, the MIA group showed more rightward turns (32.72 +/-1.23) than leftward turns (30.44 +/- 1.15). In age group PND59, the comparison between left and right turns reached significance in the CG ($p = 0.033$), but not in the MIA group ($p = 0.67$). Control animals showed more rightward (30.89 +/- 1.74) than leftward turns (28.44 +/- 1.63). This indicates a rightward turning bias in the CG group that was absent in the MIA group, suggesting a reduction of asymmetry in the MIA group.

3.4. Strength of lateralization

To further analyze whether the groups showed any differences in the strength of lateralization independent of the direction of lateralization, we determined the absolute LQ for each animal as a measure of lateralization strength. We then conducted a 2 × 2 repeated-measures ANOVA with the between-subjects factors experimental group (MIA, CG) and age group (PND39, PND59). Neither the two main effects (all p's > 0.18) nor the interaction ($p = 0.42$) reached significance.

3.5. Direction of lateralization

To test whether the groups showed any differences in the direction of lateralization independent of the strength of lateralization, we classified individual animals as showing a rightward turning preference (positive LQ) or a leftward turning preference (negative LQ). We then used a Kruskal-Wallis test with four groups (PND39 MIA, PND39 CG, PND59 MIA, PND59 CG) to investigate whether there were any differences in turning bias direction between these groups (see Table 1). The results indicated a significant difference in turning bias between the four groups ($\chi^2 = 11.68$; $p = 0.009$). In the PND39 age group, the MIA group showed a rightward asymmetry (72 % of animals had a rightward preference) while the CG showed a leftward preference (69 % of animals showed a leftward preference). In the PND59 age group, the CG group showed a strong rightward preference, with 100 % of animals showing a rightward turning bias. Average LQ in this group was 4.04 (+/- 2.09), with a range between 1.37 and 7.69. In contrast, there was only a slight rightward preference in the MIA group (58 % of animals showed a rightward turning bias). Average LQ in this group was 0.65 (+/- 4.33), with a range between -7.14 and 6.85.

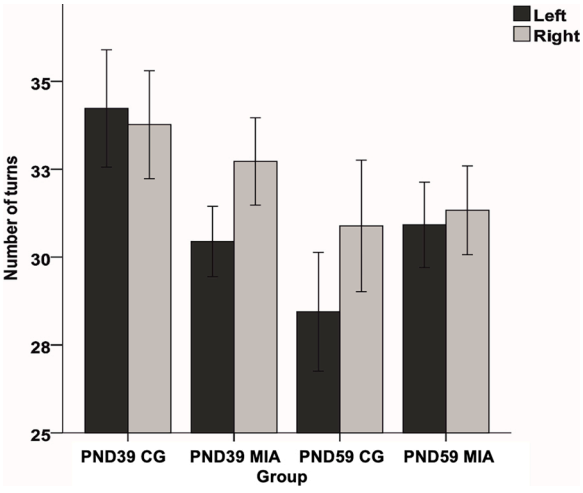

Fig. 1. Total number of left (black) and right (grey) turns per group is given as mean ± standard error. The groups are from left to right: PND39 CG, PND39 MIA, PND59 CG, and PND59 MIA.

3.6. Association between dopamine D2 receptor mRNA expression and laterality

We calculated partial correlation coefficients between relative DRD2 mRNA expression (ΔCt values) and LQ and absolute LQ with the control variables age group and treatment group. The effect failed to reach significance for LQ ($r = 0.04$; $p = 0.80$). However, there was a statistical trend for absolute LQ ($r = -0.28$; $p = 0.063$), indicating that there was a tendency of higher ΔCt values (lower relative DRD2 mRNA expression) being associated with lower lateralization strength.

4. Discussion

This study aimed to investigate whether MIA, a neurodevelopmental model of schizophrenia, induces atypical asymmetries and whether these atypical asymmetries are reflected in reduced dopaminergic signaling. Therefore, adolescent, and adult rat offspring that underwent MIA during gestation were tested on the open field test for turning bias, as well as strength and direction of lateralization. Moreover, we examined relative DRD2 mRNA expression in the offspring's PFC via rtPCR to examine the modulating role of dopamine. Lastly, a potential association between DRD2 mRNA expression and laterality was investigated.

Concerning the turning bias, we found significantly more rightward than leftward turns in the adolescent MIA group. In adults, CG animals showed more rightward turns than MIA offspring. This might indicate that in adult offspring, the rightward turning bias of the CG is absent in the MIA offspring suggesting reduced asymmetry after MIA. However, our result does not allow to state whether the two groups show significantly different biases. In this study, no effect of MIA on strength of lateralization was found but an effect of MIA and age on the direction of lateralization. In adolescents, MIA led to rightward asymmetry whereas CG showed a leftward preference. In early adulthood, the side preference shifted towards a strong rightward preference in CG (100 %) whereas the strong rightward preference of adolescents was reduced to a slight rightward preference in adult MIA (58 %). In terms of DRD2 mRNA expression in the PFC, we found significantly higher ΔCt values in the MIA group compared to CG indicating lower levels of DRD2 mRNA after MIA in the adolescents, but not in adult animals.

The analysis of ΔCt values and LQ did not reach significance. As side preference is modulated by neuronal dopamine release [14], reduced

Table 1
Number of animals with a leftward or a rightward turning preference across the 4 groups. PND39: postnatal day 39 group; PND59: postnatal day 59 group; MIA: Maternal Immune Activation Group; CG: Control group.

		Left	Right	Overall
PND39	**MIA**	5 (28 %)	13 (72 %)	18
	CG	9 (69 %)	4 (31 %)	13
PND59	**MIA**	5 (42 %)	7 (58 %)	12
	CG	0 (0%)	9 (100 %)	9

DRD2 mRNA expression could lead to reduced lateralization. Studies showed that rodents tend to turn contralateral to the striatum side containing more dopamine than the other and this turning behavior can be modulated by e.g., amphetamine, and when potentiated this behavior can lead to circling behavior [14]. This finding was confirmed in genetically modified circling rats which showed lower striatal dopamine ipsilateral to the preferred side they rotated to [15]. Interestingly, stress exposure increased dopamine release in the contralateral striatum side but only in genetically modified rats and not in wild-type controls [15]. Similarly, researchers have reported hemispheric dopaminergic asymmetries in the medial PFC consistent with the turning preferences of rats after ethanol injection. So right-turning rats showed activation in the right medial PFC [16] as well as dominant right PFC cortex activation during stress regulation [17]. Further studies should therefore examine the association between turning behavior and PFC DRD2 expression. As we investigated DRD2 mRNA expression, our study does not allow conclusions regarding potential functional effects of altered DRD2 protein expression.

In this study, adolescent CG show no turning bias but slightly more leftward turns in the open field test similar to previous results [45]. However, the increased rightward turning in the adolescent MIA group is contrary to our previous finding that prolonged early life stress leads to atypical leftward turning in adolescent rats [45]. Interestingly, in our previous study, rats that only experienced stress in the early postnatal days showed a slight (but insignificant) rightward turning bias. Thus, the results might indicate that the timing of stress is relevant for altered side preferences as prenatal or early postnatal disruptions in brain development seem to result in atypical rightward turning behavior while later postnatal disruptions lead to atypical leftward turning asymmetries. Another possible explanation might be that side preferences can change throughout development. So far, side preferences throughout development have not been investigated extensively in mammals. However, in precocial species such as domestic chicks, hemispheric lateralization has been examined quite well throughout development [52,53]. Thus, for domestic chicks, dramatic age-dependent changes in hemispheric control have been reported. For example, when they show locomotion for the first time independently and away from the mother hen (at around day 10), as an opportunity to learn about unfamiliar conspecifics, this is accompanied by increased activation of the right hemisphere, which is also in charge of social recognition processing [52, 53]. In a study investigating tail asymmetry after prenatal stress in rats, it was shown that prenatal stress led to an atypical rightward bias in neonatal rats compared to a leftward bias in controls [54]. Interestingly, when prenatally stressed rats reached adulthood, a reduction in directional preference was found in males while females even presented a reversal of side preference [54]. But most developmental studies tend to focus on the early postnatal period and adulthood leading to a lack of data for the critical time of adolescence.

The here reported turning bias in adult rats is in line with the results on handedness in humans where people diagnosed with schizophrenia show a 1.5 fold increase in non-right-handedness compared to healthy controls [5,6]. Matching this tendency in humans, our results show that adult MIA rats turn equally to both sides when navigating in the open field test with only a slight rightward preference while CG rats show a strong rightward preference.

The shift in direction of lateralization from adolescence to early adulthood highlights the crucial time of adolescence characterized by great progress in brain maturation [55]. This brain maturation process also includes naturally occurring brain asymmetries during development. A study investigating human brain asymmetries throughout development analyzing white matter networks in adolescents and young adults found that the degree of rightward asymmetry in global and local network efficiencies was decreased in young adults compared to adolescents underlining adolescents as a period of great change also in terms of asymmetry [56]. The critical time of adolescence is also influenced by the dopaminergic system [57]. Dopaminergic projections into the PFC continue to develop until early adulthood with a peak in PFC dopamine levels during adolescence in non-human primates [58] and rats [59].

The here found lower DRD2 mRNA expression in the PFC of MIA offspring is in line with the association between polymorphisms in the DRD2 gene and schizophrenia in humans [22]. This was recently supported by a study in a young Bangladeshi population where polymorphisms in the DRD2 gene showed again association with the susceptibility towards schizophrenia [60]. In the PolyI:C MIA model, altered DRD2 expression in the PFC has already been reported in adult mice [31]. Thus, the here found alteration in DRD2 mRNA expression after MIA underlines the validity of this PolyI:C MIA model for schizophrenia and allows to claim that the MIA applied in this study induced a schizophrenic-like phenotype. One reason for the failed significant difference in DRD2 mRNA expression in adult rats might be that in the study of Meyer and colleagues, only male mice showed a reduced expression while female MIA mice did not [31]. In our study, no sex differences were reported, possibly due to small sample sizes. It is thus likely that an increased sample size would lead to significant differences between treatment groups in DRD2 mRNA expression in adults as well.

As a limitation of this study, we did not analyze DRD2 mRNA expression for each hemisphere separately. Thus, in future research, it would be of interest to investigate whether the atypical turning behavior found in the MIA offspring is not only modulated by an overall altered PFC DRD2 mRNA expression but a result of atypical asymmetric expression patterns. Studying hemispheric expression levels will also enable us to investigate if hemispheric DRD2 mRNA expression is predictive of turning asymmetries and vice versa. Furthermore, it would be of interest to examine DRD2 mRNA expression levels in the context of atypical turning behavior in the medial PFC, as this region has been reported to be implicated in patients diagnosed with schizophrenia as well.

5. Conclusion

To sum up, MIA induces atypical turning behavior with different implications on strength and direction throughout development highlighting adolescents as a crucial time of change. Furthermore, MIA led to reduced DRD2 mRNA expression in the PFC in adolescent rats. Thus, our results add valuable insights into the basic understanding of the association between asymmetries and schizophrenia.

Authors contribution

Annakarina Mundorf and Nadja Freund designed the study. **Karola Hünten** and **Nadja Kubitza** performed the experiments. **Hiroshi Matsui** performed the data extraction with DeepLabCut. Statistical analyses were performed by **Annakarina Mundorf** and **Sebastian Ocklenburg**. The manuscript was written by **Annakarina Mundorf**, **Hiroshi Matsui**, **Sebastian Ocklenburg**, **Nadja Freund** and **Georg Juckel**. All authors approved the manuscript. This manuscript is our original work, and it is submitted for first publication.

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Declaration of Competing Interest

The authors report no declarations of interest.

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