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Prenatal treatment with methylazoxymethanol acetate as a neurodevelopmental disruption model of schizophrenia in mice

Kohei Takahashi[a,1], Osamu Nakagawasai[a,*], Wakana Sakuma[a,1], Wataru Nemoto[a], Takayo Odaira[a], Jia-Rong Lin[a], Hiroshi Onogi[b], Lalit K. Srivastava[c], Koichi Tan-No[a]

[a] Department of Pharmacology, Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai, Miyagi 981-8558, Japan
[b] Faculty of Health Science, Tohoku Fukushi University, 1-8-1 Kunimi, Aoba-ku, Sendai, Miyagi 981-8522, Japan
[c] Department of Psychiatry, Douglas Mental Health University Institute, McGill University, Montreal, QC, Canada

HIGHLIGHTS

- MAM mice showed schizophrenia-like behaviors.
- MAM mice exhibited neuroanatomical changes in the hippocampus.
- DA concentrations and DOPAC/DA increased in the mPFC of MAM treated mice.
- MAM-induced PPI deficits and social withdrawal improved with atypical antipsychotics.
- Atypical and typical antipsychotics improved hyperactivity by MK-801 in MAM mice.

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ABSTRACT

Methylazoxymethanol (MAM)-treated pregnant rat at gestation day (GD) 17 has been shown to be a valuable developmental animal model for schizophrenia. Yet, this model remains to be established in mice. In the present study, we examined behavioral, cytoarchitectural, and neurochemical changes in the offspring of MAM-treated mice and validated the model's face, construct and predictive validities. We found that in contrast to a single injection of MAM to dams at GD 15, 16 or 17, its daily administration from GD 15 to 17 led to deficits in prepulse inhibition (PPI) of startle in the post-pubertal offspring. In addition, we observed behavioral deficits in working memory and social interactions, as well as an increase in locomotor activity induced by the NMDA antagonist MK-801 in GD15-17 MAM offspring. These animals also showed a reduction in the volume of the prefrontal cortex (PFC) and hippocampus, neuroanatomical changes such as discontinuities and heterotopias in the hippocampus, and an increase of DA level and DOPAC/DA ratio in the medial PFC. Atypical antipsychotic drugs clozapine, risperidone, and aripiprazole, but not the typical drug haloperidol, reversed the deficit in PPI and social withdrawal in the offspring of MAM-treated dams. In contrast, MK-801-induced hyperactivity in MAM mice was reversed by both and typical or atypical antipsychotic drugs. Taken together, the treatment of pregnant mice with MAM during GD 15–17 offers a new approach to study neurobiological mechanisms involved in the pathogenesis of schizophrenia.

1. Introduction

Schizophrenia is a frequent psychiatric disorder that appears during the late adolescent stage or early adulthood and is regarded as a neurodevelopmental disorder in which alterations start early in development. Epidemiological research (Waddington et al., 1999) suggest that during the second trimester of pregnancy, the fetus could be exposed to environmental factors that increase the risk of developing schizophrenia (Waddington et al., 1999). During this period, extensive neuronal cell migration to the cortex occurs in the human brain

Abbreviations: ANOVA, analysis of variance; BrdU, 5-bromo-2′-deoxyuridine; CA, Cornu Ammonis; DA, dopamine; DG, dentate gyrus; GD, gestation day; MAM, methylazoxymethanol; mPFC, medial prefrontal cortex; NVH, neonatal ventral hippocampal lesions; SEM, standard error of the mean

* Corresponding author.
E-mail address: osamun@tohoku-mpu.ac.jp (O. Nakagawasai).

[1] These authors contributed equally to this work.

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(Sidman and Rakic, 1973). The current view is that during adolescence brain maturation could be inhibited by the primal developmental alterations and thus contribute to the pathophysiology of schizophrenia (Rapoport et al., 2005). A number of animal models have been described to explore this hypothesis.

Current schizophrenia models are not intended to serve as the complete animal equivalent of the human disorder. Rather, they are often designed to test specific causative or mechanistic hypotheses.

In accordance with the neurodevelopmental hypothesis of schizophrenia, animal models have been proposed using pre- or early postnatal brain damages to induce schizophrenia-like behavioral changes (Lipska and Weinberger, 2000; Boksa, 2004; Laplante et al., 2005). Prenatal toxin treatment can induce alterations in the structures of the brain, with a relative specificity given by the neurogenic timetable (Cattabeni and Di Luca, 1997). To induce neurodevelopmental damages in the hippocampus and cortex, methylazoxymethanol (MAM) can be used to briefly interfere with brain cellular proliferation during late gestation, a time when the neurons that will migrate to these areas are undergoing major cell division (Bayer and Altman, 2004). MAM administration to pregnant rats at gestation day (GD) 17 was recently used as an experimental model for schizophrenia (Moore et al., 2006). This treatment leads to aberrant methylation of DNA and interference with neurogenesis (Hoareau et al., 2006) and induces many characteristics consistent with schizophrenia, including neurochemical changes, behavioral changes [in prepulse inhibition (PPI) of startle reflex, working memory and social interaction] and increased locomotor activity to psychotropic drugs (Gomes et al., 2016). Furthermore, MAM-treated rats display an increase in the firing of dopamine (DA) neurons that is driven by hyperactivity in the ventral hippocampus (Chen et al., 2014; Gomes et al., 2016). Thus, this model is appropriate for studying the link between altered embryonic neurogenesis and transition to schizophrenia-like deficits in the adult.

Given the general advantage of mice models (for example, the availability of transgenic strains) the MAM-treated mice could enable researchers to analyze genetic influences in the pathophysiology of schizophrenia. Guo et al. (2013) have reported the effect of MAM given to adolescent mice. More recent studies have reported establishment of models based on pregnant mice treated with MAM at GD16 or 17 (Chalkiadaki et al., 2018; Huo et al., 2018). However, these models fail to exhibit many schizophrenia-like phenotypes, such as behavioral and brain changes, in the offspring.

Our validation experiments using GD 16 or 17 MAM-treated mice did not reveal significant schizophrenia-related behaviors in the offspring, e.g., deficits in prepulse inhibition of startle. Therefore, we developed a protocol in which mice are treated with MAM on GD15-17. According to species comparison of Carnegie stages of embryonic development (https://embryology.med.unsw.edu.au/embryology/index.php/Mouse_Development), GD15.5 of mice may be in accordance with GD17 of rats, regarding the morphological development of the embryo. In addition, the latest predictive model (Workman et al., 2013) proposes that equivalent maturation stages of brain development between mouse and rat differ by 1–2days, with the latter maturing later. We anticipated that a prenatal treatment with MAM during this period would induce neuronal loss (Gaspard et al., 2008; Alsiö et al., 2013) and behavioral, pathological and neurochemical changes relevant to schizophrenia. Our data shows that a number of these changes induced by prenatal MAM treatment are detectable in the offspring at post-pubertal period and can be reversed by typical and atypical antipsychotic drugs. We believe that the mice MAM model may serve as a new heuristic paradigm to further investigate the neurodevelopmental bases of schizophrenia.

2. Materials and methods

All experiments were performed following the approval of the Ethics Committee of Animal Experiment in Tohoku Medical and Pharmaceutical University, and according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Efforts were made to minimize suffering and to reduce the number of animals used. Measurement of the behaviors and post-mortem analyses were done in blinded manner.

2.1. Animals

Pregnant female ddY mice were obtained from Japan SLC (Shizuoka, Japan) on GD 11–13 and were housed individually in plastic cages (height: 12 cm, width: 17 cm, length: 27 cm). Our choice of this strain of mice was partly based on previous work showing that ddY mice are more vulnerable to the development of behavioral disturbance by stress or phencyclidine treatments (Mouri et al., 2012). This strain is also more responsive to treatment with antipsychotics than other strains (Sugimoto et al., 2008). The dams were injected once daily with saline or MAM (10 mg/kg, i.p.) for 3 days, between GD 15 and 17 (dams treated with higher dose of MAM, 20 mg/kg, died almost immediately after delivery and the pups didn't survive). Within 10 days after birth, all female offspring were culled. The remaining pups were weaned 21 days after birth and were housed in groups of 4–6 mice. Only the male pups were included in the studies, and each animal was tested only for one behavior at pre-pubertal age (5 weeks) and then one behavior at post-pubertal age (8 weeks). The onset of pubertal age has been described previously (Palanza et al., 2001; Naert et al., 2013). The experiments were performed on several groups of saline or MAM-treated animals. They consisted of: (1) behavioral testing (n = 492), (2) measurement of body and brain weights (n = 10), (3) determination of neurotransmitter concentrations (n = 14), and (4) immunohistochemistry (n = 28). The animals were housed in plastic cages with free access to food and water under conditions of constant temperature (22 ± 2 °C) and humidity (55 ± 5%), on a 12 h light-dark cycle (lights on at 07:00).

2.2. Drugs

MAM (Wako Pure Chemical Industries Ltd, Osaka, Japan) and MK-801 (Sigma-Aldrich, St-Louis, USA) were dissolved in saline. Haloperidol (Sigma-Aldrich), Clozapine (Sigma-Aldrich), Risperidone (Sigma-Aldrich) and Aripiprazole (Wako Pure Chemical Industries Ltd) were dissolved in 0.5% Tween80 (Wako Pure Chemical Industries Ltd). These drugs were administered intraperitoneally (i.p.) at a volume of 0.1 ml/10 g of mouse body weight. The antipsychotic drugs were administered 30 min before the behavioral tests.

2.3. PPI of the acoustic startle response

Sensorimotor gating processes which are frequently impaired in schizophrenia patients were evaluated using the PPI of the startle response which is one of the paradigms to detect this deficit in patients (Braff et al., 2001). Tests were conducted using the commercial software package SR-LAB system (SR-LAB, San Diego Instruments, San Diego, USA) that comprised two sound-attenuating chambers each equipped with a cylindrical Plexiglas animal enclosure (length: 6.7 cm; inner diameter: 3.8 cm). Ventilation was provided by a small electric fan that also generated a 70 dB background noise. Tone pulses were presented by a speaker positioned 24 cm directly above the animal enclosure. A piezoelectric accelerometer affixed to the animal enclosure frame was used to detect and transduce motion resulting from the animal's response. Tone pulse parameters were controlled by a computer using a SR-LAB and interface assembly that also digitized, rectified and recorded stabilimeter readings.

Measures of both acoustic startle response (ASR) and PPI were obtained in a single session. Mice were placed in the Plexiglas enclosure and allowed to acclimatize to the environment for 5 min before being tested during 42 discrete trials. When antipsychotic drugs or vehicle

were administered, mice were placed in the Plexiglas enclosure 30 min after the administration. On the first two trials, the magnitude of the ASR to a 120 dB tone lasting 50 ms was measured. These first two startle tones were presented in order to habituate the animals to the testing procedure. Therefore, the ASR magnitude of these two trials was omitted from the statistical analysis of the mean ASR amplitude. On the subsequent 40 trials, the startle tone was either presented alone or 100 ms after the presentation of a 30 ms duration prepulse. Prepulse intensity ranged from 3 to 15 dB above background noise and was varied randomly between trials in 3 dB steps. Measures were taken at each of the five prepulse intensities during five trials; animals were randomly presented with the startle tone alone during another ten trials; null trials (background tone alone) were conducted during the other five trials. The same stimulus condition was never presented on more than two consecutive trials. The interval between each trial was programmed to a variable time schedule with an average duration of 15 s (range 5–30 s). A measure of startle response amplitude was derived from the mean of 100 digitized data points collected from stimulus onset at a rate of 1 kHz. Prepulse effectiveness in suppressing the startle response was expressed as a percentage based on the mean amplitude of response to the startle tone alone (10 startle tones) relative to those recorded under the five prepulse conditions (5 startle tones/condition): PPI = 100 - [(mean startle amplitude for prepulse + pulse trials/mean startle amplitude for pulse-alone trials) × 100]%. The five different prepulse intensities were averaged and used for statistical analysis.

2.4. Locomotor activity

Positive symptoms of schizophrenia develop around adulthood (Tandon et al., 2009) and are exacerbated by NMDA glutamatergic transmission in brain (Lahti et al., 1995). An increase in locomotor activity has been proposed as an animal behavioral equivalent to the positive symptoms seen in schizophrenia patients (Tandon et al., 2009). The locomotor activity of mice was evaluated using the multichannel activity-counting system SUPERMEX (Muromachi Kikai Co., Tokyo, Japan). This instrument can monitor even minute movements in all three planes of motion (vertical and horizontal) as one movement, owing to its infrared sensor with multiple Fresnel lenses that can be moved close enough to the cage to capture multidirectional locomotor alterations in a single mouse. Thus, vertical movement such as jumping as well as horizontal movements such as walking and running could be counted. The Supermex instrument was connected to a behavioral analyzing system (CompACT AMS) (Muromachi Kikai Co.) which interpreted the movements as one count. Measurements of activity were conducted between 11:00 and 15:00 during the light phase. Mice were divided into two groups (saline and MAM), placed in activity boxes for 60 min. Mice were then injected with saline or MK-801 (0.1 mg/kg, i.p.) and locomotor activity was recorded for another 60 min.

2.5. Social interaction test

Social withdrawal is frequently observed as a negative symptom of schizophrenia (Mueser and McGurk, 2004) that can be modeled in rodents. The general design of the model was adapted from Tanda et al. (2009). The test was performed in a Plexiglas cage (height: 18 cm, width: 26 cm, length: 30 cm). The measurement of social interaction was done by video recording without any observer in the room. Experiments were performed at a light intensity of 35 lux. The arena was placed in a dimly lit room and was cleaned after each test. Two unfamiliar mice that had approximately the same weight and had received an identical prenatal treatment were placed simultaneously in the opposite corners of the arena and the behavior of each mouse was recorded for a 10 min period. Behaviors such as sniffing, grooming and chasing at a distance closer than 2 cm of one another were counted as social interaction. Frequency as well as the time spent in these behaviors were analyzed.

2.6. Y-maze test

In addition to positive and negative symptoms, schizophrenia patients consistently present cognitive deficits in working memory (Heinrichs and Zakzanis, 1998). We assessed cognitive deficits in MAM-treated using spontaneous alternation in a Y-maze. The Y-maze apparatus consisted of three compartments (height: 25 cm, width: 3 cm, length: 40 cm) radiating out from the center. The mice were placed in one of the compartments and allowed to move freely for 8 min. Experiments were performed at a light intensity of 35 lux. An arm entry was defined as three legs entering one of the arms, and the sequence of entries was manually recorded. An alternation was defined as entry into all three arms on consecutive trials. Thus, the maximum number of alternations was the total number of entries minus 2, and the percent alternation was calculated as (actual alternations/maximum alternations) × 100. The percent spontaneous alternation behavior of the mouse was taken as a measure of spatial short-term memory.

2.7. Measurement of body and brain weights

We examined regional brain weights in MAM mice as schizophrenia patients consistently show a decrease in cortical and hippocampal volumes (Brugger and Howes, 2017). On postnatal day 56, mice were weighed and then killed by decapitation. The brain (including the cerebellum) was removed and weighed. On a separate group of saline and MAM treated mice, the prefrontal cortex (PFC), hippocampus and striatum were dissected out and weighed. The PFC corresponded to an area that included the rostral pole of the brain, and was delimited medially by the interhemispheric fissure, laterally by the corpus callosum and caudally extended to Bregma +2.68. The striatum and hippocampus were dissected from the remaining bloc of tissue.

2.8. Immunohistochemical study (cell proliferation and neurogenesis)

Some studies performed on postmortem brains revealed that the proliferation of hippocampal neural stem cell was significantly reduced in schizophrenic patients (Allen et al., 2016; Iannitelli et al., 2017). On postnatal day 42, 5-bromo-2′-deoxyuridine (BrdU) (Nacalai Tesque, Inc, Kyoto, Japan; 75 mg/kg i.p.) was injected three times every 24 h for analysis of neurogenesis. These mice were sacrificed 2 weeks after the first BrdU injection. On postnatal day 56, BrdU was injected three times every 2 h for analysis of cell proliferation. These mice were sacrificed 24 h after the first BrdU injection. Brains were fixed with 4% paraformaldehyde. The brains were cut into 40 μm sections from bregma −1.60 mm to −2.0 mm using a cryostat (MICROM HM560, Mikron Instrument, Inc., Simi Valley, USA). For BrdU immunodetection, tissue sections were first incubated at 37 °C for 30 min in HCl (2 N) followed by primary antibody addition. The primary antibodies used were rat anti-BrdU monoclonal antibody (1:100; Harlan SeraLab, Loughborough, UK), mouse anti-doublecortin (DCX) monoclonal antibody (1:50; Santa Cruz Biotech, Santa Cruz, CA) and mouse anti-neuronal nuclear antigen (NeuN) monoclonal antibody (1:500; Millipore, Temecula, USA). The following fluorescence conjugated secondary antibodies were used: goat anti-rat IgG Alexa Fluor 568 (1:200; Molecular Probes, Eugene, USA) and goat anti-mouse IgG Alexa Fluor 488 (1:200; Molecular Probes). DAPI was used to identify nuclei. Finally, sections were washed and coverslipped with Dako fluorescence mounting medium (Dako, Carpinteria, USA). Labeled sections were analyzed using a confocal laser-scanning microscope (A1Rsi: Nikon, Tokyo, Japan). Eight sections per mouse were used, and two images (640 × 640 μm) of the DG region of the hippocampus were obtained from each section. We counted the number of BrdU+/DCX + cells and BrdU+/NeuN + cells in the DG for analysis of cell proliferation and neurogenesis using NIS-Elements AR analysis system, respectively. A mean number of eight

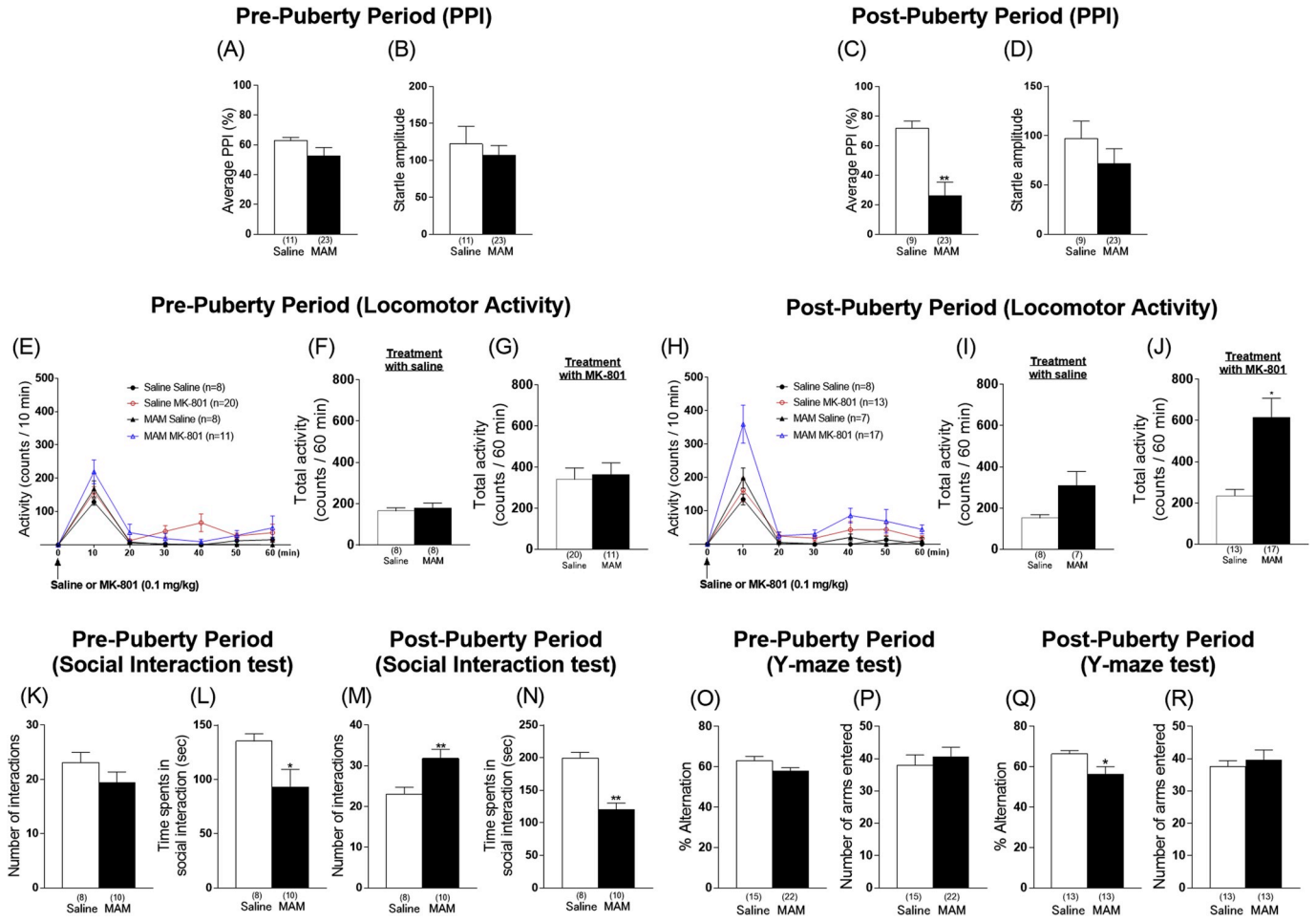


Fig. 1. Behavioral changes in mice exposed to MAM at GD 15–17. Average PPI and acoustic startle response in pre-pubertal (A and B) and post-pubertal (C and D) mice treated prenatally with saline or MAM. PPI deficits in MAM mice developed at post-pubertal, but not pre-pubertal age. Locomotor activity in pre-pubertal (E–G) and post-pubertal (H–J) mice treated prenatally with saline or MAM. (E) and (H) show the temporal profiles of MK-801-induced locomotion. (F, G, I and J) Analysis of total activity scores following MK-801 treatment reveals that MAM mice become more active than control animals only at post-pubertal period. Social interaction in pre-pubertal and post-pubertal mice treated prenatally with saline or MAM (K–N). Social withdrawal in MAM mice began development in pre-pubertal age. Working memory in the Y-maze in pre-pubertal and post-pubertal mice treated prenatally with saline or MAM (O–R). Memory deficits in MAM mice developed in post-pubertal, but not pre-pubertal age. Bars represent means ± S.E.M. *: $p < 0.05$ and **: $p < 0.01$ vs. prenatal treatment with saline group.

images were analyzed for each mouse, and each group contained 4–5 mice.

2.9. Immunohistochemical study (cytoarchitectural and neuromorphometric alteration)

One of the structural abnormality observed in the brains of schizophrenic patients is the hippocampal cellular disarray such as selective decrease in the number of pyramidal cells in the cornu ammonis (CA)2 area and neuron of discontinuities and heterotopias (Benes et al., 1998; Heckers, 2004; Nelson et al., 1998). Brain sections were prepared as described in the preceding section (cell proliferation and neurogenesis). Antigen retrieval was performed for RGS-14 staining. Briefly, free-floating sections were incubated at 80 °C for 30 min in 50 mM sodium citrate (pH = 9.0). We used the following primary antibodies: rabbit anti-Prox1 monoclonal antibody (1:500; Abcam Ltd., Cambridge, UK), mouse anti-RGS14 monoclonal antibody (1:100; NeuroMab, Davis, USA), and rat anti-Ctip2 monoclonal antibody (1:500; Abcam Ltd). The fluorescence conjugated secondary antibodies were: goat anti-mouse IgG Alexa Fluor 488 (1:200; Molecular Probes), goat anti-rabbit IgG Alexa Fluor 568 (1:200; Molecular Probes) and goat anti-rat IgG Alexa Fluor 647 (1:200; Molecular Probes). For analyses, eight sections per mouse were used and two images (640 × 640 μm) of the dorsal and ventral hippocampus were obtained from each section. We counted the number of DAPI positive cells in each sector of the hippocampus i.e. CA1, CA2, CA3 and DG. An average of eight images from each of the three mice per group was used for the analysis. Heterotopias, defined as a group of misplaced neurons, were counted in the hippocampus from MAM and control animals. Discontinuities were defined as a disruption in the CA layers characterized by the presence of interruption in the neuronal layer continuity and dispersed neurons on any side of the cellular layer. Heterotopias, defined as a group of misplaced neurons, were counted in the dorsal hippocampus from MAM and control animals.

2.10. DA and metabolite measurements with high-performance liquid chromatography (HPLC)

It has been suggested that a disturbance in the dopaminergic system, such as an increased presynaptic DA function in the various brain regions, underlies schizophrenia (Kesby et al., 2018). Mice were divided into two groups (saline and MAM) and sacrificed by decapitation on postnatal day 56. Each brain was rapidly removed and the medial PFC (mPFC), striatum and hippocampus were dissected on an ice-cold plate. These brain tissues were homogenized by sonication in ice-cold 0.1 M perchloric acid with an internal standard (Isoproterenol)

added at a concentration of 100 ng/ml. The homogenates were centrifuged at 10000 × *g* then each supernatant was filtered through a 0.45 μm pore size membrane filter. The filtrate was used for the quantification of DA (Sigma-Aldrich) by HPLC coupled with electrochemical detection. The HPLC system was comprised of a CCPM pump, an auto-sampler equipped with a cooling plate maintained at 4 °C, and CO-8010 column oven (Tosoh, Tokyo, Japan). Separation was achieved on a TSK gel ODS-100s (Tosoh, 250 × 4.6 mm i.d.). The mobile phase was 95% 50 mM sodium acetate, 10 mM citric acid, 0.15 mM EDTA, 0.45 mM SOS and 5% acetonitrile adjusted to pH 3.5 with glacial acetic acid then filtered through a 0.45 μm membrane. The flow rate was 1.0 ml/min. The electrochemical detection was accomplished using an electrochemical detector (model EC8020, Tosoh) with a glass working electrode at a potential of 1700 mV. The ratio of DOPAC/DA and HVA/DA were used as indices of DA turnover.

2.11. Statistical analysis

Normality and homoscedasticity assumptions were verified prior to the use of any parametric tests (Shapiro-Wilk normality test and equality of variances F-test). Results are expressed as mean ± standard error of the mean (SEM). The statistical significance of differences was determined by the Student's t-test for two-group comparisons. One-way, two-way or three-way analysis of variance (ANOVA) followed by Tukey-kramer test were employed for multigroup comparisons. In some cases, when a main effect was significant without interaction effect, we did an exploratory and limited pairwise post-hoc comparison consistent with our a priori hypothesis. Brain weights were analyzed by ANCOVA with body weight as a covariate. $p < 0.05$ represented a significant difference.

3. Results

3.1. Acoustic startle and PPI

There was no significant differences in the startle amplitude measured at either pre- or post-pubertal periods between mice treated prenatally (GD 15–17) with saline or MAM. MAM-treated mice displayed significant average PPI deficits at the post-pubertal period, but not pre-pubertal period [Fig. 1 (A-D) Pre-pubertal: Student's t-test of average PPI: t (32) = 1.19, p = 0.24, (A); startle amplitude: t (32) = 0.61, p = 0.55, (B). Post-pubertal: Student's t-test of average PPI: t (30) = 3.01, p = 0.0053, (C); startle amplitude: t (30) = 0.94, p = 0.35, (D)]. These results indicated that the prenatal administration of MAM at GD 15–17 induced schizophrenia-like behavioral deficits in PPI at adulthood, similar to that reported for MAM-treated rats at GD 17 (Gomes et al., 2016). In preliminary experiments, we found that mice prenatally treated with single injection of MAM (10 mg/kg) on either GD 15, GD 16 or GD 17 did not display PPI deficits during the post-pubertal period [Fig. S1 GD 15 (A): Student's t-test: average: t (27) = 0.88, p = 0.39. GD 16 (B): Student's t-test of average PPI: t (49) = 0.029, p = 0.98. GD 17 (C): Student's t-test of average PPI: t (35) = 1.28, p = 0.21]. Based on these results, we chose GD 15–17 as the time period to treat pregnant mice with MAM for further behavioral, pathological and neurochemical studies.

3.2. Locomotor activity

Changes in locomotor activity induced by the NMDA antagonist MK-801 was analyzed at both pre- and post-pubertal periods. For either periods, the activities of MAM and saline treated mice were not significant different following the saline treatment of the test. ANOVA showed significant main effects of treatment and prenatal treatment factors but not an interaction. Thus, we focused our analysis on the effects of MAM and drug treatments. A significant increase in total locomotor activity by treatment with MK-801 was only observed at post-pubertal age in MAM treated mice compared to saline treated mice. [Fig. 1 (E-J) Three-way ANOVA: prenatal treatment: F (1, 43) = 0.93, p = 0.76, treatment: F (1, 43) = 8.80, p = 0.005, time: F (3, 143) = 35.32, p < 0.0001, prenatal treatment × treatment: F (1, 43) = 0.004, p = 0.95, time × prenatal treatment: F (3, 143) = 1.67, p = 0.17, time × treatment: F (3, 143) = 0.21, p = 0.91, time × prenatal treatment × treatment: F (3, 143) = 0.68, p = 0.58, (E); Student's t-test: t (14) = 0.50, p = 0.62, (F); t (29) = 0.26, p = 0.80, (G); Three-way ANOVA: prenatal treatment: F (1, 41) = 4.88, p = 0.033, treatment: F (1, 41) = 9.28, p = 0.004, time: F (2, 86) = 35.99, p < 0.0001, prenatal treatment × treatment: F (1, 41) = 1.61, p = 0.21, time × prenatal treatment: F (2, 86) = 3.55, p = 0.031, time × treatment: F (2, 86) = 1.18, p = 0.31, time × prenatal treatment × treatment: F (2, 86) = 0.88, p = 0.43, (H); Student's t-test: t (19) = 1.75, p = 0.096, (I); t (22) = 2.56, p = 0.018, (J)]. These results indicated that prenatal MAM treatments induced MK-801-induced hyperactivity in mice during the post-pubertal period.

3.3. Social interaction test

The effect of prenatal MAM on social behavior was evaluated at pre- and post-pubertal periods between pairs of unfamiliar mice that received the same prenatal treatments [Fig. 1 (K–N)]. Compared to saline-treated mice, MAM-treated mice showed significantly reduced time spent in social interactions at both developmental periods [Student's t-test: t (14) = 2.38, p = 0.032, (L); t (18) = 5.91, p < 0.0001, (N)]. Moreover, at the post-pubertal period, the number of interactions significantly increased in MAM treated mice compared to saline treated mice [Student's t-test: t (14) = 1.38, p = 0.19, (K); t (18) = 3.12, p = 0.0059, (M)]. These results indicate that prenatal MAM treatments induced social deficits in mice at both pre- and post-pubertal periods.

3.4. Y-maze test

Memory functions were evaluated in saline and MAM-treated mice at pre- and post-pubertal periods in the Y-maze test [Fig. 1 (O-R)]. Total arm entries were not significantly different between saline and MAM treated mice at both periods [Student's t-test: t (35) = 0.57, p = 0.57, (P); t (24) = 0.58, p = 0.57, (R)]. However, spontaneous alternation was significantly decreased in MAM-treated mice compared to saline-treated mice at the post-pubertal, but not the pre-pubertal period [Student's t-test: t (35) = 1.82, p = 0.077, (O); t (24) = 2.48, p = 0.021, (Q)]. The data indicates that prenatal MAM treatments induced post-pubertal cognitive deficits in mice.

3.5. Measurement of body and brain weights

Our data show that the weight of the PFC and hippocampus, but not the striatum, were significantly reduced in MAM-treated mice compared to saline controls on postnatal day 56 [Fig. 2 (A-E) Student's t-test: PFC: t (8) = 3.26, p = 0.012, Fig. 2 (A); striatum: t (8) = 1.53, p = 0.17, Fig. 2 (B); hippocampus: t (8) = 4.51, p = 0.002, Fig. 2 (C); whole brain: t (8) = 4.92, p = 0.0012, Fig. 2 (D); whole body: t (8) = 3.05, p = 0.016, Fig. 2 (E)]. Total brain weight was also significantly reduced by approximately 9.5% in MAM treated mice while the average body weight was smaller by 16.2%. However, an analysis by ANCOVA revealed that the body weight contribution to brain weight was not significant [ANCOVA: whole brain: F (1, 6) = 11.23, p = 0.015, whole body: F (1, 6) = 0.013, p = 0.91].

3.6. Cytoarchitectural alterations in the hippocampus of MAM treated mice

We have performed multiple immuno-labeling in order to evaluate the pyramidal/granule cell density and morphology in the dorsal and ventral hippocampus [Fig. 3 (A and B)]. Anti-Ctip2, anti-RGS14 and anti-Prox1 antibodies were used to identify the CA1, CA2 and DG areas,

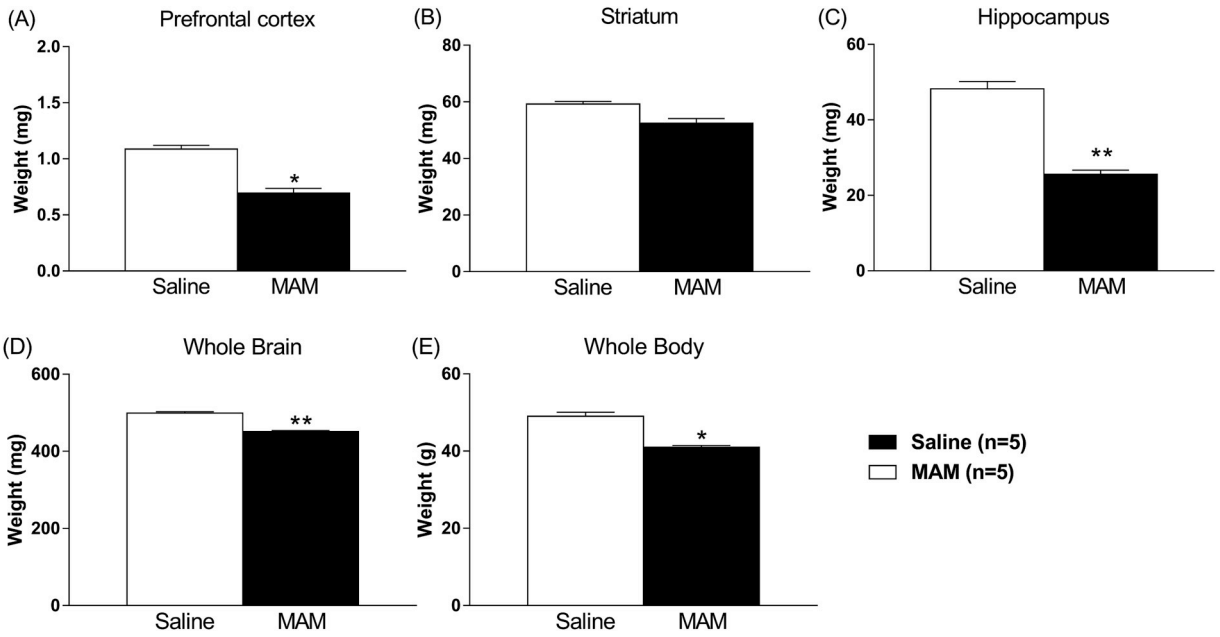


Fig. 2. Prefrontal cortex (PFC) (A), striatum (B), hippocampus (C), whole brain (D) and whole body (E) weight comparisons at postnatal day 56 between mice treated prenatally with saline or MAM. The weight of the PFC, hippocampus and whole brain, but not the striatum, were significantly reduced in MAM-treated mice compared to saline controls on postnatal day 56, while body weight contribution to brain weight was not significant. Bars represent means ± S.E.M. *: $p < 0.05$ and **: $p < 0.01$ vs. prenatal treatment with saline group.

respectively. The pyramidal cell density in CA2 was significantly decreased in MAM-treated mice compared to saline treated mice, whereas the subfields CA1, CA3 or DG in both the dorsal and ventral hippocampus showed no significant differences [Fig. 3 (C and D) Two-way ANOVA: prenatal treatment: $F(1, 32) = 20.43$, $p < 0.0001$, brain region: $F(3, 32) = 282.1$, $p < 0.0001$, prenatal treatment × brain region: $F(3, 32) = 11.66$, $p < 0.0001$, Post-hoc test: CA1: $p > 0.9999$, CA2: $p < 0.0001$, CA3: $p > 0.9999$, DG: $p = 0.75$, (C); prenatal treatment: $F(1, 32) = 9.27$, $p = 0.0046$, brain region: $F(3, 32) = 67.87$, $p < 0.0001$, prenatal treatment × brain region: $F(3, 32) = 3.32$, $p = 0.032$, Post-hoc test: CA1: $p > 0.9999$, CA2: $p = 0.0007$, CA3: $p > 0.9999$, DG: $p = 0.77$, (D)]. Quantitative analyses of discontinuities and heterotopias in the dorsal hippocampus [Fig. 3 (E, F and H)] showed that, compared to control saline-treated mice. Main effect of prenatal treatment was significant by ANOVA, while interaction was not significantly different. Thus, we focused on the effect of MAM, but not brain regions. The mice treated with MAM exhibited more discontinuities in CA1, CA2 and CA3 compared to control mice [Fig. 3 (G) Two-way ANOVA: prenatal treatment: $F(1, 24) = 26.59$, $p < 0.0001$, brain region: $F(2, 24) = 0.39$, $p = 0.68$, prenatal treatment × brain region: $F(2, 24) = 0.86$, $p = 0.44$, Post-hoc test: CA1: $p = 0.015$, CA2: $p = 0.013$, CA3: $p = 0.025$, (G)]. In addition, the number of heterotopias was also significantly increased in the MAM mice [Fig. 3 (I) Student's t-test: $t(8) = 2.43$, $p = 0.042$, (I)]. These results indicate that the prenatal treatment with MAM induced a decrease in pyramidal cell density in the CA2 area which was accompanied with an increase in discontinuities and heterotopias.

3.7. Cell proliferation and neurogenesis in the DG of the hippocampal formation

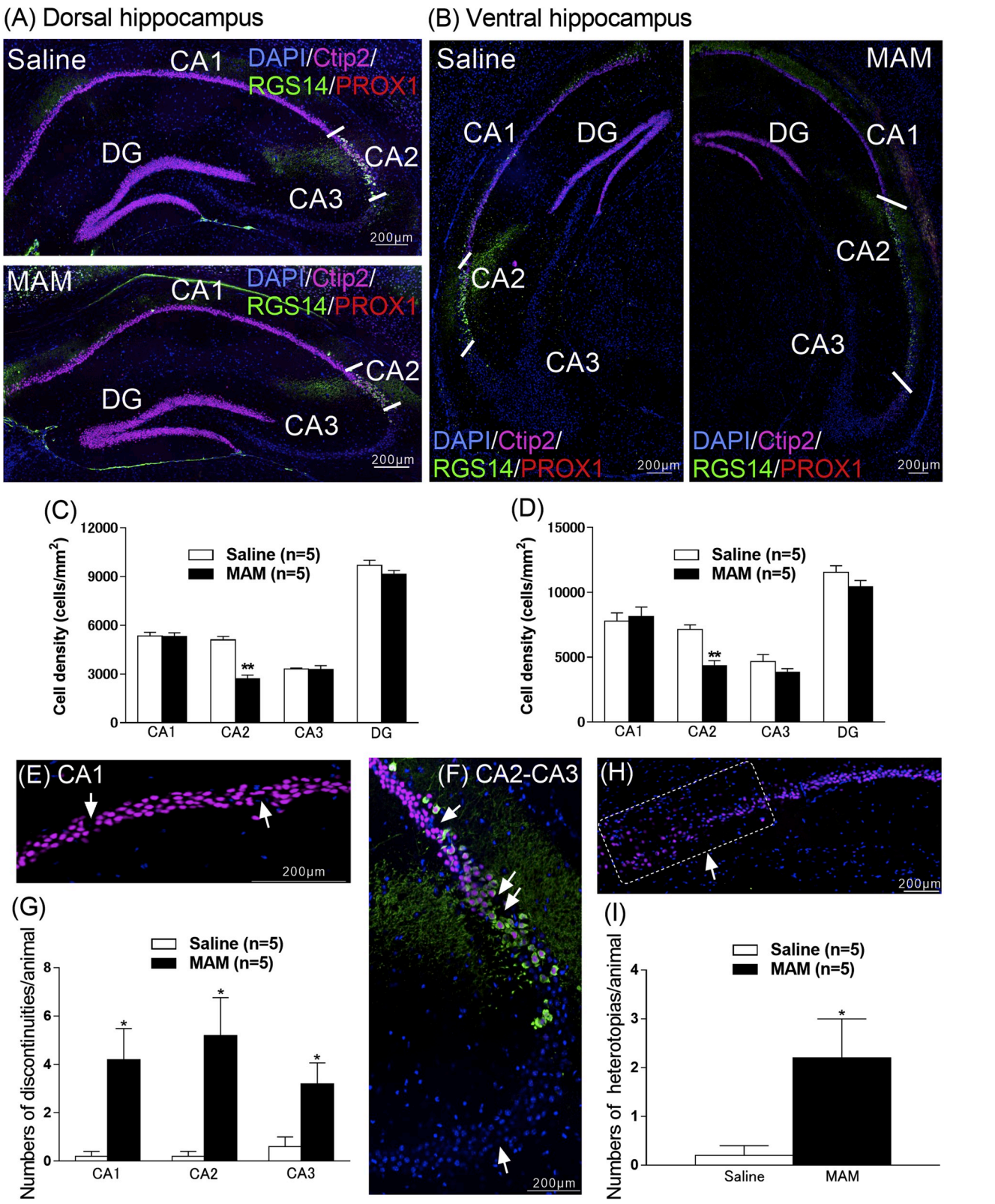
To determine the rate of hippocampal cell proliferation and neurogenesis in our model, animals were injected with BrdU. Anti-DCX antibody was used to identify immature neurons while anti-NeuN antibody was used to identify mature neurons in the DG area. The incorporation of BrdU into a cell indicates that it was dividing at the time of the BrdU injection. MAM-treated mice had a significantly lower number of both BrdU+/DCX + cells and BrdU+/NeuN + cells compared to the saline-treated group [Fig. 4 (B) and (D) Student's t-test: $t(8) = 8.52$, $p < 0.0001$, (B); $t(6) = 5.70$, $p = 0.0013$, (D)]. These results indicate that the prenatal treatment with MAM in mice induced a reduction in cell proliferation and neurogenesis in the DG of the hippocampus.

3.8. Alterations of dopaminergic system in the brain of MAM-treated mice

Tissue levels of DA, its metabolites and their turnover in the mPFC, striatum and hippocampus of mice treated prenatally with saline or MAM are shown in Fig. 5. ANOVA showed a significant main effect of prenatal treatment but not of interaction in the content and turnover in the mPFC area. Thus, we focused on the effect of MAM only. In the mPFC, the DA concentration and DOPAC/DA ratio in MAM treated mice was significantly increased compared to the saline treated group [Two-way ANOVA: prenatal treatment: $F(1, 36) = 12.56$, $p = 0.0011$, concentration: $F(2, 36) = 19.52$, $p < 0.0001$, prenatal treatment × concentration: $F(2, 36) = 0.60$, $p = 0.55$, Post-hoc test: DA: $p = 0.038$, DOPAC: $p = 0.059$, HVA: $p = 0.12$, (A); Two-way ANOVA: prenatal treatment: $F(1, 24) = 6.53$, $p = 0.017$, turnover: $F(1, 24) = 7.08$, $p = 0.014$, prenatal treatment × turnover: $F(1, 24) = 2.33$, $p = 0.14$, Post-hoc test: DOPAC/DA: $p = 0.032$, HVA/DA: $p = 0.36$, (B); Two-way ANOVA: prenatal treatment: $F(1, 36) = 1.54$, $p = 0.22$, concentration: $F(2, 36) = 120.7$, $p < 0.0001$, prenatal treatment × concentration: $F(2, 36) = 1.28$, $p = 0.29$, (C); prenatal treatment: $F(1, 24) = 0.11$, $p = 0.75$, turnover: $F(1, 24) = 77.22$, $p < 0.0001$, prenatal treatment × turnover: $F(1, 24) = 0.19$, $p = 0.66$, (D); prenatal treatment: $F(1, 36) = 3.19$, $p = 0.083$, concentration: $F(2, 36) = 10.29$, $p = 0.0003$, prenatal treatment × concentration: $F(2, 36) = 0.46$, $p = 0.64$, (E); prenatal treatment: $F(1, 24) = 1.63$, $p = 0.21$, turnover: $F(1, 24) = 3.64$, $p = 0.068$, prenatal treatment × turnover: $F(1, 24) = 0.25$, $p = 0.62$, (F)]. These results indicate that the treatment with MAM induced an increase in the tissue levels of DA and a disturbance in the homeostasis of the dopaminergic system.

3.9. Effect of antipsychotic drugs on MAM-induced PPI disruption

We evaluated whether haloperidol (a typical antipsychotic), risperidone and clozapine (atypical antipsychotics) or aripiprazole (dopamine-serotonin stabilizer) reversed the PPI deficits (average PPI) in MAM-treated mice. MAM-induced PPI deficits were reversed by all tested compounds except for haloperidol [Fig. 6 (A) Two-way ANOVA: average PPI: prenatal treatment: $F(1, 78) = 18.17$, $p < 0.0001$, antipsychotic: $F(3, 78) = 1.36$, $p = 0.26$, prenatal treatment × antipsychotic: $F(3, 78) = 0.66$, $p = 0.58$, startle amplitude: prenatal treatment: $F(1, 78) = 3.30$, $p = 0.073$, antipsychotic: $F(3, 78) = 0.82$, $p = 0.49$, prenatal treatment × antipsychotic: $F(3, 78) = 0.91$,



(caption on next page)

Fig. 3. Alteration of pyramidal/granule cell density and cytoarchitecture in different areas of the hippocampus in mice exposed to MAM at GD 15–17. Representative tissue sections used for cell counting in the dorsal (A) and ventral (B) hippocampus. Color of the neuronal layer in the different areas after merging of labeled images: CA1 and DG (magenta), CA2 (white), CA3 (blue). An example of labeling with individual antibodies is shown on Fig. S2. Cells were counted in the pyramidal/granule cell layer (C and D). Bar designated total was the average of all hippocampal subregions and DG. Coronal slices at Bregma −2.2 mm to −3.0 mm of the dorsal and ventral hippocampus: CA1, CA2 and CA3 from MAM treated mice stained with anti-Ctip2 antibody, anti-RGS14 antibody, anti-Prox1 antibody and DAPI. The pyramidal cell density in CA2 was significantly decreased in MAM-treated mice compared to saline treated mice, whereas the subfields CA1, CA3 or DG in both the dorsal and ventral hippocampus showed no significant differences. Arrows point to discontinuities in the dorsal hippocampus (E and F). Discontinuity differences between MAM and saline treated mice in hippocampal subfields CA1, CA2 and CA3 (G). The prenatal treatment with MAM exhibited more discontinuities in CA1 and CA2. The arrow points to heterotopia in the dorsal hippocampal CA1 area (H). Comparison between the number of heterotopias in the hippocampus of MAM treated mice and controls (I). The prenatal treatment with MAM induced an increase in heterotopias in the dorsal hippocampus. Bars represent means ± S.E.M. *: $p < 0.05$ and **: $p < 0.01$ vs. prenatal treatment with saline group.

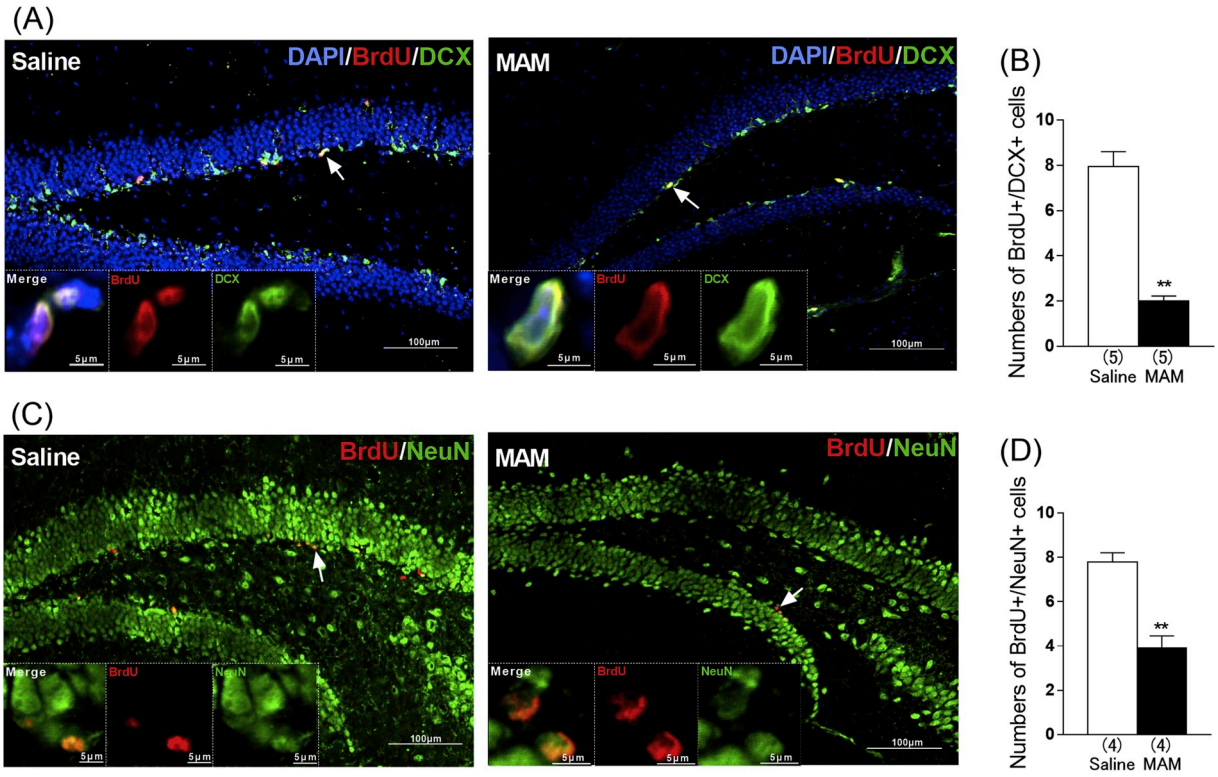


Fig. 4. Microscopy images of BrdU (red), DAPI (blue), and DCX or NeuN (green) immunostaining in the DG region of the hippocampus. The prenatal treatment with MAM in mice induced a reduction in cell proliferation and neurogenesis in the DG of the hippocampus. Bars represent means ± S.E.M. **: $p < 0.01$ vs. prenatal treatment with saline group.

$p = 0.44$, (A)]. Risperidone (0.03 mg/kg), clozapine (0.5, 1 and 3 mg/kg) and aripiprazole (1 and 5 mg/kg) attenuated the MAM-induced PPI deficit for the average PPI, respectively [Fig. 6 (B-D) Two-way ANOVA: average PPI: prenatal treatment: $F(1, 75) = 10.28$, $p = 0.0020$, antipsychotic: $F(2, 75) = 4.21$, $p = 0.019$, prenatal treatment × antipsychotic: $F(2, 75) = 4.076$, $p = 0.021$, startle amplitude: prenatal treatment: $F(1, 75) = 0.00047$, $p = 0.98$, antipsychotic: $F(2, 75) = 1.73$, $p = 0.19$, prenatal treatment × antipsychotic: $F(2, 75) = 0.18$, $p = 0.84$. Post-hoc test (average PPI): 0.01 mg/kg: $p = 0.99$; 0.03 mg/kg: $p = 0.0027$, (B); Two-way ANOVA: average PPI: prenatal treatment: $F(1, 102) = 6.76$, $p = 0.011$, antipsychotic: $F(3, 102) = 7.97$, $p < 0.0001$, prenatal treatment × antipsychotic: $F(3, 102) = 3.79$, $p = 0.013$, startle amplitude: prenatal treatment: $F(1, 102) = 0.30$, $p = 0.59$, antipsychotic: $F(3, 102) = 0.59$, $p = 0.62$, prenatal treatment × antipsychotic: $F(3, 102) = 0.45$, $p = 0.72$. Post-hoc test (average PPI): 0.5 mg/kg: $p = 0.034$; 1 mg/kg: $p = 0.0041$; 3 mg/kg: $p < 0.0001$, (C); Two-way ANOVA: average PPI: prenatal treatment: $F(1, 70) = 1.91$, $p = 0.17$, antipsychotic: $F(3, 70) = 2.12$, $p = 0.11$, prenatal treatment × antipsychotic: $F(3, 70) = 3.077$, $p = 0.033$, startle amplitude: prenatal treatment: $F(1, 70) = 2.86$, $p = 0.095$, antipsychotic: $F(3, 70) = 0.26$, $p = 0.85$, prenatal treatment × antipsychotic: $F(3, 70) = 0.21$, $p = 0.89$. Post-hoc test (average PPI): 0.2 mg/kg: $p = 0.68$; 1 mg/kg: $p = 0.027$; 5 mg/kg: $p = 0.013$, (D)]. These results indicate that prenatal MAM-induced PPI deficits in mice were improved with atypical antipsychotics but not with the typical antipsychotic drug haloperidol.

3.10. Effect of antipsychotic drugs on MK-801-induced hyperactivity in MAM-treated mice

We evaluated whether haloperidol, risperidone, clozapine or aripiprazole reversed the hyperactivity by MK-801 in MAM-treated mice. MAM-induced hyperactivity by MK-801 were reversed by all tested compounds without influence on control group [Fig. 7 (A-D) Haloperidol: Two-way ANOVA: prenatal treatment: $F(1, 47) = 6.076$, $p = 0.017$, antipsychotic: $F(3, 47) = 19.69$, $p < 0.0001$, prenatal treatment × antipsychotic: $F(3, 47) = 3.86$, $p = 0.015$, Post-hoc test: 0.05 mg/kg: $p = 0.0002$, 0.1 mg/kg: $p < 0.0001$, 0.5 mg/kg: $p < 0.0001$, (A); Risperidone: Two-way ANOVA: prenatal treatment: $F(1, 32) = 1.89$, $p = 0.18$, antipsychotic: $F(2, 32) = 4.12$, $p = 0.026$, prenatal treatment × antipsychotic: $F(2, 32) = 3.99$, $p = 0.028$, Post-hoc test: 0.01 mg/kg: $p = 0.0097$, 0.03 mg/kg: $p = 0.034$, (B); Clozapine: Two-way ANOVA: prenatal treatment: $F(1, 40) = 4.75$, $p = 0.035$, antipsychotic: $F(3, 40) = 3.30$, $p = 0.030$, prenatal

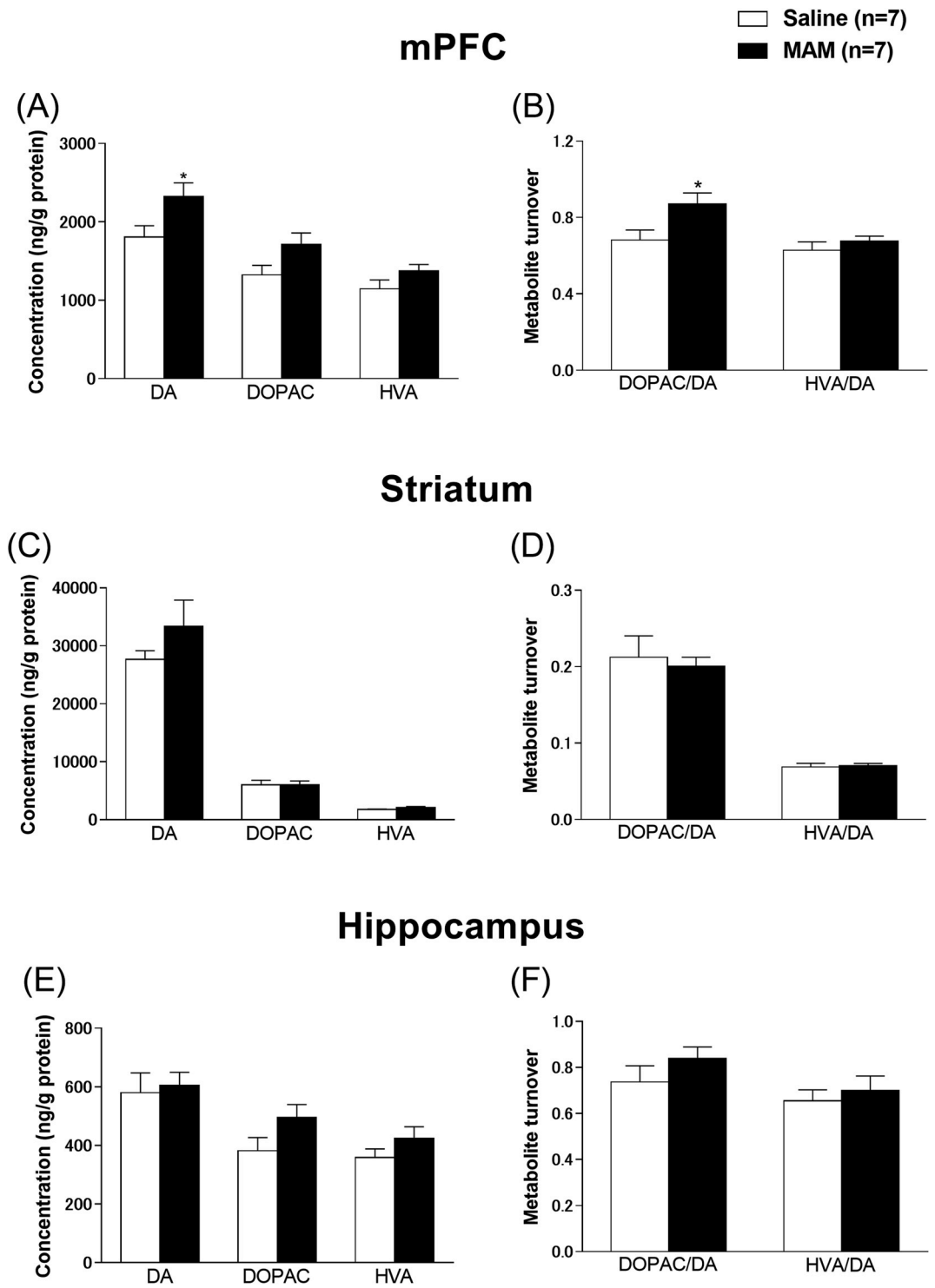


Fig. 5. Alternation of DA, DOPAC and HVA concentrations and DOPAC/DA and HVA/DA in the mPFC, striatum and hippocampus in MAM mice. Concentrations are indicated as ng per g fresh weight of brain tissue. The treatment with MAM induced an increase in the tissue levels of DA and a disturbance in the homeostasis of the dopaminergic system in the mPFC. Bars represent means ± S.E.M. *: $p < 0.05$ vs. prenatal treatment with saline group.

treatment × antipsychotic: $F(3, 40) = 2.47$, $p = 0.049$, Post-hoc test: 0.5 mg/kg: $p = 0.14$, 1 mg/kg: $p = 0.0091$, 3 mg/kg: $p = 0.18$, (C); Aripiprazole: Two-way ANOVA: prenatal treatment: $F(1, 45) = 2.71$, $p = 0.11$, antipsychotic: $F(3, 45) = 11.47$, $p < 0.0001$, prenatal treatment × antipsychotic: $F(3, 45) = 2.95$, $p = 0.043$, Post-hoc test: 0.2 mg/kg: $p = 0.0006$, 1 mg/kg: $p = 0.0001$, 5 mg/kg: $p < 0.0001$, (D)]. These results indicate that prenatal MAM-induced hyperactivity by MK-801 were improved with typical and atypical antipsychotics.

3.11. Effect of antipsychotic drugs on MAM-induced deficits in social behavior

We evaluated whether haloperidol, risperidone, clozapine or aripiprazole reversed the social deficits in MAM treated mice. Haloperidol (0.05–0.5 mg/kg) did not significantly improve MAM-induced social deficits [One-way ANOVA: $F(3, 25) = 5.88$, $p = 0.0035$, Post-hoc test: 0.05 mg/kg: $p = 0.47$, 0.1 mg/kg: $p = 0.66$, 0.5 mg/kg: $p = 0.0020$,

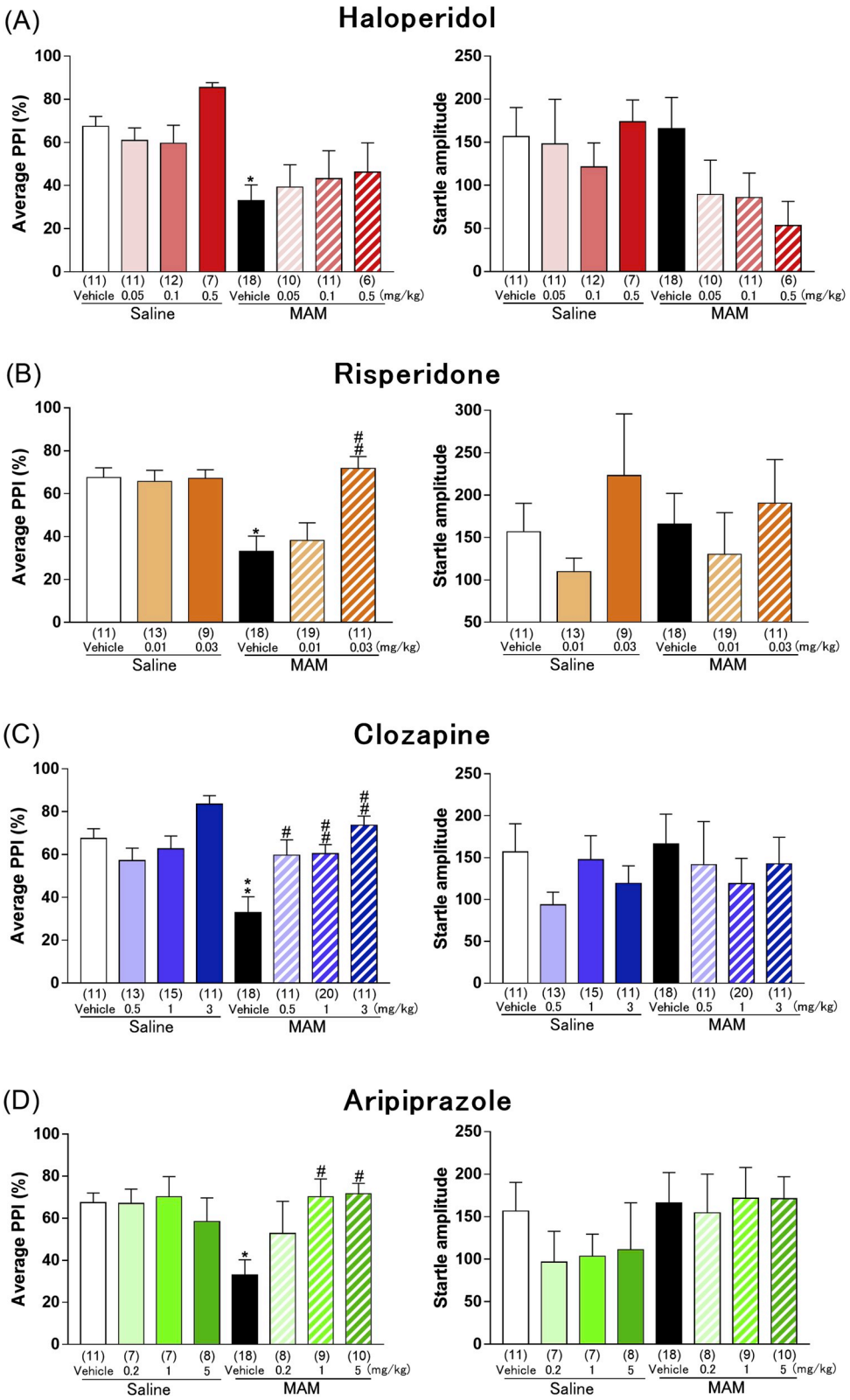


Fig. 6. Effect of haloperidol (A), risperidone (B), clozapine (C) and aripiprazole (D) on average PPI and startle amplitude in saline and MAM-treated mice. Prenatal MAM-induced PPI deficits in mice were improved with atypical antipsychotics but not with the typical antipsychotic drug haloperidol. Bars represent means ± S.E.M. *: $p < 0.05$ and **: $p < 0.01$ vs. vehicle-treated saline group. #: $p < 0.05$ and ##: $p < 0.01$ vs. vehicle-treated MAM group.

Fig. 8 (A)]. Risperidone at 0.01 mg/kg improved the social deficits in MAM mice [One-way ANOVA: $F(2, 19) = 19.27$, $p < 0.0001$, Post-hoc test: 0.01 mg/kg: $p < 0.0001$, 0.03 mg/kg: $p = 0.075$, Fig. 8 (B)]. Clozapine at 0.5 mg/kg also improved the MAM-induced social deficit [One-way ANOVA: $F(3, 23) = 7.52$, $p = 0.0011$, Post-hoc test: 0.5 mg/kg: $p = 0.0046$, 1 mg/kg: $p = 0.090$, 3 mg/kg: $p = 0.99$, Fig. 8 (C)]. Similarly, aripiprazole at 0.2 mg/kg improved the MAM-induced social deficits [One-way ANOVA: $F(3, 24) = 4.72$, $p = 0.010$, Post-hoc test:

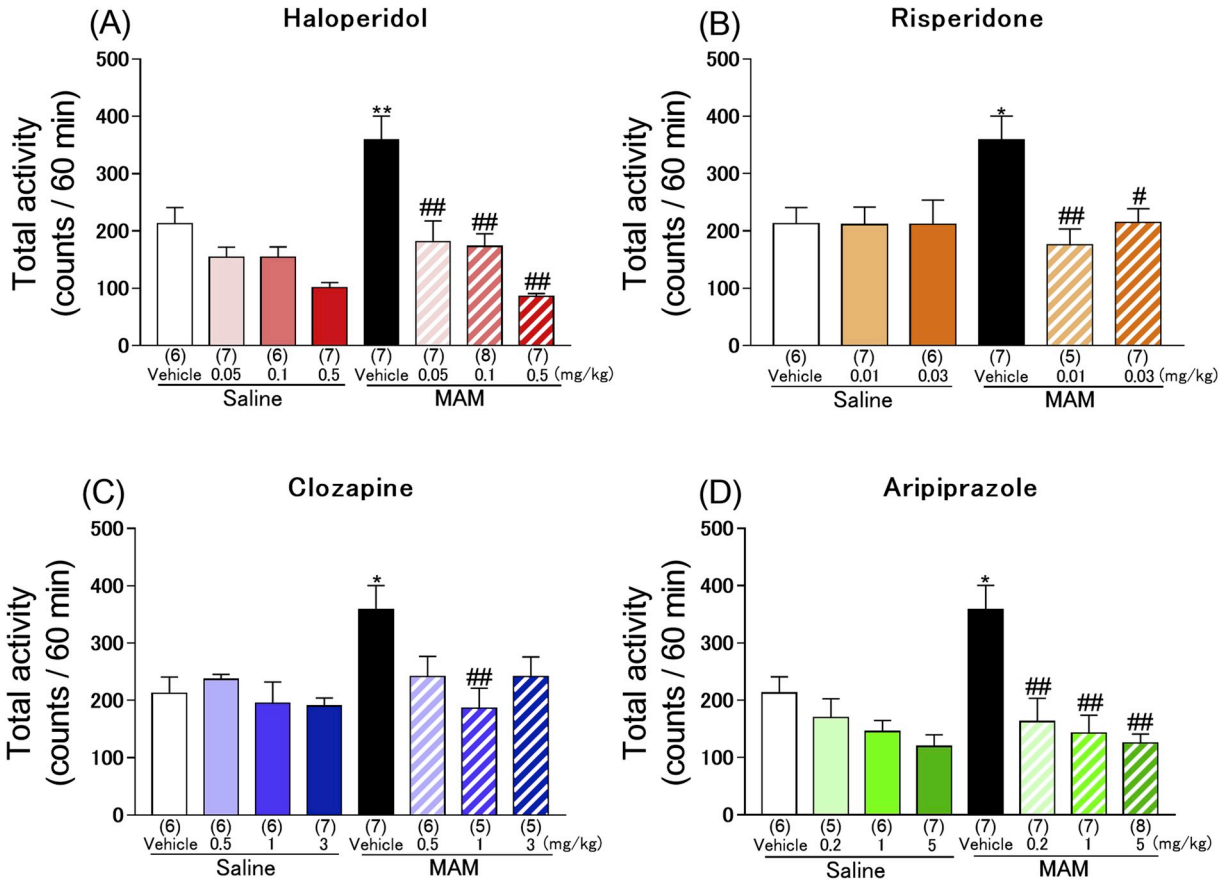


Fig. 7. Effect of haloperidol (A), risperidone (B), clozapine (C) and aripiprazole (D) on hyperactivity in saline and MAM-treated mice. Prenatal MAM-induced hyperactivity by MK-801 in mice were improved with typical and atypical antipsychotics. Bars represent means ± S.E.M. *: $p < 0.05$ and **: $p < 0.01$ vs. vehicle-treated saline group. #: $p < 0.05$ and ##: $p < 0.01$ vs. vehicle-treated MAM group.

0.2 mg/kg: $p = 0.045$, 1 mg/kg: $p = 0.87$, 5 mg/kg: $p = 0.99$, Fig. 8 (D)]. These results indicate that prenatal MAM-induced social withdrawal were improved with atypical antipsychotics but not with the typical antipsychotic drug haloperidol.

4. Discussion

In the present study, we have examined the face, constructive and predictive validities of GD 15-17 MAM-treated mice to determine whether this model exhibits schizophrenia-like features. We initially evaluated the face validity with respect to behavioral changes such as PPI, locomotor activity, social interaction and working memory.

First, we found that mice exposed to MAM at GD 15–17 present PPI deficits during post-pubertal period, which is one of the endophenotypes of schizophrenia patients (Braff et al., 2001) and also a valid marker for sensorimotor gating in rodent models (Geyer et al., 2001). Interestingly, PPI deficits in prenatal MAM-treated rats were also observed only at post-pubertal period (Gomes et al., 2016), a finding that parallels the onset of the disorder in schizophrenia patients (Neumann et al., 1995). Secondly, we found that MK-801-induced locomotor activity was enhanced in MAM-treated mice at post-pubertal period in a similar fashion to what was observed with the neonatal ventral hippocampal (NVH) lesion rat model (Al-Amin et al., 2000). Clinical studies suggest that the antagonism of NMDA glutamatergic transmission in brain exacerbates positive symptoms of schizophrenia (Lahti et al., 1995). Thirdly, we found that GD 15–17 MAM treated mice spent less time in social interactions at both pre- and post-pubertal ages [Fig. 1 (I-L)]. Such changes have previously been observed at both pre- and post-pubertal periods in MAM-treated rats (Hazane et al., 2009) as well as in NVH lesioned rats (Sams-Dodd et al., 1997). Social withdrawal is considered as one of the negative symptoms of schizophrenia (Mueser and McGurk, 2004) which is frequently observed before the onset of positive symptoms and is persistent (Olin and Mednick, 1996). Fourthly, we found that mice prenatally treated with MAM presented working memory deficits in the Y-maze test at post-pubertal period [Fig. 1 (M-P)]. Working memory deficits in the Y-maze test has also been reported at post-pubertal period for rats treated with MAM (Moore et al., 2006) and for NVH lesioned mice (Naert et al., 2013). In agreement, schizophrenia patients consistently present cognitive deficits involving working memory (Heinrichs and Zakzanis, 1998). From our results we can infer that treating mice with MAM on GD 15–17 can lead to behavioral deficits of relevance to positive and negative symptoms as well as cognitive dysfunctions.

Meta-analysis studies reveal that schizophrenia patients present a decrease in the volume of prefrontal cortex and hippocampus (Brugger and Howes, 2017). In the present study, the treatment with MAM resulted in a marked decrease in brain tissue weight for both the PFC and hippocampus which appears to be consistent with a previous study in rat MAM model (Flagstad et al., 2004). Studies on postmortem hippocampi of schizophrenic patients suggest that there may be a selective decrease in the number of pyramidal cells in the CA2 area which are essential for social memory (Benes et al., 1998; Hitti and Siegelbaum, 2014). In accordance, social memory deficits have been observed in patients with schizophrenia (Okruszek et al., 2017). Our MAM-treated mice also displayed a decrease in pyramidal cell density in the CA2 area compared to the control group (Fig. 3). Changes in hippocampal structure in schizophrenia patients are consistently reported in postmortem and neuro-imaging studies (Heckers, 2004; Nelson et al., 1998). Neuronal discontinuities and heterotopias in the dorsal hippocampus observed in schizophrenia patients have also been observed in

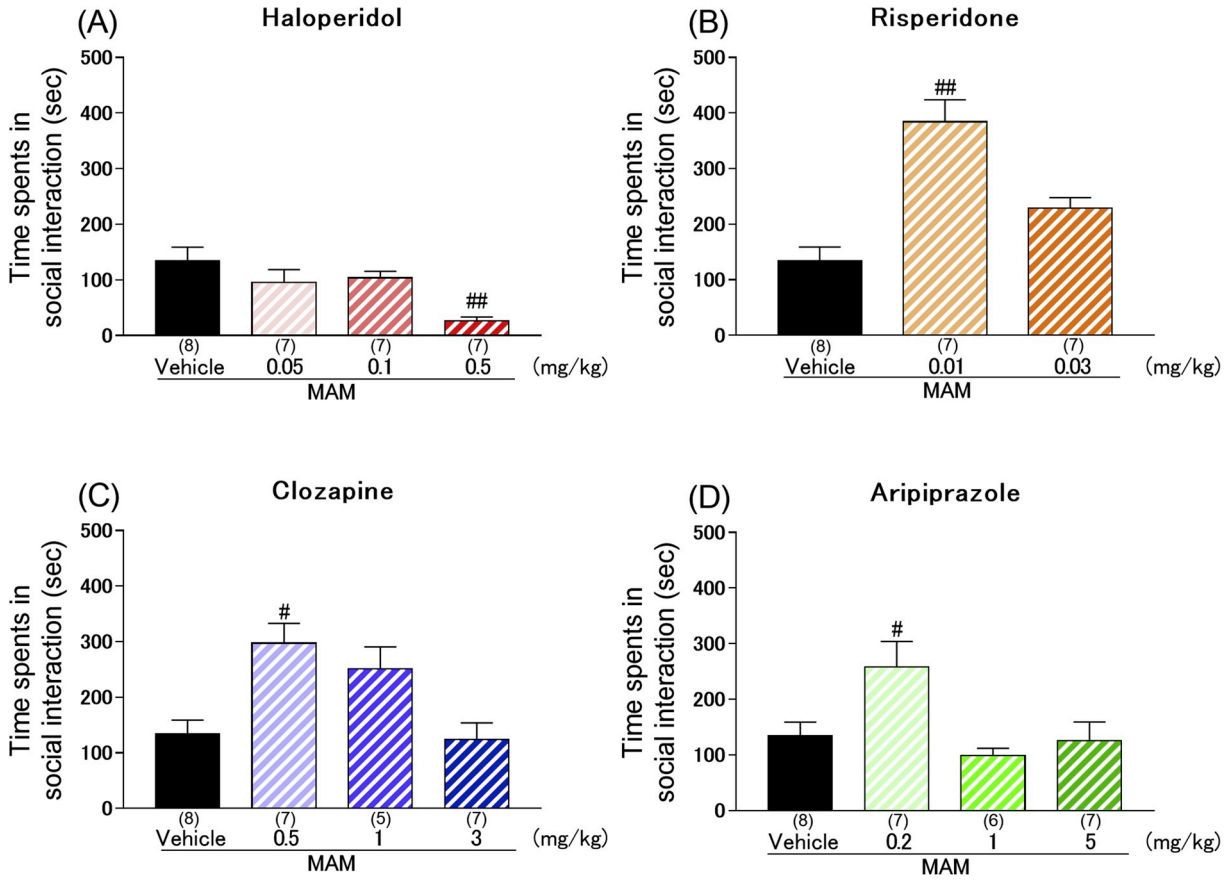


Fig. 8. Effect of haloperidol (A), risperidone (B), clozapine (C) and aripiprazole (D) on social withdraw in saline and MAM-treated mice. Prenatal MAM-induced social withdrawal in mice were improved with atypical antipsychotics but not with the typical antipsychotic drug haloperidol. Bars represent means ± S.E.M. #: $p < 0.05$ and ##: $p < 0.01$ vs. vehicle-treated MAM group.

the offspring of MAM-treated rat dams (Singh, 1977; Chevassus-Au-Louis et al., 1998). Consistent with these observations, we found neuronal discontinuities and heterotopias in the dorsal hippocampus in the offspring of MAM-treated pregnant mice.

Our studies show that prenatal MAM-treated mice display a decrease in cell proliferation and neurogenesis in the DG compared to the control group (Fig. 5). Some studies have suggested that a reduction in hippocampal neural stem cell proliferation may be involved in the pathogenesis of schizophrenia and an association between decreased neurogenesis and schizophrenia has been demonstrated (Allen et al., 2016; Eriksson, 2006; Iannitelli et al., 2017). Interestingly, it has been demonstrated in rodents that a reduction in hippocampal neurogenesis may lead to PPI deficits (Osumi et al., 2015), memory deficits and social withdrawal (Iwata et al., 2008). Together, these results may indicate that the schizophrenia-like behaviors induced by prenatal MAM may be related to the reductions in cell proliferation and neurogenesis we observed.

It has been demonstrated that a dysfunction in central DA efflux may be related to the positive and negative symptoms as well as the cognitive deficits of schizophrenia (Grace, 2016). DA levels and DOPAC/DA ratio in the PFC were found to be increased in the mice offspring of MAM-treated dams, which is in agreement with the enhanced cortical DA levels seen in the offspring of pregnant rats treated with MAM (Hallman and Jonsson, 1984) or after prenatal treatment with mitotic inhibitor Ara-C in rats which is proposed as a neurodevelopmental model of schizophrenia (Brown et al., 2012). Belujon et al. (2014) reported that MAM rats leaded to a hyperdopaminergic state and abnormally increased activity in the mPFC induced suppression of the nucleus accumbens and long-term potentiation via D_2 receptor in the mPFC. Long-term potentiation in the ventral subiculum–mPFC-nucleus accumbens pathway plays a significant role in the performance of goal-directed behavior (Kelley, 2004; Goto and Grace. 2005a, 2005b). It is thus possible that the abnormal increased activity by hyperdopaminergic state in the mPFC may be associated with some abnormal behaviors related to schizophrenia. Interestingly, similar to our finding in the MAM mice, a clinical study has reported that schizophrenia patients show increased DA synthesis rate in the mPFC (Lindström et al., 1999). However, Abi-Dargham and colleagues suggest that schizophrenia patients exhibit reduced dopaminergic transmission in the dorsolateral PFC as revealed by neuroimaging studies (Abi-Dargham et al., 2012; Slifstein et al., 2015; Roffman et al., 2016). These paradoxical results may possibly be associated with the different of PFC regions studied in the rodents versus humans. Taken together, we suggest that hyperdopaminergic state in the mPFC of MAM mice may be associated with altered behaviors relevant to schizophrenia.

Other researchers have reported the effects of antipsychotic drugs on prenatal MAM rat model in multiple paradigms (Brown et al., 2013; Du and Grace. 2013, 2016; Goda et al., 2015; Grace and Gomes. 2018; Le Pen et al., 2011; Sonnenschein et al., 2018). This study was undertaken to evaluate the predictive validity of our mice model whether typical or atypical antipsychotic drugs could reverse the deficits in the PPI of startle reflex, hyperactivity by MK-801 and social withdrawal observed in MAM-treated mice. Using the PPI of startle paradigm or social interaction test, we showed that atypical antipsychotics such as clozapine, risperidone and the dopamine-serotonin stabilizer aripiprazole all reversed the MAM-induced sensorimotor gating deficits and social withdrawal. In contrast the typical antipsychotic haloperidol could not reverse the PPI deficits and social withdrawal in these mice. On the other hand, we found that hyperactivity by MK-801 in MAM mice was reversed by both typical and atypical antipsychotics, results

that are consistent with previous studies in MAM rat model (Le Pen et al., 2011). These results suggest that PPI deficits and social withdrawal may be particularly sensitive to some critical characteristics related to the "atypicality" of those antipsychotic drugs while alterations of DA neurotransmission may be responsible for hyperactivity by MK-801. Clinical studies reported that atypical antipsychotics such as clozapine or ziprasidone, but not typical antipsychotic haloperidol-treated schizophrenic patients occasionally showed normalization of PPI (Kumari et al., 1999; Braff et al., 1992) and negative symptoms (Stahl et al., 2010). Moreover, clinical efficacy of antipsychotic drugs for positive symptoms is correlated with their ability to block subcortical D_2 receptors (Creese et al., 1976; Seeman and Lee, 1975), suggesting DA signal is important. Thus, atypical antipsychotics such as clozapine, risperidone and aripiprazole probably interact with brain systems affected by the MAM treatment through their action on receptors other than DA receptors to normalize PPI deficits and social withdrawal, while all typical and atypical antipsychotics may improve hyperactivity by MK-801 via inhibition of D_2 receptors. In contrast to haloperidol, atypical antipsychotics also interact with $5HT_{2A}$, α_2 adrenergic, muscarinic and histaminergic receptors, among others (Bymaster et al., 2003; Citrome, 2014; Kusumi et al., 2015). Any or all of these receptors could play an important role for the unique action of atypical antipsychotics in our mice model. Taken together, the data suggest that the MAM mice model may be useful for the screening of novel antipsychotic drugs for negative symptoms of schizophrenia.

In conclusion, we have established a MAM model in mice that displays behavioral changes similar to what has been found in MAM treated rats including neurodevelopment-dependent onset of behavioral alterations such as PPI deficits, hypersensitivity to MK-801, social interaction deficits and cognitive deficits. Moreover, the prenatal treatment with MAM led to a decrease in brain weight (notably in the PFC and hippocampus), cytoarchitectural alterations such as discontinues and heterotopias, reductions in cell proliferation and neurogenesis, as well as an increase of DA content and turnover in the PFC. The behavioral, anatomical and neurophysiological changes are more consistent with those observed in schizophrenia patients than prenatal MAM rat models. Moreover, we found for the first time that atypical antipsychotic drugs reversed MAM-induced PPI deficits and social withdrawal. We believe that prenatal GD 15-17 administration of MAM in mice may offer a new means to study neurobiological mechanisms involved in the pathophysiology of schizophrenia.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2019.02.034.

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