Elevated glutamate, glutamine and GABA levels and reduced taurine level in a schizophrenia model using an in vitro proton nuclear magnetic resonance method

Am J Transl Res 2019;11(9):5919-5931
www.ajtr.org /ISSN:1943-8141/AJTR0097619

Original Article

Elevated glutamate, glutamine and GABA levels and reduced taurine level in a schizophrenia model using an in vitro proton nuclear magnetic resonance method

Jingyu Yang[1,2,3], Huiling Guo[1,2,3], Dandan Sun[1,2], Jia Duan[1,2], Xiaoping Rao[3,4], Fuqiang Xu[3,4,5,6], Anne Manyande[7], Yanqing Tang[1,2,8], Jie Wang[3,4,9], Fei Wang[1,2]

[1]Department of Psychiatry, [2]Brain Function Research Section, The First Affiliated Hospital of China Medical University, Shenyang, Liaoning, P. R. China; [3]State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Key Laboratory of Magnetic Resonance in Biological Systems, Wuhan Center for Magnetic Resonance, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan 430071, Hubei, P. R. China; [4]University of Chinese Academy of Sciences, Beijing 100049, P. R. China; [5]Center for Excellence in Brain Science and Intelligence Technology, Chinese Academy of Sciences, Shanghai 200031, P. R. China; [6]Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, Wuhan 430074, Hubei, P. R. China; [7]School of Human and Social Sciences, University of West London, London, UK; [8]Department of Geriatric Medicine, First Affiliated Hospital, China Medical University, Shenyang, Liaoning, P. R. China; [9]2nd Hospital of Shijiazhuang, Shijiazhuang 050051, Hebei, P. R. China

Received May 28, 2019; Accepted July 24, 2019; Epub September 15, 2019; Published September 30, 2019

Abstract: Accumulating evidence suggests that brain metabolic changes may be associated with the pathophysiology of schizophrenia. Both *in vivo* and *in vitro* studies have found glutamatergic and GABAergic abnormalities in different brain regions of individuals with schizophrenia. We report a longitudinal behavioral study in a methylazoxymethanol acetate (MAM) rat model of schizophrenia at three different age periods: prepuberty, late-puberty and early-adulthood. MAM-treated rats showed stable hypolocomotive activity, anxiety and cognitive deficits from late-puberty to early-adulthood. Therefore we detected the metabolites changes of adult MAM-treated rats using an *in vitro* proton nuclear magnetic resonance (^{1}H-NMR) method. In the MAM-treated rats, glutamate was increased in the thalamus and hypothalamus, glutamine was increased in the hippocampus and GABA was increased in the hippocampus and prefrontal cortex, while taurine showed a decrease in the striatum, temporal cortex and parietal cortex. These abnormalities may be helped further understanding the pathophysiology of schizophrenia.

Keywords: Schizophrenia, ^{1}H-NMR, glutamate, GABA, taurine

Introduction

Schizophrenia is regarded as a neurodevelopmental disorder characterized by positive symptoms, negative symptoms and cognitive deficits, which has clinical onset in late-puberty to early adulthood [1]. Because of ethical and practical limitations of human experimental biology, animal models are an important tool for investigating the pathophysiology and treatment of neuropsychiatric diseases [2]. Methylazoxymethanol acetate (MAM) is a mitotoxin which can interfere with neurogenesis in cortical and subcortical areas when it is administrated at embryonic day 17. As a neurodevelopmental model, there are a lot of evidences that the structural, functional and behavioral alterations in the MAM model are consistent with those observed in schizophrenia [3, 4]. However, there is still no study that has explored how prenatal interference alters brain metabolic status after disease onset.

Accumulating evidences suggest that brain metabolic changes may be associate with the pathophysiology of schizophrenia [5]. Glutamatergic and GABAergic metabolites are of particular interest which could stand for the majority of excitory and inhibitory neurotransmissions among brain metabolites. Evidence for the glutamate hypothesis originated from the fact that administration of N-methyl-D-aspartate (NMDA)

receptor antagonists, such as phencyclindine (PCP) and ketamine, could induce the psychotic symptoms that resemble schizophrenia in healthy volunteers [6, 7]. Kim et al. first found decreased glutamate (Glu) concentration in the cerebrospinal fluid of schizophrenia patients using the fluorometric method [8]. Postmortem analysis found abnormalities on density and subunit constitution of glutamatergic receptor in the hippocampus and thalamus [9-11], and also reduced GABA-synthesizing enzyme, mRNA and protein in schizophrenia [12-14]. Proton magnetic resonance spectroscopy (^{1}H-MRS) studies have reported elevated GABA and Glx (glutamate + glutamine) in the medial prefrontal cortex [15]. There is also evidence which showed that hypofunction of NMDA receptors on GABAergic neurons and abnormal metabolism of glutamatergic and GABAergic neurotransmission are associated with the pathophysiology of schizophrenia [16].

Additionally, taurine is a neurotransmitter and an inhibitory neuromodulator in the central nervous system which could protect neurons from glutamate-induced neuronal excitotoxicity [17]. Through different types of calcium channels, taurine can antagonize the glutamate induced elevation of intracellular free calcium ion concentrations [17]. Studies using high-performance liquid chromatography and gas chromatography-mass spectrometry showed that the schizophrenia patients had significantly lower concentrations of taurine in cerebrospinal and plasma compared with the control subjects [18, 19]. However, another study using ^{1}H-MRS found taurine to be elevated in the medial prefrontal cortex of schizophrenia patients [20].

Among the methods that detect metabolic alterations, ^{1}H-MRS has been developed as a powerful tool to measure metabolites of the regional brain in schizophrenia with the majority focusing on patients *in vivo*. Due to limited resolution and time constraint, most studies commonly use the magnetic field strength of 1.5 or 3 T [5, 21], *in vivo* ^{1}H-MRS can only detect a few molecules and locate predetermined voxels. However, based on previous findings of brain metabolic changes in schizophrenia, the abnormalities were found in multiple brain regions. Therefore, a specific technique could be adopted to simultaneously detect the metabolites in different brain regions, and the brain regions most associated with the metabolic abnormalities in schizophrenia could be selected. With much higher spectral resolution, the *in vitro* proton nuclear magnetic resonance (^{1}H-NMR) can provide the specific metabolic information of different brain regions in the whole brain simultaneously, which can improve our understanding of the findings *in vivo* [22].

In this study, we selected three different age periods of MAM vs. saline treated rats to test behavioral alterations. At adult age, the ^{1}H-NMR method was used to carry out the metabolomics analysis of extracts from nine different brain regions. To our knowledge, this is the first time the neurochemical changes in multiple brain areas of MAM model with *in vitro* ^{1}H-NMR have been explored. The findings in the current study may contribute to further investigations of the pathophysiology of this disease.

Materials and methods

Animals

All procedures in these experiments were in accordance with the guidelines of the Animal Care and Use Committees at the Wuhan Institute of Physics and Mathematics, Chinese Academy of Science. The pregnant Sprague Dawley dams were obtained from VITAL RIVER (Beijing, China). The dams were randomly treated with either MAM 22 mg/kg or vehicle (0.9% saline solution) intraperitoneally (*i.p.*) on gestational day 17 (day 0 was defined as the day of plug observed) [23, 24]. Pups were weaned 21 days after birth, and the males were kept in groups of three individuals with unlimited access to a diet of pellets and water. Animals were housed in a room under controlled temperature (21 ± 2°C) with a 12-h light/dark (lights at 8:00 am-8:00 pm) cycle.

Animal behavioral studies

In order to investigate the alterations of behaviors during the animal's growth, a list of animal behavioral experiments (Open field test, OFT; Morris water maze, MWM) of the MAM or saline treated male rats were studied in three different age periods (n = 11 in each group), which are considered as prepuberty (PD32-39), late-puberty (PD50-57) and early-adulthood (PD80-87).

Open field test (OFT): The apparatus used was a black box (100 × 100 × 30 cm^3) with a video camera above the arena. The bottom of the box was divided into 25 (5 × 5) squares, and the central four squares were considered as the central zone. On the experimental day, a rat was placed in the center of the bottom, video recorded and timed at the same time. After 5 min, the rat was returned to its home cage. The chamber was cleaned with 70% ethanol to avoid any information left before the next test. The total distance traveled, mean speed, entries to the center area and time spent in the center area were analyzed.

Morris water maze (MWM): The water maze apparatus was a circular black pool (diameter = 180 cm, height = 50 cm) filled with water (approximately 24 ± 1.0°C). A platform (diameter = 10 cm, height = 26 cm) was hidden 2 cm below the surface of the water in a fixed position (the center of the southwest quadrant). The room contained several visual cues on the room walls. A computerized video system was utilized to record and analyze the behaviors of the rat during the experiment.

The day before behavioral testing, the rats were exposed to the pool without the platform for 60 s to adapt to the experiment. In acquisition trial, the rats were placed in the water facing the wall and free to swim until they found the platform and stayed on it for 3 s. If an animal failed to find the platform within 60 s, it was guided to the platform and remained for 10 s. Each rat was subjected to 4 trials per day for 4 days, and the starting position was changed in each trial (starting on the N side, followed by E, S, W sides). In probe trial, the 5th day of the experiment, the platform was removed and the rats were allowed to swim freely for 90 s. Time to the platform (escape latency) in each acquisition trial and probe trial was calculated. Percentage time in the target quadrant (quadrant time) and the number of times the subject crossed the platform location (platform crossing) were calculated during the probe trial.

^{1}H-NMR studies

Brain samples collection: After the behavioral studies in early-adulthood periods, the animals were anesthetized (sevoflurane) and microwaved (1.0 kw, 19 s) using a commercial head focused microwave machine (Tangshan Nanosource Microwave Thermal Instrument Manufacturing Co. Ltd.) to prevent postmortem chemical changes [25]. The cooked brain tissue was quickly removed from the skull and manually divided into nine different brain regions, such as midbrain (MID), thalamus (THA), hypothalamus (HYP), hippocampus (HIP), striatum (STR), temporal cortex (TC), prefrontal cortex (PFC), parietal cortex (PC) and occipital cortex (OC) according to the anatomical landmarks [26, 27]. The tissues were placed into a 2 ml pre-weighed EP tube, reweighed and immediately frozen in liquid nitrogen. At the end, all samples were stored in -80°C refrigerator for further analysis.

Samples extraction: HCl/methanol (300 μL, 0.1 M HCl) and ethanol (600 μL, 60%) were added separately to the samples and homogenized twice (20 Hz, 90 s, Tissuelyser II, QIAGEN, Germany) [28]. The mixture was centrifuged (4°C, 11,000 g, 10 min) and the supernatant was placed into a 5.0 ml EP tube. The extraction process was repeated twice with 700 μl ethanol each time. All supernatants were collected together and desiccated with the centrifugal drying apparatus (Thermo Scientific 2010, Germany) for 3 h. Samples were further dried with a freeze dryer for 24 h.

Phosphate buffer (pH = 7.2, 540 μL) and double distilled water (60 μl, 120 mg/L 3-(Trimethylsilyl) propionic-2, 2, 3, 3, d4 acid sodium salt (TSP, 269913-1G, Sigma Aldrich) in D2O) were added to dissolve the dried product and the mixture was vortexed for 30 s. TSP was set as the internal standard. The mixed solution was centrifuged (4°C, 14,000 g, 10 min) with high-speed centrifuge, and the supernatant (500 μl) was transferred to a 5 mm NMR tube for the ^{1}H-NMR study.

^{1}H-NMR study: One dimensional (1D) NMR spectra were acquired on a BrukerAvance III 600 MHz NMR spectrometer equipped with an inverse cryogenic probe (BrukerBiospin, Germany). All data were collected at 298 K with a standard WATERGATE pulse sequence. The 90° pulse length of each individual sample was adjusted to 10.1 μs. The scan was performed with the following parameters: Dummy scans (DS), 8; Acquisition scans (NS), 128; Acquisition time (AQ), 1.8 s; Relaxation delay (D1), 3.0 s; Spectral width, 20 ppm; Data points, 32 k. Finally, it took about 10 minutes for a sample to be load-

ed and scanned. For a random sample, a series of two-dimensional (2D) NMR spectra were also collected to assign the metabolites in the ^{1}H-NMR spectra (online spectral databases). The 2D spectra include the following: ^{1}H-^{1}H correlation spectroscopy (COSY), J-resolved spectroscopy (JRES), ^{1}H-^{1}H total correlation spectroscopy (TOCSY), ^{1}H-^{13}C heteronuclear single quantum correlation (HSQC) and ^{1}H-^{13}C heteronuclear multiple bond correlation (HMBC) 2D-NMR spectra data (online spectral databases). All the NMR data collections were completed using the TOPSPIN software (V3.0, BrukerBiospin, Germany).

Statistical analysis

Behavioral experiments: For analysis of OFT, all data were analyzed using two-factor mixed ANOVA, with group (MAM vs. Control) as between-subject factor and age period (prepuberty, late-puberty, early-adulthood) as within-subject factors. Post hoc analysis was performed using the Bonferroni test. For analysis of the acquisition trial of MWM, all data of the four trials were used as an average on each training day. The effect of the prenatal MAM treatment on escape latency (s), was analyzed using two-way repeated measures (RM) ANOVA with days as RM. The Student's *t*-test was used to further test the difference between the two groups on each training day. For analysis of the probe trial of MWM, all data were analyzed by two-factor mixed ANOVA, with group (MAM vs. Control) as between-subject factor and age period (prepuberty, late-puberty, early-adulthood) as within-subject factors. The criterion for statistical significance was a probability value of 0.05. All results are presented as means ± SEM.

NMR data: For Fourier transformation, all spectra were firstly manually phased, baseline corrected, and referenced to the internal standard TMSP (δ = 0) using Topspin software 3.2 (Bruker Biospin, Germany). The raw data were automatically loaded into *NMRSpec* software [29], a tool using MATLAB code. Then, the peak alignment and integration were completed. Most amino acids were located in the region of 1.1-4.2 ppm, thus the other regions were removed before spectral alignment. Furthermore, ethanol was difficult to completely remove during the sample lyophilizing, that could affect the spectral analysis of the relevant spectral regions (δ: 1.15-1.22 and 3.597-3.687), thus the relevant ethanol signals were also discarded. All spectral data were divided into 0.04 ppm spectral buckets and automatically integrated in *NMRSpec*. The bucketed spectral data and integrated areas were normalized with the tissue weight and related peak area of TSP.

The absolute concentration (μmol/g wet weight) of the metabolite was calculated based on the following equation:

$$Cm = (A_m * C_{TSP} * V_{TSP} * 9)/(R_m * n_H)$$

Where A_m is the related peak area of the target metabolite; C_{TSP} and V_{TSP} are the concentration and volume of TSP; 9 is the number of protons in TSP; n_H is the number of protons of the metabolites within the peaks area of A_m; R_m is the ratio between the area of partial NMR signal of the selected metabolites during actual detection and the area of the whole proton signal in the standard spectrum. During sample detection, many signals overlapped, thus it was impossible to use them to calculate the absolute concentration. It was better to select a pure signal which did not overlap with other signals or part of the related signal. For example, R_m values of the metabolites Asp, taurine, Gly and creatine were equal to 1, whereas the signals of NAA (0.5871), GABA-4 (0.5728), Glu-4 (0.9001), Gln-4 (0.3901) and myo-instol (0.4933) overlapped with the other signals.

Data analysis was performed using SPSS software (Version 22) and codes in MATLAB. Student's *t*-test were employed to determine the significant differences in metabolite concentrations. Probability value of 0.05 was considered statistically significant. Metabolite concentrations were presented as means ± SEM.

Results

Behavioral experiments

Open field test: For the spontaneous locomotor activity analysis, the total travel distance was significantly reduced in MAM rats (**Figure 1A**, F = 19.578, P<0.001). There was no age period effect as well as group × age period interaction displayed. The group difference was present in late-puberty (P<0.001) and early-adulthood (P = 0.004) compared to control rats following post hoc analysis. However, there was no sig-

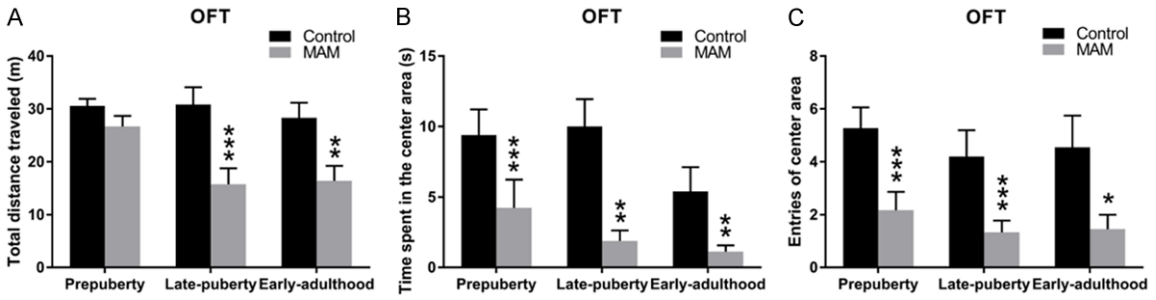

Figure 1. Effect of MAM vs. saline treatment on performance in the open field test (OFT) at three age periods. Each column represents the mean (± S.E.M.) on the experiment day of OFT. *P<.05 compared with control rats at the same age period; **P<.01 compared with control rats at the same age period. ***P<.001 compared with control rats at the same age period.

nificant difference between the two groups in prepuberty (P = 0.257). Total travel distance of MAM rats was significantly reduced in late-puberty (P = 0.045) and early-adulthood (P = 0.035) compared to prepuberty, but there was no difference between late-puberty and early-adulthood. There was no difference in total travel distance between any two age periods in control rats. Thus, MAM rats showed impairment in spontaneous locomotor activity from late-puberty to early-adulthood.

For the anxiety reactivity analysis (**Figure 1B** and **1C**), MAM rats showed less time spent in the center area (F = 26.696, P<0.001) and fewer entries to the center area (F = 31.251, P<0.001). There was no age period effect as well as group × age period interaction displayed. Post hoc analysis showed that the group difference was consistently present in the three age periods: prepuberty, time spent in the center area (P<0.001), entries of center area (P<0.001); late-puberty, time spent in the center area (P = 0.012), entries to center area (P<0.001); early-adulthood, time spent in the center area (P = 0.012), entries to center area (P = 0.031). There was no difference between any two age periods in MAM and control rats, indicating that MAM rats exhibited stability of anxiety state in all age periods.

Morris water maze: In the acquisition trial, MAM rats showed deficits in spatial learning ability from late-puberty to early-adulthood, but not in prepuberty (**Figure 2A**). The two-way ANOVA with day as repeated measures revealed an effect of group on escape latency in late-puberty (F = 0.323, P = 0.004) and early-adulthood (F = 7.250, P = 0.015), but not in prepuberty (F = 0.148, P = 0.704). Further analysis of each training day using Student's *t* test, showed that MAM rats spent more time to find the platform on day 1 (F = 0.777, P = 0.002) in late-puberty, day 2 (F = 3.960, P = 0.016) and day 4 (F = 7.274, P = 0.002) in early-adulthood. There was no difference on any day in prepuberty. The two-way ANOVA with day as repeated measurements also showed an effect of training days in three age periods (prepuberty, F = 4.529, P = 0.006; late-puberty, F = 5.331, P = 0.003; early-adulthood, F = 12.792, P<0.001), which indicates the learning process of the test. There was no interaction between groups and training days in any age periods.

In the probe trial, MAM showed a slight differential in reference memory in early-adulthood. Overall analysis showed MAM rats spent more time to find the platform (F = 6.372, P = 0.003, **Figure 2B**). An age period effect was present (F = 6.372, P = 0.003), but not a group × age period interaction (F = 0.439, P = 0.647). Post hoc analysis revealed no group difference in the three age periods (prepuberty, P = 0.421; late-puberty, P = 0.184; early-adulthood, P = 0.951). Overall analysis displayed no group effect on the time of swimming in the target quadrant (F = 0.727, P = 0.397, **Figure 2C**). There was also no age period effect (F = 1.094, P = 0.342) and group × age period interaction (F = 2.718, P = 0.074). Post hoc analysis showed that there was group difference present in early-adulthood (P = 0.019). MAM rats spent less time in crossing the platform location (F = 4.891, P = 0.031, **Figure 2D**). An age period effect (F = 10.028, P<0.001) was also found, but no group × age period interaction (F = 0.462, P = 0.633). The difference was present in early-adulthood (P = 0.049) by the post hoc analysis.

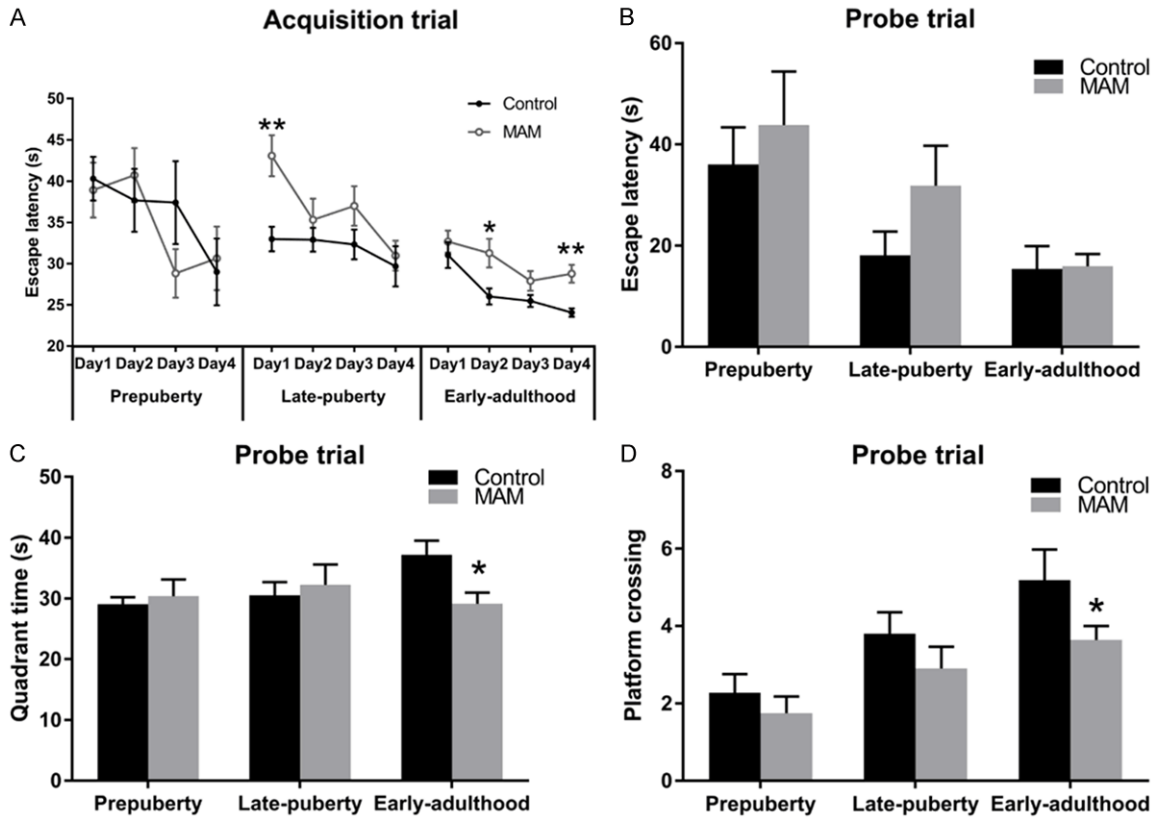

Figure 2. A. Effect of MAM vs. saline treatment on performance in the acquisition trial of the Morris water maze (MWM) at three age periods. The two-way ANOVA with day as repeated measures revealed an effect of group on escape latency in late-puberty ($F = 7.250$, $P = 0.015$) and early-adulthood ($F = 8.603$, $P = 0.008$). Each point represents mean (± S.E.M.) of the four trials on the same experiment day. *P<.05 compared with control rats on the same experiment day by Student's t test; **P<.01 compared with control rats on the same experiment day using Student's t test. B-D. Effect of MAM vs. saline treatment on performance in the probe trial of the Morris water maze at three age periods. Each column represents the mean (± S.E.M.) of four consecutive trials. *P<.05 compared with control rats at the same age period.

NMR data

In the current study, there were nine different brain regions involved. In order to show the results clearly, only a sample from the brain region (occipital cortex) were selected for illustration. At first, the metabolites in the ^{1}H-NMR spectrum were identified using related published works and 2D-NMR spectra. The identification of these metabolites is shown in (**Figure 3**). Furthermore, the average NMR spectrum of the extracts in the represented brain region (occipital cortex) in the MAM-treated and control groups were also calculated and illustrated (**Figure 3**). During the extraction of the brain samples, methanol and ethanol were involved, which were detected using ^{1}H-NMR methods. During the lyophilization procedure, these two chemicals were evaporated. However, the results showed that it was very difficult to totally erase them. Thus, the NMR signal regions related to ethanol and methanol were discarded for further analysis.

In order to primarily estimate the difference of the metabolites in different brain regions, the differences of the average ^{1}H-NMR spectra in the MAM-treated and control groups were calculated (**Figure 4**). The different metabolites in these brain regions were identified. However, there is no information of the standard deviation of these metabolites in this figure. Thus, it was very difficult to verify the major differences between brain regions in the MAM group compared with the control group.

Statistical analysis of the metabolites

The absolute concentrations of all detected metabolites in different brain regions were cal-

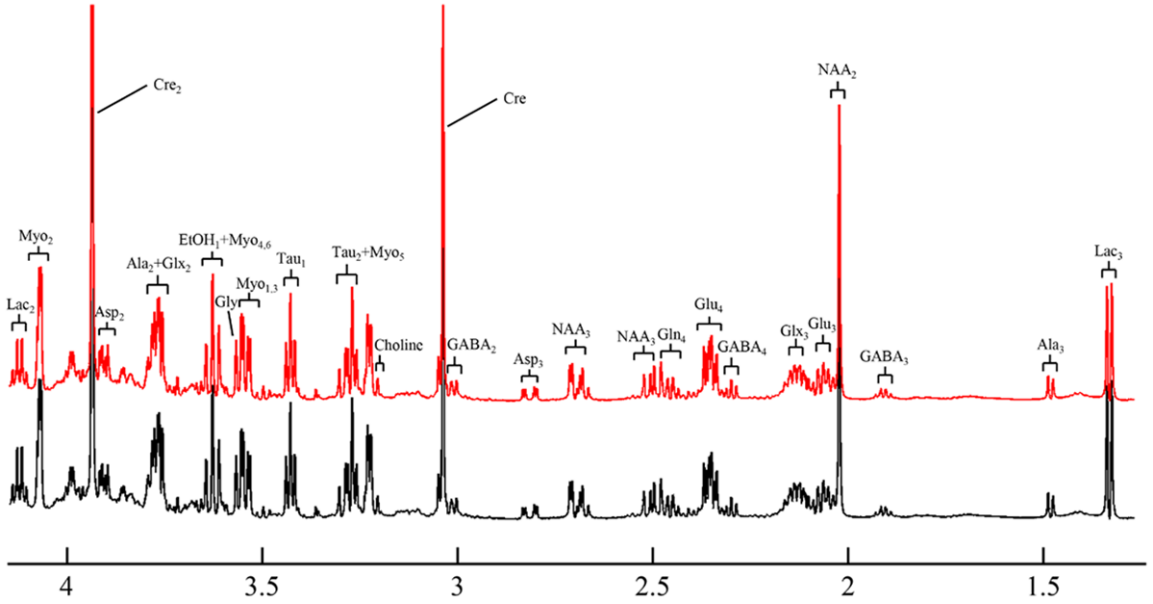

Figure 3. The average normalized ^{1}H-NMR spectra of the metabolites of occipital cortex of MAM *vs.* saline treatment. Red line: control; black line: MAM. Note: Lowercase, position of the hydrogen signal which is connected with the carbon position; Lac, lactate; Myo, myo-institol; Cre, creatine; Asp, aspartic acid; Ala, alanine; Glx, glutamine + glutamate; EtoH, ethanol; Gly, glycine; Tau, taurine; GABA, gamma amino acid butyric acid; NAA, N-aceytl aspartate; Gln, glutamine; Glu, glutamate.

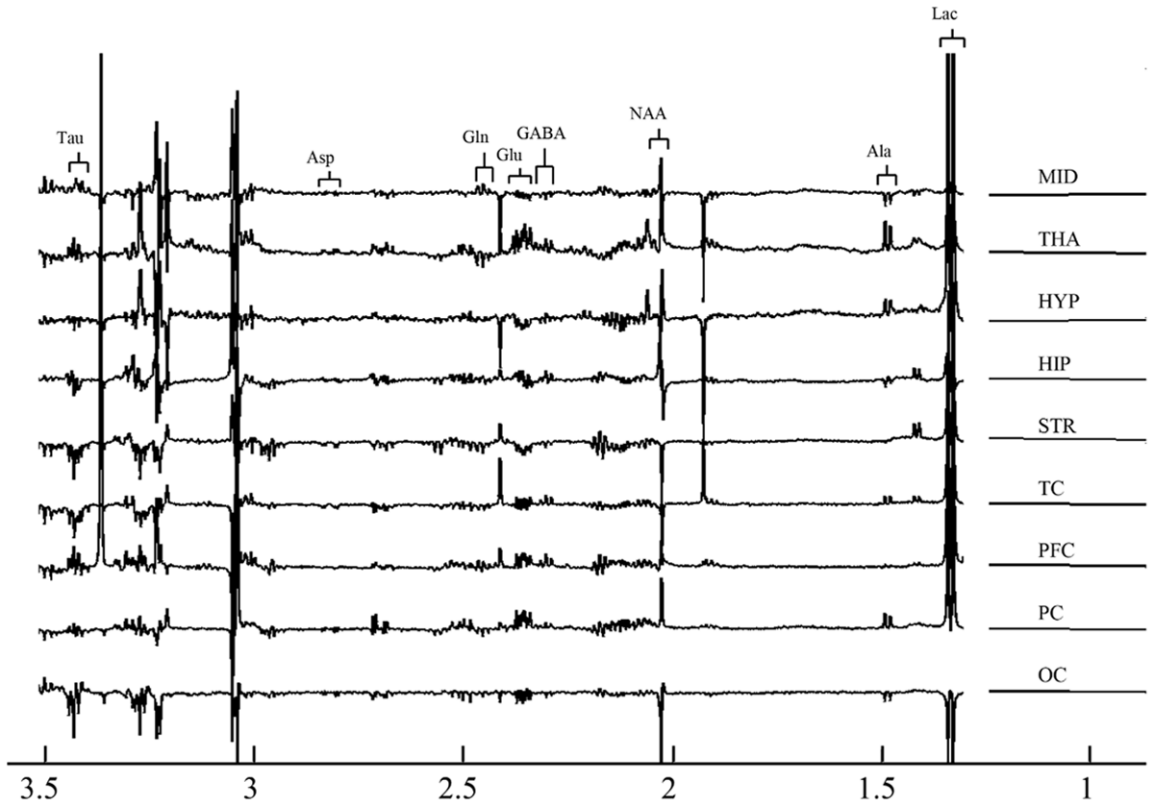

Figure 4. Differences in ^{1}H-NMR spectra of the metabolites of different brain regions of MAM *vs.* saline treatment.

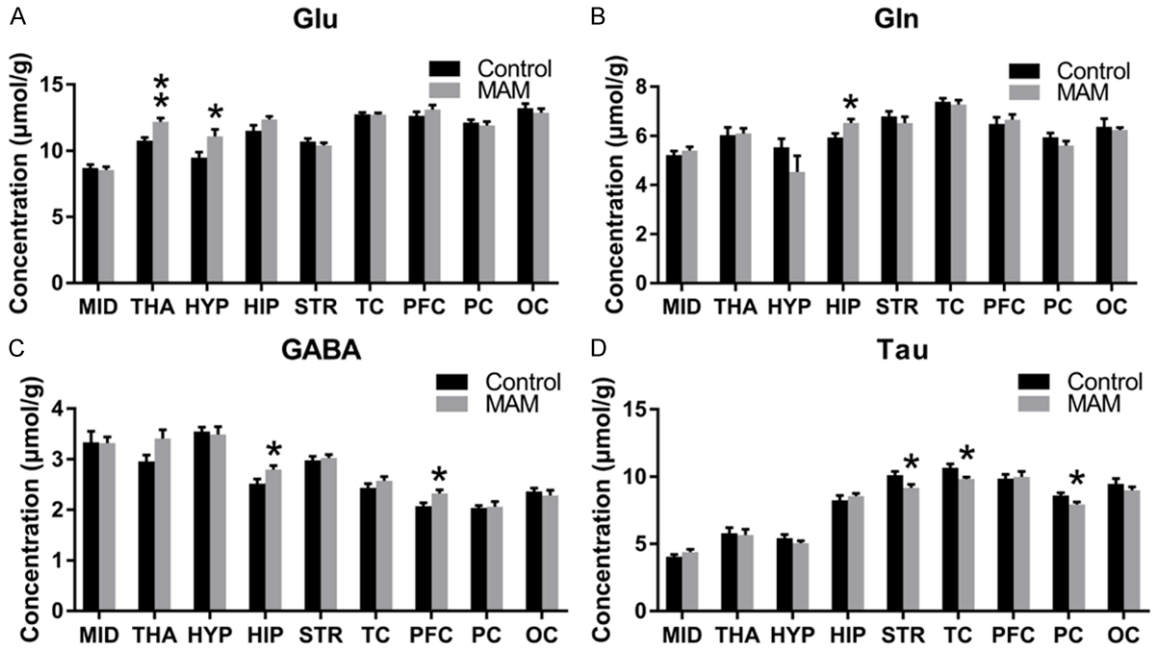

Figure 5. Differences of metabolite concentration in nine brain regions of MAM vs. saline treatment. Each column represents the mean (± S.E.M.) of metabolite concentration of each group in certain brain region. *P<0.05 compared with control rats at the same brain region; **P<.01 compared with control rats at the same brain region. ***P<.001 compared with control rats at the same brain region.

culated. Then the metabolites in different brain regions of these two different groups were compared. For precise analysis, group differences were found in Glu, Gln, GABA and taurine. Glu was significantly increased in the thalamus (F = 0.476, P = 0.004) and hypothalamus (F<0.001, P = 0.025, **Figure 5A**). Gln was elevated in the hippocampus (F≤0.001, P = 0.015, **Figure 5B**). GABA was found raised in the hippocampus (F = 0.040, P = 0.029) and the prefrontal cortex (F = 0.201, P = 0.203) of MAM rats (**Figure 5C**). Taurine decreased in the striatum (F = 0.044, P = 0.026), temporal cortex (F = 5.438, P = 0.020) and parietal cortex (F = 0.067, P = 0.028, **Figure 5D**).

Discussion

Behavioral patterns of MAM-treated rats

Gestational MAM exposure model has been suggested to produce similar behavioral abnormalities to those seen in schizophrenia. A previous study screened the MAM-treated rats in three different age periods: prepuberty (PD28-42), late-puberty (PD56-70) and adulthood (PD98-112). The results indicated that the MAM rats had elevated locomotor activity in late puberty and adulthood, but not in prepuberty. The anxiolytic effect had different age patterns which was present in prepuberty and adulthood, but had disappeared in late puberty [30]. Another study found that MAM rats had the same locomotor activity with control rats in late-puberty (PD58-60) [31]. Elevated anxiety-like behaviors of MAM rats were observed both in peripuberty [32] and adulthood [33] in other studies. However, there are few studies that confirmed whether the MAM model, like schizophrenia, had onset in late-puberty or adulthood. Our findings are consistent with the clinical observation that anxiety is highly prevalent in schizophrenia patients [34]. In the current study, MAM-treated rats showed hypoactivity from late-puberty to early-adulthood and heightened anxiety levels throughout the three age periods. These abnormalities might be correlated to hypersensitivity to stress [35] around puberty and resulted in the dopaminergic hyper responsivity which occurs in adulthood [32].

Cognitive deficits are believed to be core symptoms of schizophrenia. One study assessed MAM rats on an attentional set-shifting task which is a rodent analog of the Wisconsin card sort task. They found that MAM rats required

more time to learn the task [36]. Furthermore, another study examined the performance on Morris water maze of juvenile (PD12-21) and adolescent (PD28-45) MAM-treated rats. Both groups presented deficits in learning the location of the platform [37]. In the present study, we found MAM-treated rats had similar performance compared with the control during prepuberty, but the spatial learning ability were impaired from late-puberty and persisted in early-adulthood.

Overall, the behavioral abnormalities indicate that the MAM rats were in a relatively constant onset state from late-puberty to early-adulthood. This provided favorable evidence for our further study of metabolic changes using the MAM treated animal model.

Metabolic changes in adult MAM-treated rats

Glutamatergic and GABAergic metabolites: The glutamate hypothesis of schizophrenia highlighted that NMDA receptor hypofunction might be a key mechanism that could help explain major clinical and pathophysiological aspects of schizophrenia [38]. The NMDA receptor hypofunction was associated with reduced parvalbumin-containing GABA interneurons [39], leading to the imbalance between neuronal excitation and inhibition [40, 41], which may contribute to the disinhibition of cortical excitatory neurons resulting in excess glutamate release [41]. Mice with reduced expression of NMDA receptor manifested schizophrenia-like symptoms, and these kinds of symptoms can be alleviated by haloperidol or clozapine [42]. The long-time treatment with antipsychotic drugs could induce adaptive changes on glutamate receptor expression in different brain regions of rats [43]. Postmortem analysis found reduction of glutamate in the hippocampus and prefrontal cortex [44] and alteration of the density and subunit constitution of glutamate receptor in the thalamus and hippocampus of schizophrenia patients [9-11]. A recent meta-analysis which included 59 *in vivo* ^{1}H-MRS studies revealed that there was a broad elevation of glutamatergic metabolites (glutamate, glutamine or glutamate plus glutamine) across several brain regions of schizophrenia patients and individuals at high risk, such as the thalamus, basal ganglia, medial frontal cortex and medial temporal cortex [45].

GABA is the main inhibitory neurotransmitter which is formed by the decarboxylation of glutamate catalyzed by glutamate decarboxylase (GAD). Postmortem analysis found a subclass of GABA interneurons in the prefrontal cortex with significant low expression of GAD67 mRNA [13], which is consistent with other studies [12, 14]. However, in ^{1}H-MRS studies, there have been inconsistent results of GABA levels in schizophrenia individuals, with reports showing a reduction [46], unchanged [47, 48] and elevation [15, 49, 50], mainly located in the striatum, dorsal caudate nucleus, anterior cingulate, medial prefrontal cortex, temporal cortex, parietal cortex and occipital cortex. With the *in vitro* ^{1}H-NMR method, we observed elevated GABA concentrations in the hippocampus and prefrontal cortex of MAM-treated rats. Our result is not exactly consistent with these former postmortem findings. That might be due to two main factors: the postmortem metabolite changes in the bio-system and the different living systems. However, all findings support that the GABA disorders do occur in schizophrenia.

Taurine: Increasing evidences suggest that the taurine-induced neuroprotective effect may be mediated by its antagonism to the glutamate-induced excitotoxicity [17]. In an analysis of a rodent model of maternal immune activation (MIA) which is associated with schizophrenia, taurine was significantly reduced in hippocampus of the adult offspring [51]. Moreover, in another MIA model with longitudinal observation, the reduction of taurine in the prefrontal cortex was found in adult offspring but not adolescent offspring [52]. *In vitro* ^{1}H-MRS study that used a rodent model of neonatal maternal deprivation revealed increased taurine in the hippocampus and prefrontal cortex, which reflect that the increased taurine might protect the neonatal brain after more severe damage [53]. At present, fewer taurine *in vivo* ^{1}H-MRS studies have been reported, which only found elevated taurine in plasma [54] and the medial prefrontal cortex [20] of schizophrenia individuals. Our results suggest that taurine is significantly reduced in the striatum, temporal cortex and parietal cortex of adult MAM-treated rats, this could probably be a decompensation state for excess glutamate.

Abnormal metabolic state in corticolimbic system: Our findings revealed that there was meta-

bolic dysfunction in the corticolimbic system, such as the thalamus, hypothalamus, hippocampus, striatum, prefrontal cortex, temporal cortex and the parietal cortex. Structural changes in the corticolimbic system are important features of schizophrenia. In this pathway, a major function of the NMDA receptor is to drive GABA interneurons, thus maintaining the inhibition of primary neurons in the limbic system. NMDA receptor antagonists could inhibit the activation of GABA interneurons, which in turn could increase glutamate release [55] and lead to excessive excitatory activity release [56]. Further, evidence showed that the sensitivity to NMDA receptor antagonists of rats developed during adolescence and continued into early adulthood [56] and NMDA receptor hypofunction was present in adolescence of the MAM model [57], which suggests that early developmental interference with the nervous system does not begin to take effect until adolescence, which is consistent with our findings of behavioral changes. We postulate that the impairment of the NMDA receptor, which was caused by interference during the early developmental period, damaged GABAergic neurons, which led to the disinhibitory effect on downstream neurons. In the beginning, the nervous system could compensate for these damages through some mechanisms, and taurine might be one of these compensatory mechanisms. However, with the continuous maturation of the nervous system, the level of metabolites in the brain gradually become a decompensation state, as we observed with the elevation of Glu, Gln, GABA and the reduction of taurine, which eventually leads to damage of the cortical limbic system. The mechanism by which the imbalance in brain function and metabolic status and the role played in the pathophysiology of schizophrenia needs further research.

According to our experimental design, the detection of metabolites cannot be realized in prepuberty and late-puberty stage. Thus, we did not conduct dynamic monitoring of metabolic alterations during the growth process. In future experiments, we will conduct multi-point monitoring to better explain this problem.

The present study demonstrated the effect of gestational exposure to MAM on the behavioral changes in different developmental periods and the metabolic alteration in different brain regions of adult rats. MAM-treated rats showed significant hypoactivity and high levels of anxiety mainly from late-puberty to early-adulthood, and slightly cognitive impairment in early-adulthood. Metabolic analysis of adult MAM-treated rats showed Glu was increased in the hypothalamus and thalamus, GABA was increased in the prefrontal cortex and hippocampus, and Gln was increased in the hippocampus. However, taurine showed a significant decrease in the striatum, temporal cortex and parietal cortex. These findings may have some significance when studying how metabolic abnormalities in different brain regions of schizophrenia patients are involved in the pathophysiological mechanism of the disease. In conclusion, the behavioral changes of MAM rats, as a neurodevelopmental model, manifested in a time-dependent pattern and the metabolic changes in the corticolimbic system could be critical to the pathophysiology of schizophrenia.

Acknowledgements

This work was supported by the China Postdoctoral Science Foundation (2018M640265) and the Youth Innovation Promotion Association of Chinese Academy of Sciences (Y6Y00-21004).

Disclosure of conflict of interest

None.

Address correspondence to: Jie Wang, 30 West of Xiao Hong Shan Street, Wuchang, Wuhan 430071, Hubei, P. R. China. Tel: +86-27-87197653; Fax: +86-27-87199543; E-mail: jie.wang@wipm.ac.cn; Fei Wang, Department of Psychiatry and Radiology, The First Affiliated Hospital, China Medical University, 155 Nanjing North Street, Shenyang 110001, Liaoning, P. R. China. Tel: 8624-83283405; Fax: 8624-83283405; E-mail: fei.wang@cmu.edu.cn

References

[1] Gomes FV, Rincon-Cortes M and Grace AA. Adolescence as a period of vulnerability and intervention in schizophrenia: insights from the MAM model. Neurosci Biobehav Rev 2016; 70: 260-270.

[2] Nestler EJ and Hyman SE. Animal models of neuropsychiatric disorders. Nat Neurosci 2010; 13: 1161-1169.

[3] Lodge DJ and Grace AA. Gestational methylazoxymethanol acetate administration: a de-

velopmental disruption model of schizophrenia. Behav Brain Res 2009; 204: 306-312.
[4] Moore H, Jentsch JD, Ghajarnia M, Geyer MA and Grace AA. A neurobehavioral systems analysis of adult rats exposed to methylazoxymethanol acetate on E17: implications for the neuropathology of schizophrenia. Biol Psychiatry 2006; 60: 253-264.
[5] Wijtenburg SA, Yang S, Fischer BA and Rowland LM. In vivo assessment of neurotransmitters and modulators with magnetic resonance spectroscopy: application to schizophrenia. Neurosci Biobehav Rev 2015; 51: 276-295.
[6] Allen RM and Young SJ. Phencyclidine-induced psychosis. Am J Psychiatry 1978; 135: 1081-1084.
[7] Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB Jr and Charney DS. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch Gen Psychiatry 1994; 51: 199-214.
[8] Kim JS, Kornhuber HH, Schmid-Burgk W and Holzmuller B. Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia. Neurosci Lett 1980; 20: 379-382.
[9] Gao XM, Sakai K, Roberts RC, Conley RR, Dean B and Tamminga CA. Ionotropic glutamate receptors and expression of N-methyl-D-aspartate receptor subunits in subregions of human hippocampus: effects of schizophrenia. Am J Psychiatry 2000; 157: 1141-1149.
[10] Ibrahim HM, Hogg AJ Jr, Healy DJ, Haroutunian V, Davis KL and Meador-Woodruff JH. Ionotropic glutamate receptor binding and subunit mRNA expression in thalamic nuclei in schizophrenia. Am J Psychiatry 2000; 157: 1811-1823.
[11] Meador-Woodruff JH and Healy DJ. Glutamate receptor expression in schizophrenic brain. Brain Res Brain Res Rev 2000; 31: 288-294.
[12] Volk DW, Austin MC, Pierri JN, Sampson AR and Lewis DA. Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. Arch Gen Psychiatry 2000; 57: 237-245.
[13] Hashimoto T, Volk DW, Eggan SM, Mirnics K, Pierri JN, Sun Z, Sampson AR and Lewis DA. Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. J Neurosci 2003; 23: 6315-6326.
[14] Akbarian S, Kim JJ, Potkin SG, Hagman JO, Tafazzoli A, Bunney WE Jr and Jones EG. Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. Arch Gen Psychiatry 1995; 52: 258-266.
[15] Kegeles LS, Mao X, Stanford AD, Girgis R, Ojeil N, Xu X, Gil R, Slifstein M, Abi-Dargham A, Lisanby SH and Shungu DC. Elevated prefrontal cortex gamma-aminobutyric acid and glutamate-glutamine levels in schizophrenia measured in vivo with proton magnetic resonance spectroscopy. Arch Gen Psychiatry 2012; 69: 449-459.
[16] Coyle JT. Glutamate and schizophrenia: beyond the dopamine hypothesis. Cell Mol Neurobiol 2006; 26: 365-384.
[17] Wu H, Jin Y, Wei J, Jin H, Sha D and Wu JY. Mode of action of taurine as a neuroprotector. Brain Res 2005; 1038: 123-131.
[18] Do KQ, Lauer CJ, Schreiber W, Zollinger M, Gutteck-Amsler U, Cuenod M and Holsboer F. gamma-Glutamylglutamine and taurine concentrations are decreased in the cerebrospinal fluid of drug-naive patients with schizophrenic disorders. J Neurochem 1995; 65: 2652-2662.
[19] Cao B, Wang D, Brietzke E, McIntyre RS, Pan Z, Cha D, Rosenblat JD, Zuckerman H, Liu Y, Xie Q and Wang J. Characterizing amino-acid biosignatures amongst individuals with schizophrenia: a case-control study. Amino Acids 2018; 50: 1013-1023.
[20] Shirayama Y, Obata T, Matsuzawa D, Nonaka H, Kanazawa Y, Yoshitome E, Ikehira H, Hashimoto K and Iyo M. Specific metabolites in the medial prefrontal cortex are associated with the neurocognitive deficits in schizophrenia: a preliminary study. Neuroimage 2010; 49: 2783-2790.
[21] Schwerk A, Alves FD, Pouwels PJ and van Amelsvoort T. Metabolic alterations associated with schizophrenia: a critical evaluation of proton magnetic resonance spectroscopy studies. J Neurochem 2014; 128: 1-87.
[22] Barton SJ, Howe FA, Tomlins AM, Cudlip SA, Nicholson JK, Bell BA and Griffiths JR. Comparison of in vivo 1H MRS of human brain tumours with 1H HR-MAS spectroscopy of intact biopsy samples in vitro. MAGMA 1999; 8: 121-128.
[23] Gourevitch R, Rocher C, Le Pen G, Krebs MO and Jay TM. Working memory deficits in adult rats after prenatal disruption of neurogenesis. Behav Pharmacol 2004; 15: 287-292.
[24] Le Pen G, Gourevitch R, Hazane F, Hoareau C, Jay TM and Krebs MO. Peri-pubertal maturation after developmental disturbance: a model for psychosis onset in the rat. Neuroscience 2006; 143: 395-405.
[25] Wang J, Zeng HL, Du H, Liu Z, Cheng J, Liu T, Hu T, Kamal GM, Li X, Liu H, Xu F. Evaluation of metabolites extraction strategies for identify-

ing different brain regions and their relationship with alcohol preference and gender difference using NMR metabolomics. Talanta 2018; 179: 369-376.
[26] Wang J, Du HY, Ma XX, Pittman B, Castracane L, Li TK, Behar KL and Mason GF. Metabolic products of [2-C-13]ethanol in the rat brain after chronic ethanol exposure. J Neurochem 2013; 127: 353-364.
[27] Wang J, Jiang L, Jiang Y, Ma X, Chowdhury GM, Mason GF. Regional metabolite levels and turnover in the awake rat brain under the influence of nicotine. J Neurochem 2010; 113: 1447-1458.
[28] Liu T, He Z, Tian X, Kamal GM, Li Z, Liu Z, Liu H, Xu F, Wang J, Xiang H. Specific patterns of spinal metabolites underlying alpha-Me-5-HT-evoked pruritus compared with histamine and capsaicin assessed by proton nuclear magnetic resonance spectroscopy. Biochim Biophys Acta Mol Basis Dis 2017; 1863: 1222-1230.
[29] Liu Y, Cheng J, Liu HL, Deng YH, Wang J and Xu FQ. NMRSpec: an integrated software package for processing and analyzing one dimensional nuclear magnetic resonance spectra. Chemometrics and Intelligent Laboratory Systems 2017; 162: 142-148.
[30] Kallai V, Toth A, Galosi R, Peczely L, Ollmann T, Petyko Z, Laszlo K, Kallai J, Szabo I, Karadi Z and Lenard L. The MAM-E17 schizophrenia rat model: comprehensive behavioral analysis of pre-pubertal, pubertal and adult rats. Behav Brain Res 2017; 332: 75-83.
[31] Flagstad P, Mork A, Glenthoj BY, van Beek J, Michael-Titus AT and Didriksen M. Disruption of neurogenesis on gestational day 17 in the rat causes behavioral changes relevant to positive and negative schizophrenia symptoms and alters amphetamine-induced dopamine release in nucleus accumbens. Neuropsychopharmacology 2004; 29: 2052-2064.
[32] Du Y and Grace AA. Peripubertal diazepam administration prevents the emergence of dopamine system hyperresponsivity in the MAM developmental disruption model of schizophrenia. Neuropsychopharmacology 2013; 38: 1881-1888.
[33] Du Y and Grace AA. Amygdala hyperactivity in mam model of schizophrenia is normalized by peripubertal diazepam administration. Neuropsychopharmacology 2016; 41: 2455-2462.
[34] Achim AM, Maziade M, Raymond E, Olivier D, Merette C and Roy MA. How prevalent are anxiety disorders in schizophrenia? A meta-analysis and critical review on a significant association. Schizophr Bull 2011; 37: 811-821.
[35] Zimmerman EC, Bellaire M, Ewing SG, Grace AA. Abnormal stress responsivity in a rodent developmental disruption model of schizophrenia. Neuropsychopharmacology 2013; 38: 2131-2139.
[36] Featherstone RE, Rizos Z, Nobrega JN, Kapur S and Fletcher PJ. Gestational methylazoxymethanol acetate treatment impairs select cognitive functions: parallels to schizophrenia. Neuropsychopharmacology 2007; 32: 483-492.
[37] Snyder MA, Adelman AE and Gao WJ. Gestational methylazoxymethanol exposure leads to NMDAR dysfunction in hippocampus during early development and lasting deficits in learning. Neuropsychopharmacology 2013; 38: 328-340.
[38] Olney JW and Farber NB. Glutamate receptor dysfunction and schizophrenia. Arch Gen Psychiatry 1995; 52: 998-1007.
[39] Lisman JE, Coyle JT, Green RW, Javitt DC, Benes FM, Heckers S and Grace AA. Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. Trends Neurosci 2008; 31: 234-242.
[40] Benes FM, McSparren J, Bird ED, SanGiovanni JP and Vincent SL. Deficits in small interneurons in prefrontal and cingulate cortices of schizophrenic and schizoaffective patients. Arch Gen Psychiatry 1991; 48: 996-1001.
[41] Lewis DA and Moghaddam B. Cognitive dysfunction in schizophrenia: convergence of gamma-aminobutyric acid and glutamate alterations. Arch Neurol 2006; 63: 1372-1376.
[42] Mohn AR, Gainetdinov RR, Caron MG and Koller BH. Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. Cell 1999; 98: 427-436.
[43] Tascedda F, Blom JM, Brunello N, Zolin K, Gennarelli M, Colzi A, Bravi D, Carra S, Racagni G and Riva MA. Modulation of glutamate receptors in response to the novel antipsychotic olanzapine in rats. Biol Psychiatry 2001; 50: 117-122.
[44] Tsai G, Passani LA, Slusher BS, Carter R, Baer L, Kleinman JE and Coyle JT. Abnormal excitatory neurotransmitter metabolism in schizophrenic brains. Arch Gen Psychiatry 1995; 52: 829-836.
[45] Merritt K, Egerton A, Kempton MJ, Taylor MJ and McGuire PK. Nature of glutamate alterations in schizophrenia: a meta-analysis of proton magnetic resonance spectroscopy studies. JAMA Psychiatry 2016; 73: 665-674.
[46] Thakkar KN, Rosler L, Wijnen JP, Boer VO, Klomp DW, Cahn W, Kahn RS and Neggers SF. 7T proton magnetic resonance spectroscopy of gamma-aminobutyric acid, glutamate, and glutamine reveals altered concentrations in patients with schizophrenia and healthy siblings. Biol Psychiatry 2017; 81: 525-535.
[47] Duarte JMN and Xin L. Magnetic resonance spectroscopy in schizophrenia: evidence for

glutamatergic dysfunction and impaired energy metabolism. Neurochem Res 2019; 44: 102-116.

[48] Rowland LM, Kontson K, West J, Edden RA, Zhu H, Wijtenburg SA, Holcomb HH and Barker PB. In vivo measurements of glutamate, GABA, and NAAG in schizophrenia. Schizophr Bull 2013; 39: 1096-1104.

[49] de la Fuente-Sandoval C, Reyes-Madrigal F, Mao X, Leon-Ortiz P, Rodriguez-Mayoral O, Jung-Cook H, Solis-Vivanco R, Graff-Guerrero A and Shungu DC. Prefrontal and striatal gamma-aminobutyric acid levels and the effect of antipsychotic treatment in first-episode psychosis patients. Biol Psychiatry 2018; 83: 475-483.

[50] Ongur D, Prescot AP, McCarthy J, Cohen BM and Renshaw PF. Elevated gamma-aminobutyric acid levels in chronic schizophrenia. Biol Psychiatry 2010; 68: 667-670.

[51] Winter C, Djodari-Irani A, Sohr R, Morgenstern R, Feldon J, Juckel G and Meyer U. Prenatal immune activation leads to multiple changes in basal neurotransmitter levels in the adult brain: implications for brain disorders of neurodevelopmental origin such as schizophrenia. Int J Neuropsychopharmacol 2009; 12: 513-524.

[52] Vernon AC, So PW, Lythgoe DJ, Chege W, Cooper JD, Williams SC and Kapur S. Longitudinal in vivo maturational changes of metabolites in the prefrontal cortex of rats exposed to polyinosinic-polycytidylic acid in utero. Eur Neuropsychopharmacol 2015; 25: 2210-2220.

[53] Llorente R, Villa P, Marco EM and Viveros MP. Analyzing the effects of a single episode of neonatal maternal deprivation on metabolite profiles in rat brain: a proton nuclear magnetic resonance spectroscopy study. Neuroscience 2012; 201: 12-19.

[54] Samuelsson M, Skogh E, Lundberg K, Vrethem M and Ollinger K. Taurine and glutathione in plasma and cerebrospinal fluid in olanzapine treated patients with schizophrenia. Psychiatry Res 2013; 210: 819-824.

[55] Moghaddam B, Adams B, Verma A and Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. J Neurosci 1997; 17: 2921-2927.

[56] Farber NB, Wozniak DF, Price MT, Labruyere J, Huss J, St Peter H and Olney JW. Age-specific neurotoxicity in the rat associated with NMDA receptor blockade: potential relevance to schizophrenia? Biol Psychiatry 1995; 38: 788-796.

[57] Gulchina Y, Xu SJ, Snyder MA, Elefant F and Gao WJ. Epigenetic mechanisms underlying NMDA receptor hypofunction in the prefrontal cortex of juvenile animals in the MAM model for schizophrenia. J Neurochem 2017; 143: 320-333.