MAM-E17 schizophrenia rat model

Ph.D. thesis

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1. Introduction

Schizophrenia is a severe neuropsychiatric disorder affecting approximately 1% of the population worldwide [1, 2]. It is characterized by positive, negative and cognitive symptoms [1].

In the development of schizophrenia genetic and environmental risk factors are involved [3], that collectively lead to dysfunction of several neurotransmitter systems [4], neurobiological [5] and histological alterations [6, 7]. Among numerous conceptions about the origin of schizophrenia, most evidence supports the neurodevelopmental theory [3, 8]. According to this hypothesis, certain neural circuits are damaged in the early stages of brain development, with consequences in early adulthood.

Because of the obscure etiology and complex pathophysiology of schizophrenia, the treatment of patients is unresolved yet. The National Institute of Mental Health (NIMH) emphasizes the understanding of pathophysiology of the disease as a principal research direction, in which animal models provide extensive experimental opportunity.

The appearance of schizophrenia symptoms follows a diachronic pattern. Cognitive symptoms turn up early in life [9]; therefore they can be prodromal signs of later occurrence of the disease. Early recognition allows early treatment, which improves the prognosis of schizophrenia [10]. Therefore the purpose of NIMH's Early Psychosis Prediction and Prevention (EP3) initiative is to detect the risk states for psychotic disorders to prevent the onset of psychosis in high-risk individuals and to reduce the duration of untreated psychosis.

Animal experiments preparing the preventive treatments require the extensive knowledge of the experimental model and of the diachronic appearance of the symptoms represented by the model. The present experiments were carried out on the MAM-E17 model, which is a validated, neurodevelopmental model. This model is based on the effect of methylazoxymethanol-acetate (MAM), which can briefly interrupt the brain cellular proliferation. Although many investigations have been conducted so far on this model, there is still very little data available on the age-related appearance of symptoms.

1.1. Creating the MAM-E17 model and its features

To create the model, the methylazoxymethanol esther, methylazoxymethanol-acetate (MAM) was injected intraperitoneally to the pregnant dam on 17-th day (MAM-E17 model) [11, 12]. MAM enters the fetus through the placenta, where it methylates the DNS of rapidly proliferating cells of the central nervous system [13, 14], furthermore it affects the methylation pattern of histon proteins [15].

The target of MAM-treatment is selectively the central nervous system [16] affecting the neurodevelopmental processes primarily in the cerebral cortex and more particularly in the hippocampus [17, 18]. Structural alterations induce changes in metabolic activity and neurotransmission [4] in the neuronal circuits that include the affected regions (primarily the prefrontal (PFC) and temporal cortex) [17, 19]. In the pathophysiology of schizophrenia could also be involved developmental abnormalities in the limbic and frontal cortical neuronal circuits and disturbances of the associated neurotransmitter systems [20].

1.2.1. Histological features

In MAM-E17 animals reduced total brain weight can be observed [15, 21-23], similar to schizophrenia [7, 24], and in some MAM-treated animals ventricular enlargement can be shown as well, which is a frequent phenomenon in schizophrenia [6, 7, 23, 25]. Histological studies have revealed that MAM-E17 treatment induces volume reduction in the PFC, in the perirhinal (PRH), occipital (OCC) and entorhinal cortex, as well as in the hippocampus [21, 23, 26, 27], similarly to human findings [6, 7]. There are alterations in the organization and morphology of the pyramidal cells in the PFC [17] and the hippocampus [21, 22, 28-30]. In both areas the number of parvalbumin+ GABA-ergic interneurons decreases [30-33] and consequently the gamma rhythm of the affected areas is disturbed [32]. These all can be observed in schizophrenia as well [34-40].

1.2.2. Neurophysiologic features

Electrophysiological studies (single unit analysis, field potential analysis) have shown disturbances in the structures most responsible for the pathophysiology of the disease (PFC, hippocampus, nucleus Accumbens (n.Acc) and ventral tegmental area (VTA)) [17, 31, 41, 42], leading to cognitive impairment and deficient behaviour control [43].

1.2.3. Behaviour

Behavioural disturbances in the MAM-E17 model rats can be related to disturbances in the DA-ergical inputs of the frontal cortex and limbic system [21] and reflect an imbalance between the DA-ergic and glutamatergic system [25]. With the exception of social interaction deficit, almost all behavioural symptoms appear during puberty or early adulthood [21, 25], showing strong parallelism with those observed in schizophrenia. The behavioural alterations observed in the MAM-E17 model are summarized in Table 1.

	Schizophrenia	MAM-E17 model
Positive	forced movements, hallucination, delusions, bizarre behaviour, stereotyped behaviour, psychomotor agitation, amphetamine or NMDA receptor antagonists induced psychosis	hyperactivity, hyperreactivity (stress, amphetamine, or NMDA receptor antagonists induced hyperactivity), stereotyped behaviour
Negative	emotional flattening, social withdrawal	social interaction deficit
Cognitive	attentional deficit, working memory deficit, behavioural inflexibility	attentional deficit, short-term memory deficit, working memory deficit, behavioural inflexibility
	prepulse inhibition (PPI) deficit latent inhibition (LI) deficit	

Table 1. Behavioural alterations in MAM-E17 model analogous to schizophrenia symptoms

2. Objectives

The examination of schizophrenia using animal models is essential to enhance therapeutic efficacy in treatment of the disorder. MAM-E17 model is a validated neurodevelopmental model. Animal experiments which would prepare the preventive treatments would require a comprehensive study focusing on pubertal development of symptoms, however no such kind of examination was carried out until today. Therefore, the purpose of the present experiments is the comprehensive behavioural analysis of the MAM-E17 model across multiple age-periods (prepuberty, puberty and adulthood), covering a broad spectrum of symptoms of schizophrenia.
The behaviours examined were:

1. Spontaneous locomotor activity was investigated in open field test (OPF), furthermore the stereotyped behaviour of the animals were also analysed (rearing, grooming, sniffing).
2. Anxiety was examined in elevated plus maze (EPM), moreover the results of the OPF test were also analysed for anxiety.
3. Sensorimotor gating mechanisms were monitored in prepulse inhibition paradigm (PPI) of startle reaction.
4. Motor coordination skills were evaluated in rotarod test.
5. Cognitive capabilities were investigated in 8 arm radial maze (RAM).

Histological analysis was performed to detect structural alterations in the brain of MAM-E17 model animals.

3. Materials and methods

3.1. Subjects

MAM-E17 animals were prepared in our laboratory [21, 31]. Forty-one female and 19 male Wistar rats (Charles River, Hungary) were paired. The pregnant dams were injected intraperitoneally with either MAM (MRIGlobal Chemical Carcinogen Repository, Kansas City, Missouri; 25 mg/kg dissolved in saline) or vehicle (0.9% physiological saline solution) on gestational day 17. Pups were weaned 3-4 weeks after birth.

Animals were kept in a temperature- (21±2 °C), humidity- (55 ± 10%) and light-controlled room (12:12 h light–dark cycle with lights on at 7:00 a.m.). Standard laboratory food pellets (CRLT/N Charles River Kft, Budapest, Hungary) and tap water was available *ad libitum*. The animals were cared for in accordance with institutional (BA02/2000-8/2012, BA02/2000-64/2017), national (Hungarian Government Decree, 40/2013. (II. 14.)) and international standards (European Community Council Directive, 86/609/EEC, 1986, 2010).

Our experiments were carried out in the following age-periods: prepuberty, which may overlap with early puberty (4-5 weeks = PD28-42, classified as prepuberty) late puberty overlapping with young adulthood (8-9 weeks = PD56-70, classified as late puberty) and adulthood (14-15 weeks = PD98-112).

In these experiments, only male offspring were used. The locomotor activity, anxiety, performance on rotarod and sensory-motor gating mechanisms were investigated on the same male rats at three different age-periods, in prepuberty, puberty and adulthood (control rats, n=22 and MAM-E17, n=19, respectively). In RAM task because of the learning transfer different groups of animals were used in each experiment, i.e. in each age-period (control rats: in prepuberty n=12, in puberty n=9, and in adulthood n=11; and MAM-E17 rats: in prepuberty n=14, in puberty n=9, and in adulthood n=15; respectively).

Behavioural tests were performed during the daylight period between 08:00 and 14:00 h.

3.2. Neurological investigation

To survey whether MAM-treatment has any influence on motor function, animals were submitted to neurological monitoring. Intactness of muscular tone, visual and proprioceptive placing reflexes, coordination of limbs by grid walking and grasping were examined [44].

3.3. Behavioural experiments

3.3.1. Spontaneous locomotor activity in open field test (OPF)

Spontaneous locomotor activity and general behaviour were investigated in OPF test. The apparatus consisted of a 50×50×40 cm grey wooden box with an open roof. The ground of the cage was virtually divided into 16 identical squares. Experiments were carried out in a sound attenuated, dimly illuminated (40 W, red light) room. A video camera was fixed above the arena and was connected to a monitor and video tracking motion analysis system (EthoVision; Noldus Information Technology, The Netherlands).

The test was conducted on 3 consecutive days. Each rat was individually placed into the centre of the open field arena for a 5 min session. During observation period, the system recorded the locomotor activity of the rats and allowed the automated calculation of the number of crossings of the virtual borders between the squares (number of crossings), and the distance moved by the rats. The behavioural patterns of the rats, namely number of rearing, sniffing and grooming were registered by hand.

3.3.2. Anxiety

3.3.2.1. Anxiety in elevated plus maze test (EPM)

Anxiety state was analyzed in EPM test. The EPM apparatus was constructed of grey coloured wooden planks forming a cross. The equipment consisted of two opposite open arms (50×12 cm) and two opposite closed arms (50×12×40 cm) with 40cm high walls and an opened roof. The maze was elevated to a height of 100 cm above the floor. The experiment was performed in a sound attenuated, dimly illuminated room. The data recording and analyzing system was the same, as in the OPF task.

Animals were placed into the centre of the maze (central platform), facing one of the closed arms. Trials lasted for 5 min. During this period, the time spent in the two open arms, in the ends of the two open arms and in the two closed arms was monitored. The number of visits to the open and closed arms was also analyzed. Anxiolytic effect was defined as an increased time spent or visiting number of an open terrain.

3.3.2.2. Anxiety in open field test

The ground of the OPF apparatus was virtually divided into 16 squares. The central zone consisted of the central 4 quadrants and the peripheral zone the peripheral 12 quadrants. Elevated time spent in the central zone indicated an anxiolytic effect.

3.3.3. Prepulse inhibition (PPI) of the acoustic startle reflex (ASR)

PPI testing was performed in a dimly illuminated and sound attenuated room. The startle apparatus consisted of a startle chamber containing a transparent plexiglas box (24x14x14 cm) mounted on a force transducer (Aluminium Single-Point Load Cell, Model 1004, Vishay Precision Group, Malvern USA). A speaker, hanged 24 cm above the box provided the acoustic noise pulses. The output of the transducer was attached to 1 channel of a 32 channelled preamplifier. This was connected to the 64-channel low voltage AD converter (LVC-64, Noted Bt., Pécs, Hungary). Events were recorded through the event input of the LVC-64. The experimental session consisted of the following stimuli: 1. startle pulse alone trials (SA), which comprises of a single noise pulse (120 dB, 20 ms), 2. prepulse + startle pulse trials (PS) including a prepulse noise (75 dB, 20 ms) followed by the noise pulse (120 dB, 20 ms) with 100 ms interval, 3. prepulse alone (PA), 4. no stimulus (NS) trials, when solely a background white noise (50 dB) was presented.

One day before starting the test rats were one by one placed into the startle chamber for 3 minutes whilst the background noise (50 dB white noise) was presented (habituation). On the next day, the experimental session began with 5 min acclimation period of 50 dB background noise, which was maintained throughout the session. Then 5 habituation SA trials were presented, which were not implicated in the calculation of the PPI values. This was followed by 20 blocks of 4 trial types: (SA, PS, PA, NS). The order of stimuli inside the block was pseudorandom. The interval between trials was 15±5 s. The entire test session lasted about 16.5 min.

High pass filtered (10 Hz) data were analysed off-line using shell scripts written in Linux and Matlab [45]. Whole-body startle responses of the animal in response to acoustic stimuli resulted in a positive wave in the recorded force curve. Startle magnitude was defined as the peak of the startle wave in a 0-100 ms time window according to the onset of pulse stimulus and was expressed in arbitrary units.

PPI was calculated for each individual animal as the percent decrease of ASR by the help of the next formula: PPI% = [1− (startle amplitude on prepulse + startle pulse trial/startle amplitude on pulse alone trial)] * 100.

3.3.4. Motor coordination skills in rotarod test

Motor coordination skills were examined in rotarod apparatus (Ugo Basile 47700, Italy). It consisted of a rotating spindle of 6 cm diameter, divided into compartments for several animals. Rotating speed could be controlled and the time spent on the rolling rod was measured. The experiment was performed in a dimly illuminated room (40 W red light).

Rotarod test was carried out according to the protocol described by Rozas [46]. One day before the experiment, rats were taught to the task. They were placed on the roller turning by 5 rpm for 2 min. This procedure was performed three times with 2-3 hour intervals. On the test day rats were tested at 5, 10, 15, 20, 25, 30, 35, and 40 r.p.m. for a maximum of 300 s each speed, and the length of time that each animal was able to stay on the rod at each rotation speed was recorded.

3.3.5. Cognitive skills in radial arm maze (RAM)

Cognitive capabilities were investigated in RAM test. The apparatus was composed of 8 72x10 cm runways, numbered clockwise (1-8), with 5cm high walls around and an octagonal central platform with a 27 cm in diameter. The maze was elevated to a height of 52 cm above the floor. Each arm in its last tenth contained a food cup 1 cm deep and 5 cm in diameter. During the experiment 4 food pellets (chocolate cereal balls) were placed in 4 arms of the apparatus. Rats had to find the pellets and to memorize those localities during the learning process. The orientation of the rats was aided by intra-maze and extra-maze cues. Experiments were carried out in a sound attenuated, dimly illuminated room. The data recording and analyzing system was the same, as in the OPF task.

In the course of the test period, animals were food deprived and were only allowed to eat during the task followed by a 1.5 hours ad libitum feeding period. On the first day animals were placed into the apparatus for 5 min without reward to explore the maze and habituate to the experimental conditions. On the next day (first conditioning day) rats had to learn to search the food in the maze. Four food pellets were placed into the food cups of 4 arms (arms 1, 3, 5, and 7) and rats were given 10 minutes to find them. After this, rats had to learn the location of the food. A maximum of 10 minutes were given for each animal in each trial to find the four pellets. Animals were trained as long as the performance of the control and MAM groups (measured with number of entries) did not improve anymore (criterion performance). Then the food pellets were placed into new locations (arms 1, 2, 4 and 5) and animals were required to learn the new positions (reverse conditioning paradigm). The experiment was continued, until the performance of the rats did not improve anymore.

The performance of the rats was expressed by the errors committed by them. The following errors were defined: Error I, entry into non-baited arms (in conditioning paradigm: arms 2, 4, 6 and 8, in reverse paradigm: arms 3, 6, 7 and 8); Error II, re-entry into those arms, which were already visited in the actual session; Error III, entry into non-rewarding arms, which were rewarding in the acquisition paradigm (arms 3 and 7). Error III could be only defined in the reverse paradigm.

3.4. Histology

At the end of experiments, rats received an overdose of urethane (i.p. injection of 20% urethane solution, in a dose of 1.4 g/kg bw.) and were transcardially perfused with isotonic saline followed by 10% formaldehyde solution. A week after fixation, brains were cut with the help of a freezing microtome into 60 μm serial sections and stained with Cresyl Violet. Structure and extent of the dorsal hippocampus and prefrontal cortex were analyzed in light microscope and compared to control brains by means of rat brain stereotaxic atlas [47].

Before histological preparations, the volume of the whole brains was measured with water displacement method [48]. Following this, the size of cerebrum was investigated: in anteroposterior direction the distance between anterior and posterior ends, and in mediolateral line the highest extent was measured. The area of the dorsal hippocampus was defined as the length of the mediolateral and dorsoventral segments at the level of the medial habenula, according to Moore and co-workers [21]. In case of the PFC, the thickness of the cortex was measured at the level of the rostral nucleus accumbens in the anterior cingular cortex (AC), prelimbic (PL) and infralimbic regions (IL). Brain slices were photographed with Nikon Optiphot-2 microscope, and processed with a Spot Advanced 3.5.2. Software.

3.5. Statistics

For analysis of OPF, stereotyped behaviour and rotarod results, all data were processed in a three-factor mixed ANOVA (MANOVA), with treatment (MAM vs. Vehicle) as between-subject factor and age-period (prepuberty, late puberty, adulthood) and experimental day (day1, day2, day3) as within-subject factors. Results of EPM and PPI were analyzed with mixed ANOVA, with treatment (MAM vs. Vehicle) as between-subject factor and age-period (prepuberty, late puberty, adulthood) as within-subject factor. Data from the RAM task were processed with two way ANOVA, with treatment (MAM vs. Vehicle) and age-period (prepuberty, late puberty, adulthood) as between-subject factors. For post hoc analysis, Bonferroni test was carried out. For analyzing the area of the hippocampus or the mPFC independent samples t-test was used. Statistical

significance level was established at $p<0.05$. In all of the graphs, data are presented as mean ± standard error of the mean (S.E.M.). * indicates the significance with post hoc test.

4. Results

No alteration was found in the gestational duration of MAM-injected dams, the birth was timed on 23rd-24th day, as in control rats. There was no difference in size, number or general state of offspring compared to controls (data not shown).

4.1. Neurological investigations

The investigation of muscular tone, coordination of limbs by grid walking and grasping, visual and proprioceptive placing reflexes and orientations toward visual stimuli revealed no disturbances in MAM-E17 rats and no significant differences were recorded considering data of these rats and controls (data not shown).

4.2. Behavioural experiments

4.2.1. Spontaneous locomotor activity in open field test

Analyzing data of OPF task, according to the number of crossings significant overall treatment effect was displayed (Fig.1A, $F=9.629$ $p=0.005$), which indicates increased locomotor activity of MAM-treated rats. Detailed analysis revealed that hyperlocomotion is present in late puberty ($F=10.029$ $p=0.003$), and adulthood ($F=17.812$ $p<0.001$), but not in prepuberty ($F=0.005$ $p=0.945$). Pairwise comparison of the groups on each of the days has shown no difference in prepuberty (day1 $F=0.693$ $p=0.406$; day2 $F=0.005$ $p=0.944$; day3 $F=0.385$ $p=0.536$), but there was significant difference in late puberty (day1 $F=4.025$ $p=0.047$; day2 $F=3.957$ $p=0.049$; day3 $F=6.199$ $p=0.014$) and adulthood (day1 $F=7.759$ $p=0.006$; day2 $F=9.408$ $p=0.003$; day3 $F=7.785$ $p=0.006$) on each of the days. There was an age effect ($F=84.998$ $p<0.001$), and a treatment x age interaction ($F=8.721$ $p<0.001$), which means that hyperlocomotion observed in MAM-treated rats depends on the age. A day effect ($F=25.982$ $p<0.001$), moreover an age x day interaction ($F=16.253$ $p<0.001$) was evident. Post hoc analysis revealed a decreasing activity from day to day in prepuberty (day1-day2 $p=0.005$; day1-day3 $p<0.001$; day2-day3 $p<0.001$), indicating the phenomenon of habituation, which was similar in both groups.

The overall statistical analysis revealed no effect of MAM-exposition on the grooming behaviour (Fig.1B, $F=0.972$ $p=0.335$). However a significant age effect ($F=23.623$ $p<0.001$), day

effect (F=8.184 p<0.001), moreover an age x day interaction (F=2.121 p=0.080) was detected. Post hoc analysis revealed an elevation in the number of grooming over the days of prepuberty (day1-day3 p=0.017), moreover in late puberty (p<0.001); and adulthood (p<0.001) compared to prepuberty. This phenomenon is similar to that observed in the number of crossing parameter interpreted as habituation.

In case of rearing behaviour no difference was present between the MAM-treated and control groups (Fig.1C, F=0.043 p=0.838), however a significant age (F=113.368 p<0.001) and day (F=23.982 p<0.001) effect was found, moreover an age x day interaction (F=17.861 p<0.001). The decreasing rearing activity from day to day in prepuberty (p<0.05), as well as in late puberty (p<0.001) and adulthood (p<0.001) compared to that of prepuberty is reminiscent of the previous habituation pattern.

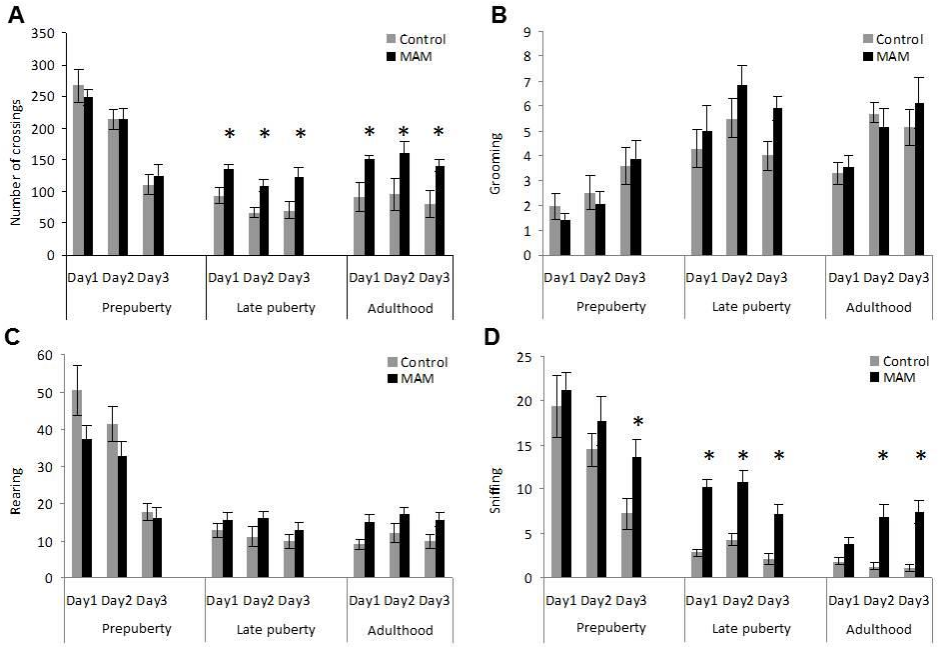

Fig. 1. Effect of MAM-E17 treatment in open field test on the number of crossings (panel A), and on the different stereotyped behaviours (panel B-D). Columns represent the average (±S.E.M.) on three experimental days in three age-periods. Gray columns: control and black columns: MAM-treated group. * P<0.05 indicates significant differences. For further explanation, see the text.

MAM-treated group displayed a significantly elevated sniffing behaviour (Fig.1D, F=13.161 p=0.002) and according to post hoc analysis this difference was present in all of the three age-periods (in prepuberty F=5.372 p=0.025; in late puberty F=14.232 p<0.001, in adulthood F=7.271 p=0.010). There was also an age- (F=108.972 p<0.001) and day effect (F=9.005 p<0.001), however, no treatment x age interaction was found (F=1.086 p=0.340). The group difference was present on each of the days of late puberty (day1 F=9.411 p=0.003; day2 F=7.547

p=0.007; day3 F=4.433 p=0.037) and adulthood, except for the first day of adulthood (day1 F=0.529 p=0.468; day2 F=5.221 p=0.024; day3 F=7.005 p=0.009), moreover it was also shown on the 3rd day of prepuberty (day1 F=0.649 p=0.422; day2 F=1.914 p=0.169; day3 F=7.165 p=0.008). A significant decrease in the activity was to observe in prepuberty over the days (day1-day2 p=0.013; day1-day3 p<0.001; day2-day3 p=0.001), and between the age-periods (prepuberty-late puberty p<0.001; prepuberty-adulthood p<0.001; late puberty-adulthood p=0.003), which can be again the sign of habituation.

4.2.2. Anxiety

4.2.2.1. Anxiety in elevated plus maze test

Time spent in the open arms was elevated in MAM-exposed rats (Fig.2A, F=9.806 p=0.003). An age effect (F=157.179 p<0.001) and a treatment x age interaction (F=4.690 p=0.012) was also found. Pairwise comparison revealed that the group difference was present only in prepuberty (F=18.693 p<0.001). There was a decrease in late puberty (p<0.001) and adulthood (p<0.001) compared to prepuberty.

The number of entries into the open arms was significantly elevated in the MAM-treated group (Fig.2B, F=11.019 p=0.002). A significant age effect (F=67.898 p<0.001), as well as a treatment x age interaction was also displayed (F=3.715 p=0.028). Post hoc test showed, that the group difference was present in prepuberty (F=15.498 p<0.001) and adulthood (F=4.081 p=0.045), but not in late puberty (F=0.059 p=0.808). Decreased activity was displayed in late puberty (p<0.001) and adulthood (p<0.001) compared to prepuberty.

4.2.2.2. Anxiety in open field test

MAM-treated rats spent significantly more time in the central zone in comparison to control rats (Fig.2C, F=10.056 p=0.005). There was also an age effect (F=30.472 p<0.001), and a treatment x age interaction (F=4.680 p=0.011), which means, that the appearance of treatment effect depends on the age-period. Post hoc analysis displayed, that the difference between the two groups was present in prepuberty (F=17.781 p<0.001) and adulthood (F=4.111 p=0.046), but not in late puberty (F=0.248 p=0.620). Pairwise comparison unfolded elevated time spent in the central zone in MAM-treated group on the 2nd (F=8.484 p=0.004) and 3rd (F=16.021 p<0.001) day of prepuberty, but in adulthood there was no difference on single days (day1 F=0.601 p=0.439; day2 F=2.565 p=0.111; day3 F=2.177 p=0.142). There was a decrease in late puberty (p<0.001) and adulthood (p<0.001) compared to prepuberty.

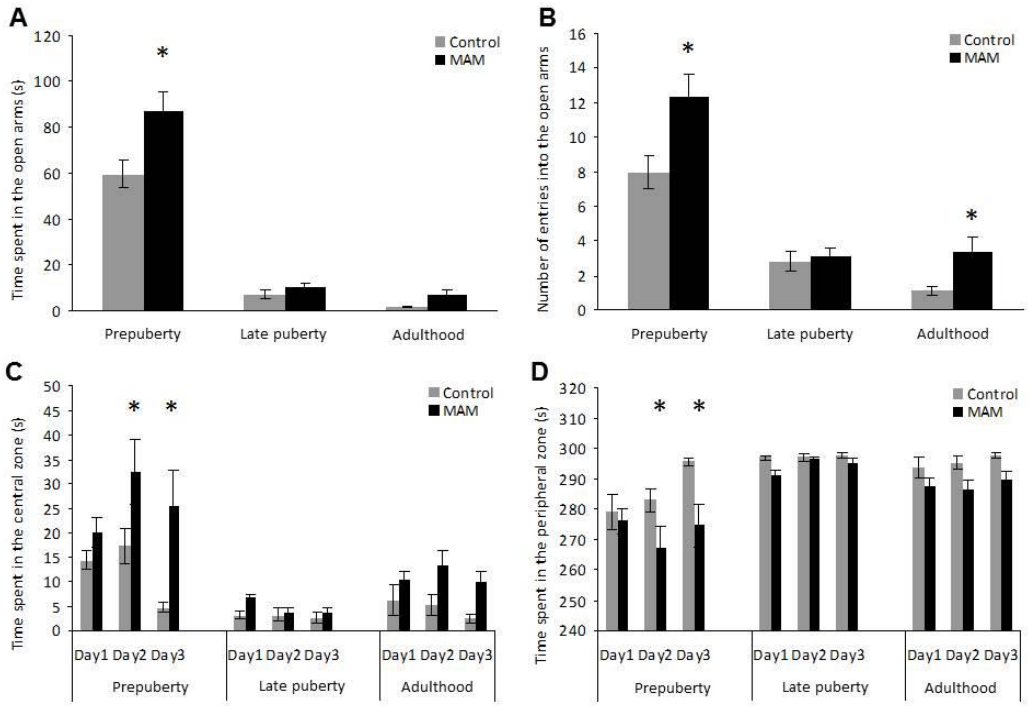

Fig. 2. Effect of MAM-E17 treatment on anxiety in EPM (panel A-B) and in OPF tests (panel C-D). Columns represent the average (±S.E.M.) on one experimental day in three age-periods in EPM task and on three experimental days in three age-periods in OPF test. Gray columns: control and black columns: MAM-treated group. * P<0.05 indicates significant differences. For further explanation, see the text.

Overall analysis displayed decreased time spent in the peripheral zone by the MAM-exposed rats (Fig.2D, F=11.247 p=0.003). An age effect was present (F=29.239 p<0.001), but there was no interaction (F=2.608 p=0.077). Analyzing the age-periods separately, post hoc test revealed difference between the two groups in prepuberty (F=14.055 p<0.001) and in adulthood (F=4.733 p=0.032). Pairwise comparison displayed difference in prepuberty on the 2nd (F=7.156 p=0.008) and 3rd day (F=13.547 p<0.001), but not in adulthood. There was an increase in late puberty (p<0.001) and adulthood (p<0.001) compared to prepuberty.

4.2.3. Prepulse inhibition of the acoustic startle reflex

Prepulse inhibition of the control rats exhibited an average of 40-60%, in all of the age-periods (Fig.3.). In contrast MAM-treated rats displayed PPI deficit in both late puberty and adulthood (~40% PPI). ANOVA revealed an overall group difference (F=7.497 p=0.012), and a treatment x age interaction (F=3.742 p=0.032). Age effect was, however, not evident (F=0.168 p=0.846). Post hoc analysis showed that the group difference was present in late puberty (F=6.456 p=0.014) and adulthood (F=11.787 p=0.001), but not in prepuberty (F=0.002 p=0.962).

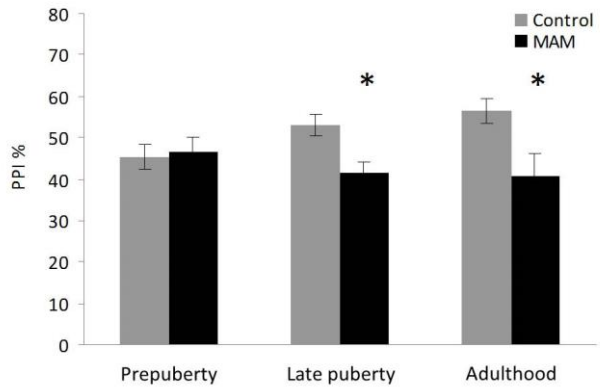

Fig. 3. Effect of MAM-E17 treatment on prepulse inhibition. Columns represent the average PPI% (±S.E.M.) on one experimental day in three age-periods. Gray columns: control and black columns: MAM-treated group. * P<0.05 indicates significant differences. For further explanation, see the text.

4.2.4. Motor coordination skills in rotarod test

MAM-treated rats spent significantly more time on the rotarod (Fig.4, F=13.638 p=0.001). There was an age effect (F=128.252 p<0.001), and a treatment x age interaction (F=6.045 p=0.002), which demonstrated the age dependency of treatment effect. According to this, the elevated performance was present in late puberty (F=10.424 p=0.002) and adulthood (F=21.606 p<0.001), but not in prepuberty (F=2.442 p=0.122). There was an overall speed effect (F=29.180 p<0.001) and an age x speed interaction (F=6.273 p<0.001), which means, that the time spent on the rotarod at each of the speed values depends on the age-period. A treatment x age x speed interaction (F=1.819 p=0.032) was also displayed. Pairwise comparison detected differences in late puberty at 5rpm (F=6.026 p=0.014) and in adulthood (5rpm F=4.893 p=0.027; 10rpm F=5.764 p=0.017; 15rpm F=5.155 p=0.024; 20rpm F=6.316 p=0.012; 25rpm F=10.911 p=0.001; 30rpm F=5.056 p=0.025; 35rpm F=4.304 p=0.038; 40rpm F=3.914 p=0.048). There was significant decrease in the performance between the age-periods (p<0.001).

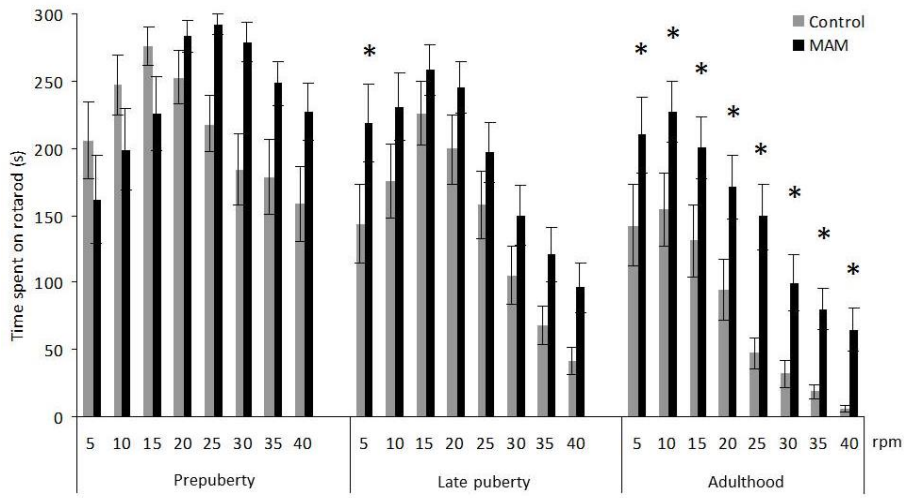

Fig. 4. Effect of MAM-E17 treatment on time spent on rotarod. Columns represent the average performance (±S.E.M.) at the single rotating speed values (rpm) on one experimental day in three age-periods. Gray columns: control and black columns: MAM-treated group. * P<0.05 indicates significant differences. For further explanation, see the text.

4.2.5. Cognitive skills in radial arm maze

In case of Error I overall analysis carried out by ANOVA revealed a significant treatment effect in the conditioning phase (Fig.5A, $F_{1,67}$=6.650, p=0.012), indicating that MAM-E17 rats committed more errors. Pairwise comparison of the data of the two groups in the single age-periods confirmed the significant difference only in puberty ($F_{1,67}$=6.263, p=0.015). No age effect ($F_{2,67}$=2.402, p=0.098) and no treatment x age interaction was detected ($F_{2,67}$=1.038, p=0.360). In the reverse paradigm, MAM-treated rats made significantly more errors in comparison to control rats (Fig.5B, $F_{1,68}$=64.603, p<0.001). Pairwise comparison of the data of MAM-treated and control groups in the single age-periods indicated diminished performance of MAM-E17 rats in prepuberty ($F_{1,68}$=19.717, p<0.001), puberty ($F_{1,68}$=36.284, p<0.001) and adulthood ($F_{1,68}$=11.237, p=0.001), as well. There was a significant age effect ($F_{2,68}$=12.332, p<0.001) but no treatment x age interaction ($F_{2,68}$=2.365, p=0.102). Pairwise comparison of the data of each of the age-groups revealed significant differences: both control and MAM-treated groups made more mistakes in prepuberty (p=0.016) and puberty (p<0.001) compared to adulthood. This means that the performance of the rats is influenced by their age-period.

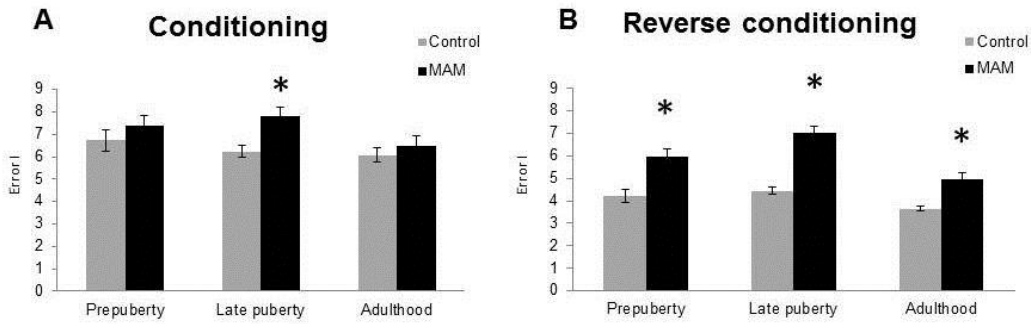

Fig.5. Effect of MAM-E17 treatment on the number of Error I in RAM task. Columns represent the average of the errors (±S.E.M.) in 11 conditioning (A) and 8 reverse conditioning (B) in three age-periods. Gray columns: control and black columns: MAM-treated group. *p<0.05 indicates significant differences. For further explanation, see the text.

In case of Error II, in the conditioning phase ANOVA revealed a significant treatment effect (Fig.6A, $F_{1,64}$=6.475, p=0.013), which means that in MAM-treated rats made more errors. Pairwise comparison of the data of the two groups in the single age-periods showed, that the difference only appeared in puberty ($F_{1,64}$=4.041, p=0.049). However, no age effect ($F_{2,64}$=2.095, p=0.131) and no treatment x age interaction was detected ($F_{2,64}$=0.468, p=0.628). In reverse paradigm, MAM-treated rats committed more errors similarly to previous results (Fig.6B, $F_{1,64}$=37.948, p<0.001). Pairwise comparison of data of the groups in the single age-periods indicated that diminished performance of MAM-E17 rats was present in prepuberty ($F_{1,64}$=14.805, p<0.001), puberty ($F_{1,64}$=16.462, p<0.001) and adulthood ($F_{1,64}$=7.369, p=0.009), as well. In this case however, neither age effect ($F_{2,64}$=3.009, p=0.056), nor treatment x age interaction ($F_{2,64}$=0.934, p=0.398) were found.

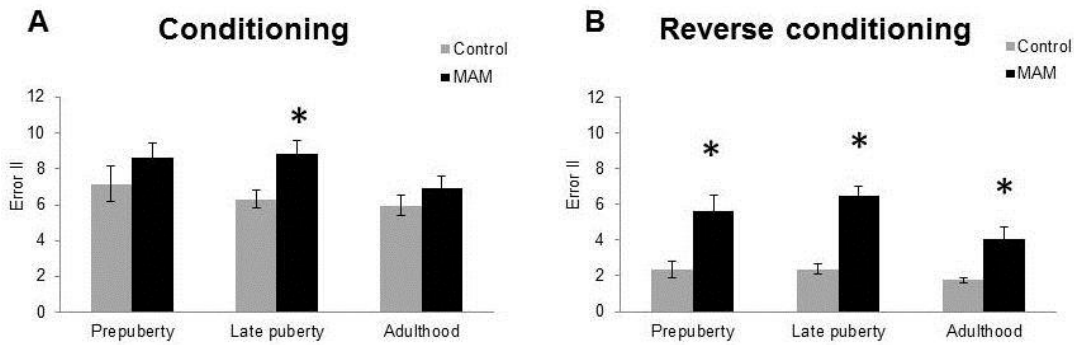

Fig.6. Effect of MAM-E17 treatment on the number of Error II in RAM task. Columns represent the average of the errors (±S.E.M.) in 11 conditioning (A) and 8 reverse conditioning (B) in three age-periods. Gray columns: control and black columns: MAM-treated group. *p<0.05 indicates significant differences. For further explanation, see the text.

Analysis of Error III in the reverse paradigm revealed a significantly higher rate of errors in MAM-E17 rats (Fig.7, $F_{1,68}$=33.792, p<0.001). Pairwise comparison of data of the groups in the single age-periods confirmed the group difference in prepuberty ($F_{1,68}$=9.153, p=0.004), puberty ($F_{1,68}$=20.537, p<0.001), as well as in adulthood ($F_{1,68}$=5.858, p=0.018). There was a significant age effect ($F_{2,68}$=7.324, p=0.001), however no treatment x age interaction was present ($F_{2,68}$=1.522, p=0.226). According to pairwise comparison of the data of each of the age-groups both groups made more errors in prepuberty (p=0.079) and puberty (p=0.001) compared to adulthood, indicating, that they had the highest performance in adulthood.

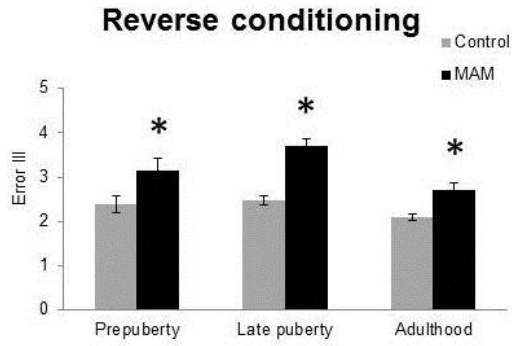

Fig.7. Effect of MAM-E17 treatment on the number of Error III in RAM task. Columns represent the average of the errors (±S.E.M.) in reverse conditioning in three age-periods. Gray columns: control and black columns: MAM-treated group. *p<0.05 indicates significant differences. For further explanation, see the text.

4.3. Histology

The total brain volumes of MAM-E17 rats were smaller (1.74 ± 0.02 ml (n=33)); than those of control rats (1.83 ± 0.03 ml (n=33)), and this difference was significant (Independent Samples Test, t=2.926, p=0.005). Analyzing the lengths of the brains significant differences were shown, as well. Anteroposterior length in control rats was 14.72 ± 0.08 mm (n=36), but in MAM-E17 rats was 13.66 ± 0.09 mm (n=34); (Independent Samples Test, t=8.753, p<0.001). Mediolateral widths in control rats was 14.64 ± 0.07 mm (n=36), while in MAM-treated rats was 14.21 ± 0.07 mm (n=33); (Independent Samples Test, t=4.318, p<0.001).

The histological analysis displayed striking alterations in case of the hippocampus (Fig.8.). In the MAM-E17 treated group a significant volume reduction was observed in the dorsal hippocampus both in mediolateral [Fig.8A,E, Independent Samples Test, t=4.994 p<0.001], and in dorsoventral direction [Fig.8A,E, Independent Samples Test, t=3.057 p=0.014]. Besides the volume reduction, disarray of pyramidal layer was observable (Fig.8F-H). Instead of the integrated compact structure, a disperse cell location and heterotopias were to note. A great number of

pyramidal cells were pushed into the stratum oriens and stratum radiatum from CA1 to CA3 region.

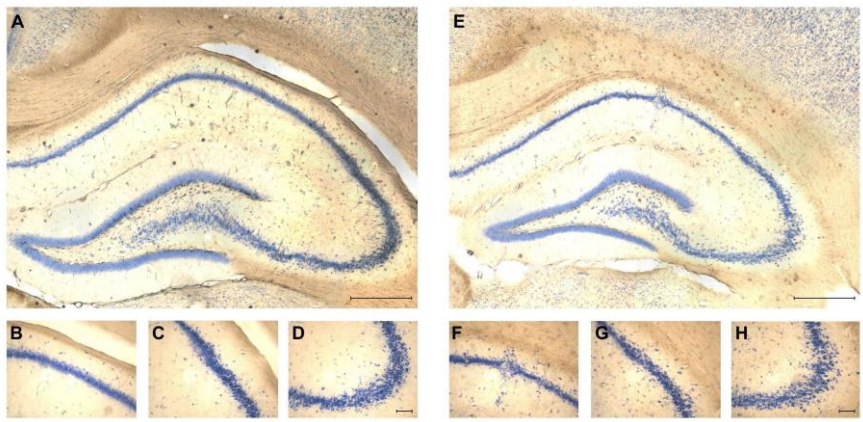

Fig. 8. Effect of MAM-E17 treatment on dorsal hippocampal structure. Panel A represents the montage and panel B-D the fine stucture of pyramidal layer of hippocampus of a control rat. Panel E displays the montage and panel F-H the fine stucture of pyramidal layer of hippocampus of a MAM-E17 treated rat. Scale bar 500 μm (A and E), and 100 μm (B-D and F-H). For further explanation, see the text.

In case of the mPFC there was no significant alteration in the MAM-treated brains compared to control brains (In MAM treated rat brains mean thickness of anterior cingular cortex (AC) was 1957.5 ± 72,56 μm, of prelimbic cortex (PL) was 1250 ± 47.43 μm, and of infralimbic cortex (IL) was 1050 ± 53.62 μm; n=10. In control rats AC was 2079.17 ± 75,94 μm, PL was 1304.167 ± 26.15 μm, and IL was 1112.5 ± 26.42 μm; n=6.). However, 6 brains of MAM-E17 rats displayed diminished cortical thickness in comparison to the average values of control brains.

5. Discussion

5.1. Neurological investigations

In the present experiments, no differences were observed regarding the time of pregnancy, offspring size, or litter size. Neurological tests revealed that MAM-E17 rats do not display any muscular tone- or motor deficiencies. In the literature there are mostly similar data to ours [16, 21, 22, 27, 29].

5.2. Behavioural experiments

5.2.1. Spontaneous locomotor activity and stereotyped behaviour

MAM-E17 rats showed significantly increased locomotor activity with pubertal onset, which remained there in adulthood. The increased locomotor activity is proposed to be equivalent to positive symptoms of schizophrenia [49], and similarly to those it first appears only in late puberty [50]. Day-to-day decreasing activity in prepuberty both in MAM-treated and control animals refers to habituation. Previous publications confirm our data [22, 25, 51, 52].

Analyzing stereotyped behaviour in late puberty and adulthood MAM-treated rats displayed significantly increased frequency of sniffing, as well as tendentiously the number of rearing was also elevated. According to our knowledge, there is only one relevant publication reporting an increase in orofacial stereotyped behaviour in adult MAM-exposed rats [21]. In our results the locomotor activity, as well as the stereotyped behaviours displayed similar elevation, with similar age-pattern. The observed phenomenon can be due to a **complex explorative-orientative behaviour, which reflects the elevated responsiveness to the environmental, particularly proximal stimuli**.

5.2.2. Anxiety

Both in EPM and OPF task an anxiolytic effect of MAM-treatment has been observed with an interesting temporal pattern: the anxiolytic effect, which was present in prepuberty, disappeared in late puberty and returned in some extent in adulthood. This phenomenon is likely due to the pubertal maturational processes, which also contributes to the emergence of several schizophrenic symptoms. **In summary, we can not conclude that MAM treated rats suffered from anxiety.**

Based on the human studies, rather an elevated anxiety could have been expected [53]. In spite of this, previous studies investigating anxiety state of MAM-E17 rats reported about contradictory results for both the EPM [54, 55], and OPF tests [22, 25, 51]. **Our results are strongly supported by the fact that in two different paradigms consistently the same behaviour, i.e. diminished anxiety was to observe. Additionally the age pattern of the anxiolytic behaviour was the same in both tasks.**

5.2.3. Prepulse inhibition of the acoustic startle reflex

PPI of MAM-E17 rats did not differ from control animals prior to puberty, however a diminished PPI was manifested in late puberty, which remained there in adulthood. This is in

line with the results of other research groups [15, 21, 25, 51]. Sensorimotor gating mechanisms ensure that from the loads of incoming sensory information solely the substantial stimuli are allowed to evoke a motor response. Patients with schizophrenia suffer from the impairment of these mechanisms [56]. In this study, the age-pattern of PPI disruption resembles to that of the explorative behaviour. Accordingly it can be assumed, that the **increased explorative activity and the impaired PPI are not independent on each other. A decrease in the filtering function indicated by a decrease in PPI may result in an increased responsiveness to environmental stimuli, which can be reflected by the increased exploratory activity.**

5.2.4. Motor coordination skills

The results of the rotarod test show that MAM-exposed rats stay significantly more time on the roller, apart from an initial period in prepuberty, compared to the control animals. Basically, rotarod test is proposed for the evaluation of motor skills [46], however, the performance is also influenced by motivational factors and motor adaptation/learning capability. It can be supposed, that the decreased performance during the initial phase, which is supported by the only existing literary data [16], can be due to learning-adaptation impairment. This is supported by neurological examinations, which demonstrated that MAM-treated animals do not have any motor deficit. Nevertheless, in our experiments, the initially decreased performance of the MAM-E17 rats gradually became increased compared to controls, and remained steady/increased throughout the experiment, in all age-periods. Hyperactivity seems to be the most likely explanation for this increased performance which is also supported by the results of the OPF test.

5.2.5. Cognitive skills

RAM task is a complex cognitive task, that is accepted by the NIMH's "Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia" (CNTRICS) initiative [57, 58]. To accomplish the RAM task the animals can apply different learning strategies: 1. The presence of intra-maze cues ensures the associative learning. 2. Spatial navigation and place learning is promoted by extra-maze cues. 3. A third possibility is the track learning [59], which means the learning of the food position pattern. The different error types in animal performance refer to different cognitive malfunctions [60].

Error I (visiting of non-baited arms) can indicate both long-term (if rats do not remember where the food pellets have to be) and working memory disturbances (if rats do not remember

where they already have been in the actual session) [60]. Error II (re-entry into already visited arms) refers to working memory deficit. **The results of Error I and Error II indicate that in the conditioning phase the performance of MAM-treated rats in prepuberty and adulthood is appropriate, indicating intact learning capabilities and working memory. In contrast, in puberty and in the reverse paradigm cognitive impairment was observed in all age groups of the animals.** Because of the similar results of Error I and Error II (in both phases), and since Error II indicates working memory only, we can suppose, that long-term memory is not affected by MAM-treatment. Accordingly, as far as we know, long-term memory deficits of MAM-exposed rats were not described in the literature yet.

Studies, carried out on MAM-E17 rats at different age-periods generally indicate impaired spatial working memory [51, 61]. Gourevitch et al., however, achieved similar results to ours in RAM test on adult rats, but with another protocol [29]. That experiment showed that the **working memory deficit** observed in MAM-treated rats is **condition-dependent**, i.e. the enhancement of the delay between the 2 phases of their experiments revealed it, while in our present experiments the deficit was induced by the altered position of the rewards during reverse paradigm.

Based on their performance during the conditioning phase the animal's spatial navigation skills are appropriate in prepuberty and adulthood. However the literature suggest that the opposite is true for all examined age-periods [51, 55, 62, 63].

In addition to spatial navigation, rats had another opportunity to accomplish the RAM task, namely the associative learning. This possibility was supported in a rewarding paradigm, similar to ours [21], while questioned in a punishment-based task [28]. A further potential explanation for the reduced performance of MAM-E17 rats is attention deficit, which is supported by numerous studies [28, 32, 64], though not all [65].

Comparing the number of errors in acquisition and reverse phase, control rats show improvement; while MAM-treated rats do not. **Presumably, the more serious working memory deficit observed in the MAM-E17 rats in reverse paradigm can indicate the incapability of following the new rule, which signifies reversal learning deficiency and generally a disturbance in behavioural/learning flexibility** [66]. This explanation is supported by our results of Error III (entering into non-baited arms in reverse paradigm, which were previously baited in conditioning paradigm) that also significantly increased in MAM-E17 rats in each of the age-periods. The elevated number of Error III means, that rats do not remember the new position of food pellet, or they are more adhered to the formerly baited arms (this is called perseveration). Numerous data in the literature support behavioural inflexibility of MAM-treated rats in different age-periods [21, 27, 28, 51, 61, 67, 68].

Cognitive disturbances of MAM-E17 rats may be temporarily more intensive during puberty, indicating increased sensitivity in this period. This may be related to the fact, that most of the disturbances induced by prenatal MAM-treatment also appear only in puberty, when ongoing brain maturational processes interfere with the pre-existent anomalies, similarly to schizophrenia [8, 69].

In addition, diminished performance in conditioning phase in puberty could be related to elevated anxiety as well, however elevated anxiety was not observed in our previous experiments on MAM-treated rats [70]. **The age-pattern of performance by MAM-treated rats in conditioning phase is parallel to that of anxiety:** anxiety was diminished in prepuberty and adulthood, but it was the same as in the control group in puberty. Since anxiety reduces cognitive performance, diminished anxiety can lead to better performance in prepuberty and adulthood.

Numerous data in the literature can be found about cognitive deficiencies in schizophrenia, namely, long-term memory disturbance [71], working memory disruption [71-73], deficient reversal learning [74], as well as perseverative behaviour [75].

5.3. Histology

The histological analysis following our experiments revealed a reduction in the brain size of MAM-E17 rats in accordance with previous studies [21, 22, 26]. **In the dorsal hippocampus in addition to the volume reduction cell dispersion and heterotopias were observed in the CA1-CA3 region**, similarly to data of literature [22, 25, 26, 28-30]. In case of the prefrontal cortex some of the MAM-E17 brains displayed diminished thickness, however in total, there was no significant reduction in comparison to the control group, while previous findings demonstrated it [21, 28]. Similar neuropathological abnormalities are frequently observed in human schizophrenic brain [6, 22, 37, 76].

This study is the first in a series of experiments that - focusing on the pubertal development of schizophrenia-like symptoms - follows the changes of behavioural parameters from prepuberty to adulthood on the same animals. In our studies age-related appearance patterns of schizophrenia-like symptoms and histological damage were demonstrated. Based on these findings, the model appears to be a useful implement in experimental studies of various drug targets, which may bring forward clinical research for the preventive treatment of patients.

6. Summary

The following results were achieved according to the examined objectives:

1. Investigating general activity and sensorimotor gating mechanisms:
   a. MAM-E17 rats showed the increase of locomotor activity and higher frequency of stereotyped behaviours with pubertal onset, which remained there in adulthood. This can be paralleled with pubertal onset of the positive symptoms of schizophrenia.
   b. PPI of MAM-E17 rats did not differ from control animals prior to puberty; however a diminished PPI was manifested in late puberty, which remained there in adulthood.
   c. Hyperactivity and enhanced stereotyped behaviour can be due to a complex explorative-orientative behaviour, which reflects the elevated responsiveness to the environmental, particularly proximal stimuli. This may be due to the disturbance of sensorimotor gating mechanisms, also indicated by PPI deficit.
2. Investigating motor coordination skills:
   MAM-E17 rats remained significantly longer on the roller during puberty and adulthood comparing to control rats, indicating, that their motor coordination skills are intact. Increased performance can be the result of hyperactivity.
3. Investigating anxiety:
   The anxiety state of MAM-E17 rats showed an interesting temporal pattern: the anxiolytic effect, which was present in prepuberty, disappeared in late puberty and reappeared in adulthood.
4. Investigating cognitive capabilities:
   a. Based on the performance of the MAM-E17 rats in the conditioning paradigm learning capabilities, long-term memory and working memory are appropriate in prepuberty and adulthood. However diminished performance in reverse paradigm indicates working memory deficit. Limiting the disturbance to the reverse paradigm may indicate that working memory deficit is condition-dependent.
   b. In reverse paradigm the incapability of following the new rule observed in the MAM-E17 rats, signifies reversal learning deficiency and generally a disturbance in behavioural/learning flexibility.
   c. Working memory deficit and deficient behavioural flexibility was present in all age-periods.
   d. In the conditioning paradigm the number of errors increases in puberty. Accordingly cognitive disturbances of MAM-E17 rats may be temporarily more intensive during puberty, indicating increased sensitivity in this period. The age-pattern of cognitive performance by MAM-treated rats in conditioning phase is parallel to that of anxiety.
5. Histological findings:
   The histological analysis revealed a reduction in the whole brain and cerebrum volume of MAM-E17 rats in accordance with previous studies. In the dorsal hippocampus in addition to the volume reduction cell dispersion and heterotopias were observed in the CA1-CA3 region.

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8. List of publications

The full publication list can be found:
https://m2.mtmt.hu/gui2/?type=authors&mode=browse&sel=10025904

8.1. Publications related to the thesis

- **Kállai V**, Tóth A, Gálosi R, Szabó I, Petykó Z, Karádi Z, Kállai J, Lénárd L. (2015) A MAM-E17 skizofrénia patkány modell (MAM-E17 schizophrenia rat model) *Psychiatr Hung;30(1):4-17.* ***(Q4)***
- **Kállai V**, Tóth A, Gálosi R, Péczely L, Ollmann T, Petykó Z, László K, Kállai J, Szabó I, Karádi Z, Lénárd L (2017): The MAM-E17 schizophrenia rat model: Comprehensive behavioral analysis of pre-pubertal, pubertal and adult rats *Behav Brain Res*; 332:75-83. ***(IF: 3.173, Q1)***
- **Kállai V**, Lénárd L, Péczely L, Tóth A, Gálosi R, Petykó Z, László K, Kállai J, Szabó I, Karádi Z, Ollmann T. Cognitive performance of the MAM-E17 schizophrenia model rats in different age-periods. Behavioural Brain Research. In press.
  Q1*, [IF: 2.770]*
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8.2. Other publications with impact factors

- Kőszegi Zs, Kovács P, Wilhelm M, Atlasz T, Babai N, **Kállai V,** Hernádi I (2006) The application of in vivo microiontophoresis for the investigation of mast cell–neuron interactions in the rat brain. *J. Biochem. Biophys. Methods, 69:227-231*
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- Ollmann T, Péczely L, László K, Kovács A, Gálosi R, Kertes E, **Kállai V**, Zagorácz O, Lénárd L (2015): Anxiolytic effect of neurotensin microinjection into the ventral pallidum. *Behav Brain Res 294:208-14.*
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Summarised impact factor: 42.101

8.3. Further publications

- Kovács P, Atlasz T, Kőszegi Zs, **Kállai V,** Molnár D, Hernádi I, Wilhelm M (2005) Changing estrogen level modifies mast cell-neuron interactions in the rat thalamus. *Magyar Idegtudományos Társaság (MITT) X., Pécs*

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- Tóth A, Petykó Z, Feldman Á, **Kállai V**, Gálosi R, Karádi K, Máthé K, Szabó I, Karádi Z, Lénárd L. (2014) Single unit analysis of auditory sensory gating in the medial prefrontal cortex (mPFC) in freely moving rats. *IBRO Workshop, Debrecen, január 16-17. p.: 95.*

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- **Kállai V**, Gálosi R, Tóth A, Petykó Z, Ollmann T, Péczely L, Kovács A, Karádi Z, Kállai J, Szabó I, Lénárd L. (2014) Magatartási vizsgálatok MAM-E17 skizofrén állatmodellen. *III. Interdiszciplináris Doktorandusz Konferencia, Pécs, április 15-17.* In: Kósa B, Springó Zs (szerk.): III. Interdiszciplináris Doktorandusz Konferencia absztraktfüzet, p. 249

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- Ollmann T, Péczely L, László K, **Kállai V,** Kovács A, Gálosi R, Kertes E, Zagoracz O, Karádi Z, Lénárd L. A ventralis pallidum neurotenzinreceptorainak szerepe a szorongás szabályozásában. In: *FAMÉ 2016.06.01-04: Magyar Farmakológiai, Anatómus, Mikrocirkulációs és Élettani Társaságok Közös Tudományos Konferenciája. Pécs, Magyarország,* Paper P.285.

- Péczely L, Ollmann T, László K, **Kállai V**, Kovács A, Gálosi R, Kertes E, Zagoracz O, Karádi Z, Lénárd L. A ventralis pallidum területén található D2 dopamin receptorok memória-stabilitásban játszott szerepének vizsgálata nem-tradicionális magatartási paraméterek segítségével. In: *FAMÉ 2016.06.01-04: Magyar Farmakológiai, Anatómus, Mikrocirkulációs és Élettani Társaságok Közös Tudományos Konferenciája. Pécs, Magyarország, P 2.95.*

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- **Kállai V**, Ollmann T, Péczely L, Gálosi R, Tóth A, Kovács A, Dusa D, Berta B, Kertes E, László K, Karádi Z, Kállai J, Szabó I, Lénárd L. A MAM-E17 skizofrénia patkánymodell: kognitív képességek vizsgálata 3 különböző életkorban. *Magyar Élettani Társaság, Szeged, Magyarország 2018. 06.27-30.*

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- Kertes E, László K, Péczely L, Ollmann T, **Kállai V,** Berta B, Lénárd L. Az amygdala centrális magjába és a globus pallidusba injektált substance P hatása a helytanulásra Morris water maze tesztben. *Magyar Élettani Társaság,* Szeged, Magyarország 2018. 06.27-30.

- Berta B, Kertes E, Péczely L, Ollmann T, **Kállai V,** Lénárd L. A ventromediális prefrontális kéreg szerepe az íz-preferenciában. *Magyar Élettani Társaság,* Szeged, Magyarország 2018. 06.27-30.