Pharmacology, Biochemistry and Behavior 99 (2011) 619–625

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Behavioral consequences of the mGlu5 receptor antagonist MTEP in immature rats

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ARTICLE INFO

Article history:
Received 1 December 2010
Received in revised form 2 June 2011
Accepted 6 June 2011
Available online 15 June 2011

Keywords:
MTEP
Sensorimotor performance
Exploratory behavior
Anxiety
Learning
Immature rats

ABSTRACT

High doses of mGluR5 antagonists have anticonvulsant effects in multiple seizure models in both adult and immature animals. Data on potential behavioral effects in immature animals are very scarce. The present study investigated whether an antagonist of mGluR5 3-((2-methyl-1,3-thiazol-4-yl)ethynyl)pyridine (MTEP) in doses proven to be anticonvulsant affects behavior in immature rats. Animals aged 12, 18 and 25 days received MTEP in doses of 20 and 40 mg/kg i.p. The sensorimotor performance was tested at 15 and 60 min after dosing. Locomotor–exploratory behavior was tested at 20 and 65 min after dosing. An elevated plus maze was used to examine an adaptive form of learning and anxiety-like behavior in 18- and 25-day-old rats at 15, 60 min and 24 h. MTEP slightly affected sensorimotor performance, regardless of age. In the open field test, MTEP decreased transiently locomotor–exploratory behavior but did not affect the habituation — a simple form of nonassociative learning. In the elevated plus maze, the drug did not impair transfer latency, an indicator of an adaptive form of learning and memory. An anxiolytic-like effect was observed at 60 min after drug administration. In conclusion, no severe impairment was observed after high anticonvulsant doses of mGlu5 antagonist MTEP in immature animals.

1. Introduction

Antagonists of mGluR5 are studied as a possible treatment in various diseases such as anxiety, depression, migraine and Parkinson (Gasparini et al., 2008). The predominant expression of mGluR5 in brain areas involved in emotional processes suggested a possible role of these receptors especially in affective disorders (for a review see Bordi and Ugolini, 1999; Gravius et al., 2010). Anxiolytic-like actions of both 2-methyl-6-(phenylethynyl) pyridine (MPEP) and 3-((2-methyl-1,3-thiazol-4-yl)ethynyl)pyridine (MTEP) were found in adult (Pietraszek et al., 2005; Varty et al., 2005) as well as in immature rodents (Hodgson et al., 2008; Mikulecká and Mareš, 2009).

Additionally, antagonists of mGluR5 subtype were also shown to have anticonvulsant effects in multiple seizure models in adult rodents (Thomsen and Dalby, 1998; Chapman et al., 1999, 2000). Clinical research demonstrated that approximately half of epilepsies start in infancy and childhood (Forsgren, 2004). Thus, we focused our attention on drugs acting at metabotropic glutamate receptors as potential anticonvulsants in immature rats. Our effort was substantiated by the fact that nearly one third of epileptic patients (including infants and children) are resistant to present pharmacotherapy, and many antiepileptic drugs exhibit unwanted side effects (Shorvon, 2010). In a previous study we showed that the antagonist of mGluR5, MPEP, suppressed PTZ-induced convulsions and cortical epileptic afterdischarges (Mareš and Mikulecká, 2004; Lojková and Mareš, 2005). The more specific mGluR5 antagonist MTEP (it is three orders more active on mGluR5 than on NMDA receptors — Cosford et al., 2003) exhibits an anticonvulsant action in the same two models of epileptic seizures in immature rats. Anticonvulsant effects decreased with age: doses of 20 and 40 mg/kg were active in 12- and 18-day-old rats, whereas only the 40-mg/kg dose was partly efficient in 25-day-old animals (Mareš, 2009; Lojková-Janečková et al., 2009).

The majority of studies performed in adult rodents assessing both beneficial and unfavorable effects of MTEP used low doses (Pietraszek et al., 2005; Simonyi et al., 2005; Varty et al., 2005); markedly higher doses of both mGlu5 antagonists were required to demonstrate anticonvulsant action in immature rats (Mareš and Mikulecká, 2004; Mareš, 2009). Therefore, the possible harmful side effects of such high doses of the mGluR5 antagonist have to be carefully considered. The present study was designed to determine the effects of an mGluR5 antagonist (MTEP) on different aspects of behavior in immature rats using doses that were shown to have anticonvulsant effects in our models of epileptic seizures. Therefore, we exposed animals to three age-appropriate behavioral paradigms: (a) sensorimotor tests to assess motor abilities (Mikulecká and Mareš, 2002; Mareš, 2009), (b) open field (OF) to assess locomotor–exploratory behavior and habituation potency (Cerbone and Sadile, 1994; Mikulecká and Mareš, 2009), and (c) an elevated plus maze (EPM) to measure an adaptive form of spatial memory and anxiety-like behavior (Itoh et al., 1990; Mikulecká et al., 2000).

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doi:10.1016/j.pbb.2011.06.007

2. Materials and methods

2.1. Animals

Experiments were performed in three age groups of male Wistar rats: 12, 18, and 25 days old. These age groups were chosen to correspond to early postnatal infants (12 days), preschool children, and early school children (18 and 25 days — Clancy et al., 2007). The animals were housed in a room with controlled temperature (22 ± 1 °C) and humidity (50–60%) and with a 12/12 hour light regime (lights on at 6:00 AM). The animals were brought to the experimental room 1 h before testing. To control for the possible confounding litter effects, a single rat from each litter was assigned to an individual group. Each experimental animal had a matched control from the same litter. To test motor performance and locomotor–exploratory behavior, all three age groups were used. Each age group consisted of controls and two groups treated with MTEP. Ten animals per group aged 12 days were used. For the 18-day-old rats, the control group consisted of 8 animals and both groups treated with MTEP contained 10 animals. For the 25-day-old rats, both MTEP treated groups consisted of 6 animals that were compared to 8 controls. To test locomotor–exploratory behavior, at each age, 10 controls and 10 MTEP treated animals for each dose group were used. The animals tested for motor performance were also part of the group tested for locomotor–exploratory behavior. In the EPM, where only 18- and 25-day-old rats were tested, naïve animals were used. Each age group consisted of 10 controls and two MTEP treated groups each consisting of 10 animals. The experiments were approved by the Animal Care and Use Committee of the Institute of Physiology ASCR and found to be in agreement with the Animal Protection Law of Czech Republic and European Community Council directives 86/609/EEC.

2.2. Drugs

MTEP (3-((2-methyl-1, 3-thiazol-4-yl) ethynyl) pyridine was purchased from Ascent Scientific (UK). A water solution (5 mg/ml) was freshly prepared at the beginning of each experiment. The two doses used (20 or 40 mg/kg i.p.) were chosen based on our recent studies on the anticonvulsant action of this drug (Mareš, 2009). Control animals received saline in a volume corresponding to the higher dose of MTEP (8 ml/kg).

2.3. Behavioral measurement

2.3.1. Sensorimotor tests

Four tests appropriate for the respective individual age groups were employed considering the time of appearance and maturation of some sensorimotor reflexes: surface righting for 12-day-old rats, negative geotaxis for 12- and 18-day-old rats, wire mesh ascending for 18- and 25-day-old rats and bar holding for all three age groups. The animal's ability to pass or fail the task was evaluated within an arbitrary pre-set period. If the pup did not successfully complete the test within the allotted time period, the score was assigned as the limit. Sensorimotor tests were performed 15 min (session 1) after MTEP administration and repeated at 60 min (session 2).

2.3.1.1. Surface righting. Pups were individually placed in a supine position on the laboratory desk, and the time to righting was recorded. The pup was tested for a maximum of 60 s.

2.3.1.2. Negative geotaxis. Pups were individually placed on an inclined (30°) surface with the head facing downward. The ability of pups to turn to 180° was recorded. The pups were tested for a maximum of 60 s.

2.3.1.3. Wire mesh ascending. The upper end of a wire mesh (45 × 15 cm inclined at a 70° angle) was connected to a small platform, and the lower end was at an edge of the laboratory desk 70 cm above the floor. Rats were placed at the bottom of the mesh and the time to reach the upper platform was measured with a limit of 120 s.

2.3.1.4. Bar holding. An animal was held by the nape and its forepaws were allowed to touch a wooden bar (25 cm long, 1 cm in diameter and suspended 25 cm above a padded soft surface). The time of fore- and hind-limb grasping was recorded with a limit of 120 s.

2.3.2. Open field test

Rats were placed in the center of the arena (48 × 48 × 30 cm). They were tested for 5 min at 20 min (session 1) and 65 min (session 2) after the drug/saline administration. The following behavioral variables were evaluated: locomotor activity expressed as the distance moved, exploratory behavior expressed as the frequency of the rearing irrespective of whether it occurred on or off the walls, and duration of grooming (nose wash, face wash, head wash, scratch and body wash and fur licking).

2.3.3. Elevated plus maze test

Two open and two closed arms of the maze (30 × 10 cm, closed arms with 30 cm high walls) connected by a central space (10 × 10 cm) were 50 cm above the floor. In a modified EPM paradigm, during the initial exposure to the EPM an animal acquires phobic avoidance of the open arms and retains strong memory for this threat for a certain time. Transfer latency, the time it takes for an animal to move from the open arm to either one of the enclosed arms, was significantly shortened when the two sessions were separated by 24 h. A number of studies validated the utility of the procedure for evaluation of short-term working and spatial memory. An animal was placed at the end of one open arm with the head directed to the periphery and the transfer latency was recorded. The criterion for scoring an entry into the enclosed arm was crossing (with all four legs) an imaginary line separating the enclosed arm from the central platform. After measurement of the transfer latency, the rat was allowed to move freely in the maze for 5 min for assessment of anxiety-like behavior. The following variables were calculated: the percent of the time spent on open arms [(open arm time/total time) × 100] and the number of entries into closed arms. The test was performed three times at 15 min (session 1), 60 min (session 2) and 24 h (session 3) after the drug administration.

After each animal exposure OF and EPM were cleaned and wiped. Both tests were recorded by a video camera and evaluated off-line using the programs EthoVision and Observer (Noldus Information Technology).

The experimental procedures are depicted in Fig. 1.

2.4. Statistics

The data set from sensorimotor performance tests did not always meet the criteria for normal distribution; thus, nonparametric tests were used: (a) a Kruskal–Wallis test for comparison in individual age groups and (b) a Wilcoxon test to compare the first and the second sessions. The OF and EPM data were analyzed by a two-way repeated-measures ANOVA with one between-group factor (treatment) and one within-subject factor (repeated session). The age differences from all behavioral tests were analyzed by a two-way ANOVA. Subsequent comparisons were performed with a Student–Newman–Keuls test. The level of significance was set at $P<0.05$, (SigmaStat® SPSS).

3. Results

3.1. Sensorimotor performance

In 12-day-old rats, a trend to a longer latency to surface righting and a significant prolongation of the time in negative geotaxis were found only 60 min after administration of either MTEP dose ($H = 9.05$, $P = 0.011$). The time spent holding the bar was not affected by MTEP in this age group.

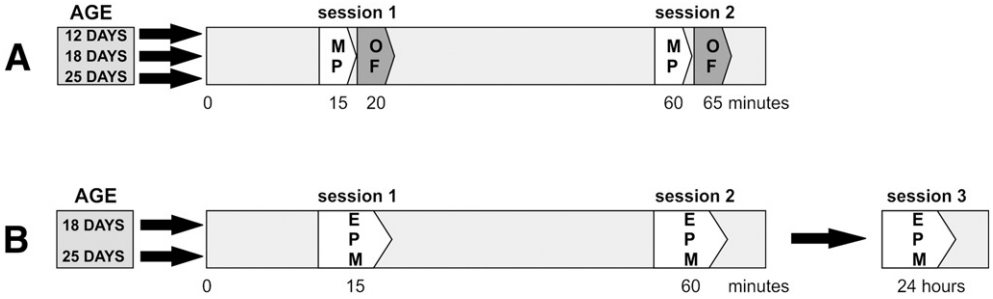

Fig. 1. Design/time diagram of MTEP experimental procedures. Animals aged 12, 18, and 25 days were used; 0 = time of drug administration. A = sensorimotor performance (MP) was tested 15 (session 1) and 60 min (session 2) after the injection. Open field (OF) test started immediately after MP (20 and 65 min after the injection, sessions 1 and 2, respectively). B = elevated plus maze (EPM) test in animals aged 18 and 25 days was performed 15 min (session 1), 60 min (session 2) and 24 h (session 3) after the injection.

In 18-day-old rats, the 20 mg/kg dose of MTEP significantly increased the latency to negative geotaxis response ($Z=2.12$, $P=0.04$) in session 1, whereas the dose of 40 mg/kg increased the latency to negative geotaxis response in session 2 ($Z=2.39$, $P=0.01$). Both doses of MTEP decreased the time to hold the bar in session 1 ($H=5.97$, $P=0.05$) but not in session 2. Both doses of MTEP increased the latency to ascending the wire mesh only in session 2 ($H=12.73$, $P=0.002$).

In 25-day-old rats, MTEP did not affect the bar holding in either session. In ascending the wire mesh, a significantly longer time was recorded in session 1 ($H=10.91$, $P=0.004$) for animals treated with the 40 mg/kg dose of MTEP (Table 1).

No age differences were found between 12- and 18-day-old rats in the negative geotaxis response and between 18- and 25-day-old animals in the wire mesh test in either session. In the bar holding test held for all age groups, there was a significant main effect of age [$F(2,78)=72.27$, $P<0.001$] and treatment [$F(2,78)=3.30$, $P=0.04$]. In session 2, there was a significant main effect of age [$F(2,78)=62.18$, $P<0.001$]: the latency to grasping increased with age in both controls and MTEP treated animals in a linear manner in both sessions.

3.2. Open field test

The MTEP did not affect the distance moved in the OF either at 20 min or 65 min after the injection in 12-day old animals. In 18-day-old animals, a main effect of session was found [$F=(1,59)=10.03$, $P=0.004$]. The post hoc test showed that animals treated with the 20 mg/kg dose walked in session 2 had a significantly shorter distance than in session 1. In 25-day-old rats, there was a significant effect of session [$F(1,59)=25.20$, $P<0.001$] and a significant interaction effect [$F(2,59)=3.45$, $P=0.04$]. The post hoc comparison revealed that both doses of MTEP decreased the distance moved in session 1. Whereas the distance moved by control animals in session 2 was significantly shorter compared to session 1, no change was observed in MTEP-treated animals (Fig. 2).

Age comparisons of distance moved in OF revealed a significant main effect of age [$F(2,89)=19.10$, $P<0.001$] and a significant age and treatment interaction effect [$F(4,89)=3.98$, $P=0.005$]. The post hoc test showed that 25-day-old control animals walked a longer distance than the two younger groups. MTEP increased the distance moved in 18- but not in 12-day-old rats. The effect in 25-day-old animals was the opposite: the distance moved was shorter in both MTEP-treated groups. No age differences were found in session 2 for control rats, but the distance moved was longer in 18- than in 12-day-old rats after the 40 mg/kg dose of MTEP. The 12-day-old animals did not display the same pattern of rearing behavior as the older animals who displayed an investigatory upright posture with scanning movements of the head oriented toward the environment. At this age, the animals only climbed with forepaws on the wall and rested for support. Thus, we analyzed rearing pattern only in 18- and 25-day-old animals. For the rearing number, in 18-day-old rats we found a main effect of treatment [$F(2,59)=6.52$, $P=0.005$] and a session effect [$F(1,59)=8.54$, $P=0.007$]. The post hoc test showed that the number of rearing was decreased in both sessions in animals treated with the 40 mg/kg dose compared to controls. Further, the 20 mg/kg dose had the same effect in the session 2. In 25-day-old rats, for the number of rearing there was a significant main effect of session with a decrease in the number [$F(1,59)=42.46$, $P<0.001$]. The post hoc comparison

Table 1
Effect of MTEP on sensorimotor performance in 12-, 18- and 25-day-old rats.

Group	Controls		20 mg/kg MTEP		40 mg/kg MTEP	
Testing	15 min	60 min	15 min	60 min	15 min	60 min
12 days	n = 10		n = 10		n = 10	
Surface righting	0.7 ± 0.2	0.4 ± 0.2	1.2 ± 0.3	0.9 ± 0.2	1.2 ± 0.1	0.8 ± 0.1
Negative geotaxis	3.9 ± 0.8	3.9 ± 0.4	8.0 ± 1.9	7.1 ± 1.0*	7.1 ± 1.1	8.8 ± 1.7*
Bar holding	8.0 ± 0.8	7.8 ± 1.2	8.8 ± 1.8	7.6 ± 0.8	10.1 ± 1.8	10.4 ± 1.6
18 days	n = 8		n = 10		n = 10	
Negative geotaxis	5.3 ± 0.9	5.4 ± 0.8	10.6 ± 5.5*	4.4 ± 0.7	5.0 ± 0.8	11.4 ± 5.5*
Bar holding	84.5 ± 17.5	76.0 ± 17.3	59.5 ± 13.3*	58.8 ± 13.2	37.1 ± 12.2*	44.2 ± 14.9
Wire mesh	26.8 ± 7.5	10.8 ± 1.0	32.9 ± 6.4	22.9 ± 13.1*	38.6 ± 6.1	37.6 ± 6.8*
25 days	n = 8		n = 6		n = 6	
Bar holding	120.0 ± 0.0[a]	112.5 ± 7.5[a]	109.6 ± 9.8[a]	113.0 ± 4.7[a]	110.4 ± 7.6[a]	110.5 ± 9.5[a]
Wire mesh	14.1 ± 5.1	22.4 ± 6.7	22.0 ± 6.8	21.5 ± 5.4	37.8 ± 7.6*	30.2 ± 7.3

Values are mean ± S.E.M.
* $P<0.05$ compared to control rats.
[a] Compared to either 18- or 12-day-old rats.

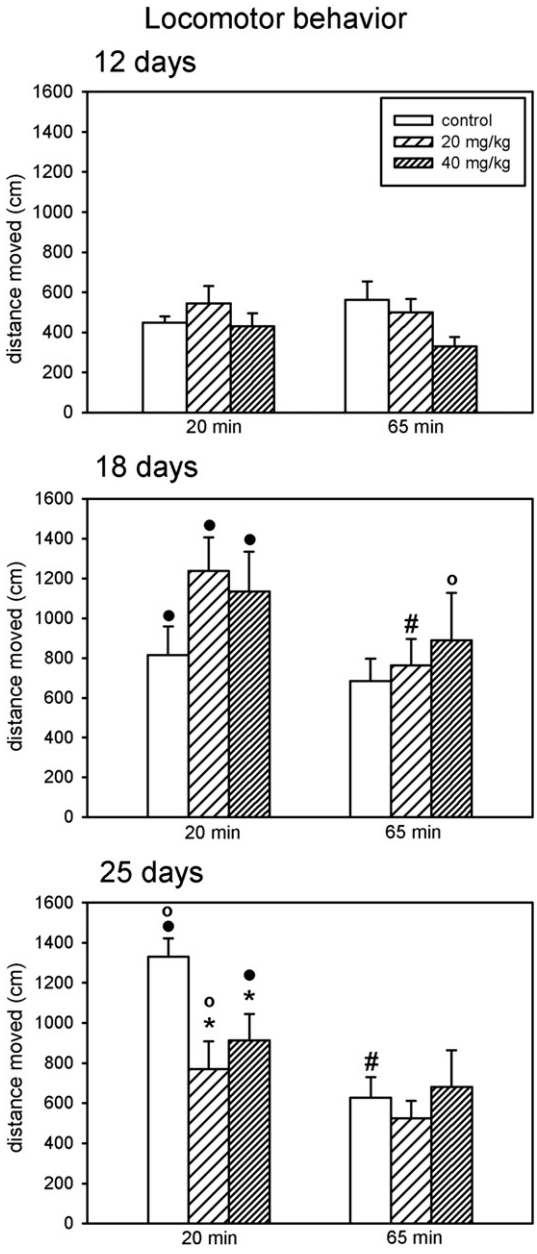

Fig. 2. Effect of MTEP on locomotor activity in the open field test in 12-, 18- and 25-day-old rats (n = 10 for each age and treated group). Abscissa: session 1 and session 2 (i.e., at 20 and 65 min after drug administration); ordinate: mean + S.E.M. for distance moved. $P<0.05$: *Compared to age appropriate control group, #Compared to session 1, ●Compared to 12-day-old rats, °Compared to 18-day-old rats.

revealed that both doses of MTEP decreased the number of rearing in session 1. In addition, a marked decrease in rearing was observed in the control animals in session 2 (Fig. 3).

An age comparison in the number of rearing revealed significant main effects of age [$F(1,59)=6.73$, $P=0.01$] and session [$F(1,59)=6.51$, $P=0.003$]. The post hoc test showed that only 18-day-old control animals had lower numbers of rearing in session 1 than 25-day-old rats. A significant age and treatment interaction was found in session 2 [$F(2,59)=0.55$, $P=0.57$]. The post hoc test showed that 25-day-old rats had higher numbers of rearing compared to 18-day-old rats in session 2.

The 12-day-old animals did not show grooming patterns (i.e., paw licking, nose washing, head washing, body and fur licking) characterized by a quiet progressive transition from one type to another. At this age, often only paw licking and nose washing could be observed. Other types of grooming behavior are rather chaotic and incomplete. For this reason, we analyzed the grooming behavior only in 18-and 25-day-old animals (Fig. 3).

In 18-day-old rats, there were significant main effects of treatment [$F(2,59)=7.34$, $P=0.003$] and session [$F(1,59)=10.49$, $P=0.003$] for grooming duration. The post hoc test showed that both doses of MTEP decreased grooming duration in session 2. In addition, control animals exhibited a significant increase in grooming duration in session 2.

In 25-day-old rats, significant main effects of treatment [$F(2,59)=17.34$, $P<0.001$], session [$F(1,59)=28.20$, $P<0.001$] and interaction [$F(2,59)=7.99$ $P=0.002$] were observed. The post hoc test showed decreased duration of grooming in both groups treated with MTEP in session 2. In control animals, a marked increase in grooming duration was observed in session 2 (Fig. 3).

No age-specific differences were found in the duration of grooming behavior in session 1. There were significant main effects of age [$F(1,59)=13.91$, $P<0.001$], treatment [$F(2,59)=24.43$, $P<0.001$] and interaction [$F(2,59)=5.88$, $P<0.005$] in session 2. The post hoc test showed that 25-day-old animals had a higher duration of grooming behavior in session 2.

3.3. Elevated plus maze test

In 18-day-old rats, the transfer latency was significantly shorter in session 2 and session 3 (i.e., 60 min and 24 h after injection, respectively) than in session 1 in controls and MTEP-treated rats [$F(2,89)=12.60$, $P<0.001$]. There were main significant effects of treatment [$F(2,89)=3.60$, $P=0.04$] and session [$F(2,89)=53.11$, $P<0.001$]. The post hoc test revealed that MTEP-treated animals spent longer times in the open arms than controls in session 1 and session 2, but not in session 3. In the test performed 24 h after drug administration, all animals spent shorter times on the open arms. For closed arm entries, there were significant main effects of treatment [$F(2,89)=6.69$, $P=0.004$] and session [$F(2,89)=3.77$, $P<0.02$]. The post hoc test showed that the animals treated with the 20 mg/kg dose of MTEP had a higher number of closed arm entries in session 2 than controls (Fig. 4).

In 25-day-old rats, the transfer latency was significantly shorter with repeated exposure to EPM [$F(2,89)=34.77$, $P<0.001$]. There were significant main effects of treatment [$F(2,89)=6.80$, $P=0.004$] and session [$F(2,89)=12.28$, $P<0.001$] in the time spent in the open arms. The post hoc test showed that animals treated with the 20 mg/kg dose of MTEP spent more time than the other two groups in the open arms in the session 2 (60 min after drug administration). For closed arms entries, there was a significant main effect of treatment [$F(2,89)=4.05$, $P=0.02$] and a significant interaction effect [$F(2,89)=2.96$, $P=0.02$]. Subsequent analysis showed that control animals had a lower number of closed arm entries in session 2 and session 3 than in session 1. Conversely, the number of closed arm entries significantly increased in session 2 in animals treated with the 20 mg/kg dose (Fig. 4).

The comparison of both age groups studied revealed that 25-day-old animals had a shorter transfer latency at all three intervals: session 1 [$F(1,59)=4.82$, $P=0.03$], session 2 [$F(1,59)=7.80$, $P=0.007$], and session 3 [$F(1,59)=12.90$, $P<0.001$]. Further, the 25-day-old animals spent less time in open arms compared to 18-day-old animals at all intervals: session 1 [$F(1,59)=18.39$, $P<0.001$], session 2 [$F(1,59)=33.38$, $P<0.001$], and session 3 [$F(1,59)=15.40$, $P<0.001$]. There was no age difference in the number of closed arm entries except session 2 when we found the main effects of age [$F(1,59)=6.92$, $P=0.01$] and treatment [$F(2,59)=15.30$, $P<0.001$]. The post hoc test showed that 25-day-old rats had lower numbers of closed arm entries than 18-day-old rats.

4. Discussion

Given the importance of metabotropic glutamate receptors (mGluRs) in a wide variety of neurophysiological processes, the potential for adverse effects of mGluRs antagonists has to be carefully considered. The majority of studies assessing both the beneficial and

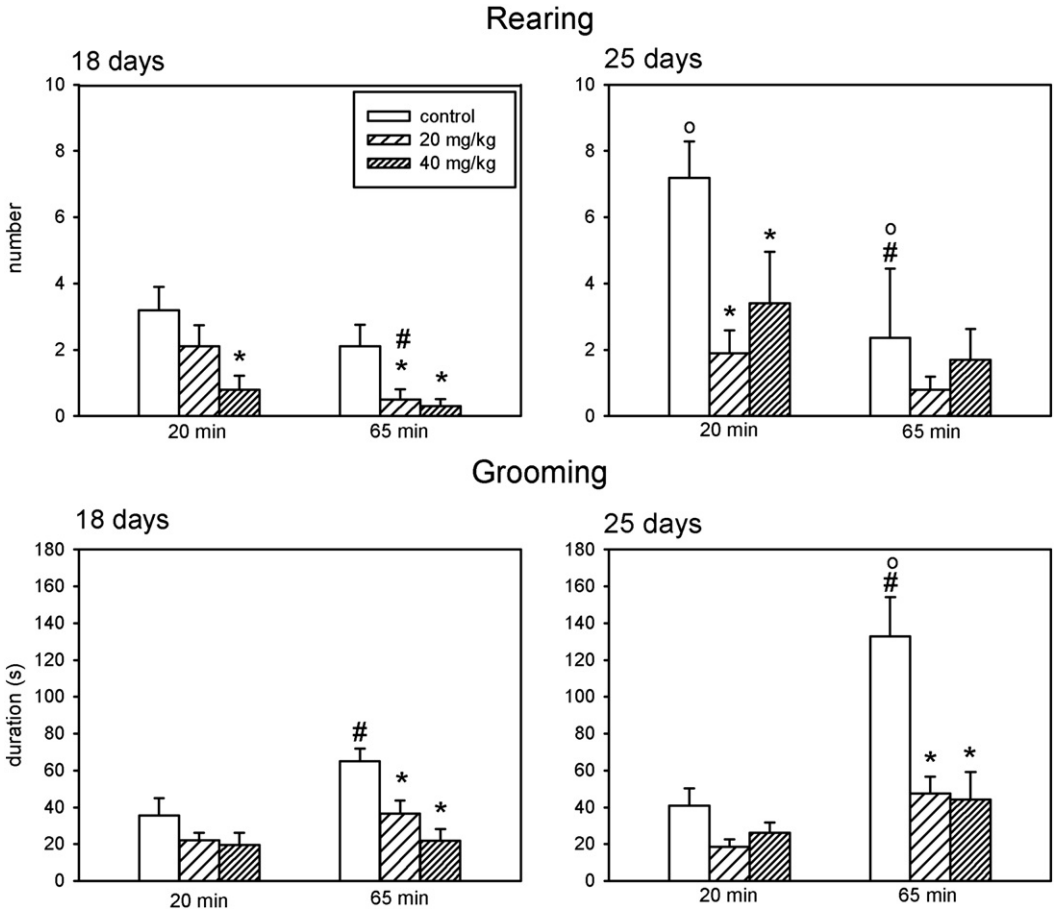

Fig. 3. Effect of MTEP on rearing and grooming in the open field test in 18- and 25-day-old rats (n = 10 for each age and treated group). Abscissa: session 1 and session 2 (i.e., at 20 and 65 min after drug administration); ordinate: mean + S.E.M. $P<0.05$: *Compared to age appropriate control group, #Compared to session 1, °Compared to 18-day-old rats.

unfavorable effects of mGluRs antagonists have been performed in adult animals (for reviews see Pietraszek et al., 2005; Varty et al., 2005). Our study investigated the possible side effects of a specific mGluR5 antagonist (MTEP) on behavioral responsiveness in immature rats. It was focused on high doses demonstrated to be effective in models of epileptic seizures (Mareš, 2009; Lojková-Janečková et al., 2009). Behavioral studies in developing animals are necessary because the level of maturation in the central nervous system can affect behavioral responsiveness in different testing paradigms. The expression of the mGluR5 subtype of group I metabotropic glutamate receptors was demonstrated in the cerebral cortex and hippocampus at the age of 12 days (Lopez-Bendito et al., 2002); therefore, we had to expect effects from their antagonist.

Although the sensorimotor performances of animals treated with MTEP were slower than in controls, all animals irrespective of the age were still able to perform the tasks successfully. These results are in agreement with our previous study using MPEP, the antagonist of mGluR5, where the same doses (20 and 40 mg/kg) slightly affected sensorimotor abilities in immature rats (Mareš and Mikulecká, 2004). Developmental differences occurred in bar holding, one of the most demanding tests of motor coordination (Gramsbergen et al., 1999), with a linear increase in time holding the bar: the oldest animals were able to hold the bar for a longer time.

Locomotion (expressed as distance moved in the open field) measured 20 min after drug administration was not affected by either MTEP doses in 12- and 18-day-old rats. Conversely, the MTEP suppressed locomotion in 25-day-old rats. This result seems to indicate a higher sensitivity to MTEP in 25-day-old animals. Animals at 18- and 25-days-old are able to perform a rearing posture, considered to be an indicator of exploratory behavior (Geisler et al., 1993). Both MTEP doses decreased this pattern, suggesting an inhibition of exploratory behavior. As for grooming behavior, distinguishable at about 20 days of age and reflecting the maturation of motor capabilities (Piggins and Merali, 1992; Geisler et al., 1993), both MTEP doses suppressed this pattern particularly with repeated exposure at 65 min after administration.

With repeated exposure to the open field, a decrease in locomotion and rearing and an increase in grooming (particularly expressed in control 25-day-old rats) indicate behavioral habituation as a consequence of the retention of information acquired during the first exposure. This result is in agreement with a developmental study demonstrating that habituation occurs in intact rats at the end of the 4th week of postnatal life (Cerbone and Sadile, 1994). This behavioral phenomenon failed to occur in immature rats treated with MTEP similar to the administration of another mGluR5 antagonist MPEP (Mikulecká and Mareš, 2009).

Studies in adult animals demonstrated that mGluR5 plays a critical role in hippocampal-dependent spatial learning. It was shown that mGluR5 overexpression in CA3 may reflect a short-term memory process, while the delayed mGluR5 overexpression in CA1 may correspond to processes underlying long-term memory (Riedel et al., 2000). Data from various tests revealed a discrepancy among the studies; mGluR5 was found to impair or not affect learning and memory (Simonyi et al., 2005).

The EPM paradigm can be also used to assess an adaptive form of spatial learning and memory (Itoh et al., 1990; Mikulecká et al., 2000). In our study, repeated exposure of the controls as well as the MTEP-treated animals led to a gradual decrease in the transfer latency; this trend was more distinct in 25-day-old animals than in 18-day-old animals. Thus, MTEP did not affect the transfer latency, the time required for the animal to move from the end of an open arm to either of the closed arms, which is considered to be an index of spatial learning and memory. A close relationship between memory mechanisms and anxiety/fear was

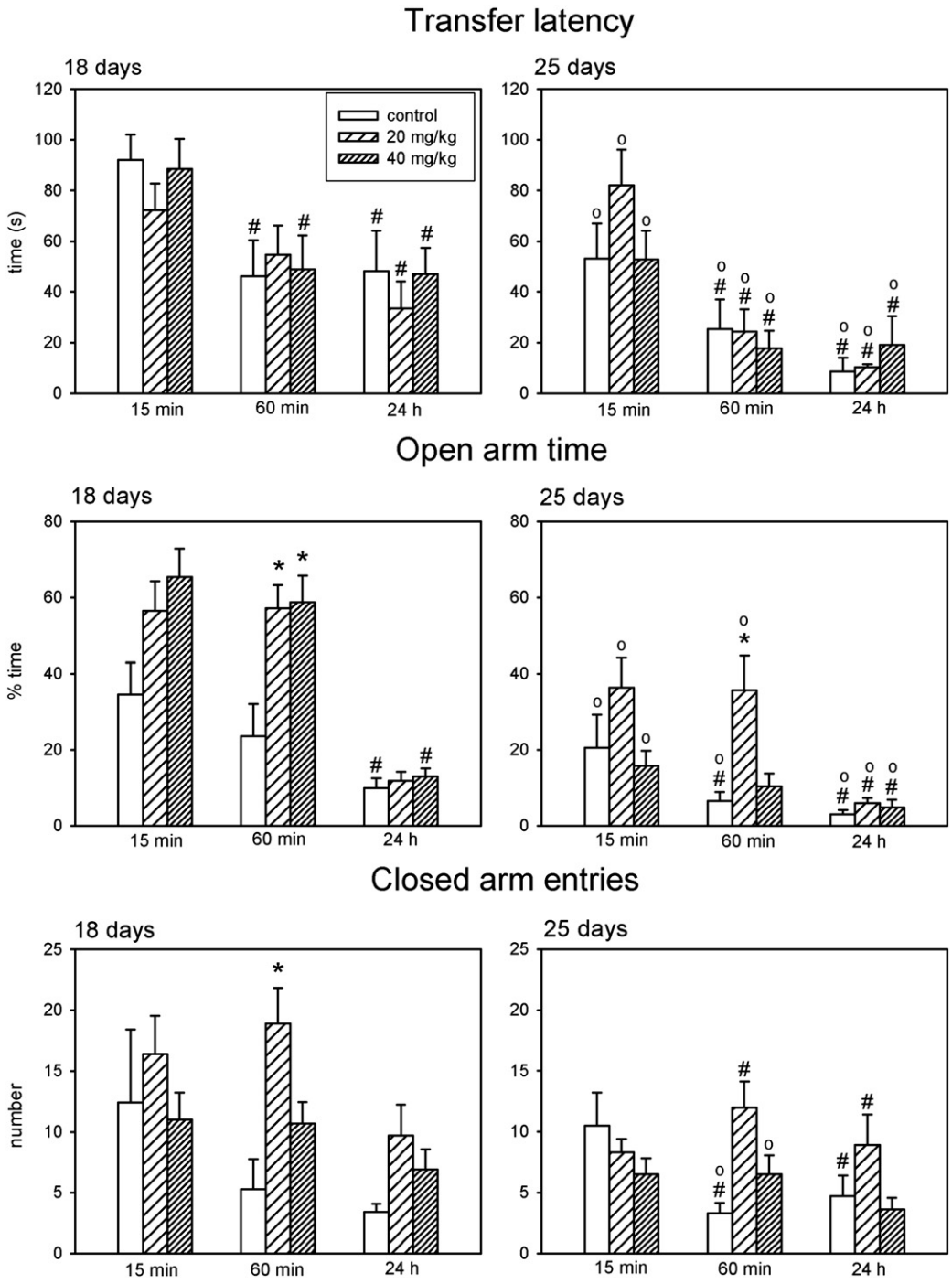

Fig. 4. Effect of MTEP on behavior in the elevated plus maze. Left graphs: 18-day-old rats, right graphs: 25-day-old rats (n = 10 for each age and treated group). From top to bottom: transfer latency (s), time spent (%) in open arm, and number of closed arm entries. Abscissa: session 1, session 2, and session 3 (i.e., at 15 min, 60 min and 24 h after drug administration); ordinate: mean + S.E.M. $P<0.05$: *Compared to appropriate control group, #Compared to session 1, °Compared to 18-day-old rats.

demonstrated. Prior knowledge of the EPM induces a phobic-like response, thus reducing the tendency to explore the open arms (File, 1993; Treit et al., 1993; Viana et al., 1994; Rodgers et al., 1996). Based on the factor analysis of behavioral variables from the EPM test (Wall and Messier, 2001) we used the percentage of open time arm entries as a measure of anxiety-like behavior and the total number of closed arm entries as a measure of locomotion, which has been shown to be a more reliable measure of locomotion than the total number of arm entries. In 18-day-old rats, both doses of MTEP increased the time spent in open arms whereas only the lower dose had a similar effect at 60 min in 25-day-old rats. This might indicate an anxiolytic-like effect of MTEP that probably faded with time. The anxiolytic-like effect of MTEP can be supported by the finding that MPEP, a similar mGluR5 antagonist, was found to exert an anxiolytic-like effect in the light–dark paradigm (Mikulecká and Mareš, 2009) and in ultrasound vocalizations (Hodgson et al., 2008) in immature rats. The number of entries in the closed arms was not significantly affected by MTEP except for an increase in 18-day-old-rats 60 min after drug administration. This latter finding could be explained as a stimulatory effect of MTEP on locomotion.

Taken together, our data received for immature rats indicate that anticonvulsant doses of MTEP slightly impaired sensorimotor performance, decreased locomotor–exploratory activity, but did not affect the habituation — a simple form of nonassociative learning. In the EPM, the drug did not impair transfer latency, an indicator of an adaptive form of

learning and memory, but did induce an anxiolytic-like effect. A simple interpretation of these findings is that high doses of mGlu5 receptor antagonist exerting anticonvulsant actions did not result in severe alterations of the behavior in immature animals. The finding on the sensorimotor performance is in distinct contrast to NMDA and AMPA receptor antagonists that compromise motor abilities (Mareš et al., 1997; Mikulecká and Mareš, 2002) as well as with our data on the classical antiepileptic drug phenytoin which seriously deranges the motor performance of immature rats (Pometlová et al., 1981). To have an anticonvulsant drug (even if anticonvulsant action is only moderate) with positive psychotropic effects (anxiolytic in the case of mGluR5 antagonists) would be very useful for pediatric epileptology. To strengthen the hope that such a drug may be found among the antagonists of the mGluR5 type of receptors, experiments with chronic administration of antagonists as well as effects in a model of chronic epilepsy are necessary.

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