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Chronic neonatal *N*-methyl-D-aspartate receptor blockade induces learning deficits and transient hypoactivity in young rats

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Abstract

A blockade of *N*-methyl-D-aspartate (NMDA)-type of glutamate receptor in rodents is believed to provide a pharmacological model of schizophrenia-related psychosis. Since neurodevelopmental abnormality, at least partly, could contribute to the pathogenesis of schizophrenia, the aim of this study was to recapitulate cognitive impairments accompanying this disorder in rats by a chronic neonatal treatment with a noncompetitive NMDA antagonist MK-801. Rat pups were treated with a low dose of MK-801 (0.05 mg/kg sc) chronically from early postnatal period (PD 7–49) known to be critical for glutamatergic system maturation. Locomotor activity in the "open-field" test, anxiety level in the elevated plus-maze test, and learning capacity in food rewarded spatial task were examined in young animals. Chronic MK-801 treatment produced a decrease of spontaneous motor and exploratory activity in 16- to 28-day-old rats. At the same time, a hyperlocomotion in response to acute administration of MK-801 was observed as well. Spatial learning of MK-801-treated rats was found to be negatively affected. Treated rats were able to respond to stress stimuli in the adequate manner but their anxiety level was found to be lower than in controls. Behavioral disturbances appeared to be temporary, and no such abnormalities could be detected at the age of 16 weeks. Thus, even mild chronic neonatal blockade of NMDA receptors may lead to a specific pattern of cognitive abnormalities presumably resulting from impairments of sensory information processing at the cortical–basal ganglia level.

Keywords: MK-801; Neonatal treatment; NMDA receptor; Rat pups

1. Introduction

A number of studies have suggested an important role of the glutamatergic system in the brain maturation and developmental processes as well as in neuro- and psychopathological changes (Parsons et al., 1998; Shi et al., 2001; Corner et al., 2002). The *N*-methyl-D-aspartate (NMDA) type of glutamate receptors has attracted a particular interest due to its role in excitotoxicity accompanying stroke, hypoxic ischemia, epilepsy, and a variety of other neurological disorders (Rothman and Olney, 1986; Coyle, 1997; Lynch and Guttmann, 2002; Planells-Cases et al., 2002). Furthermore, NMDA receptor/channel is known to play an important role in developmental changes, such as neuronal migration, survival, establishment of appropriate connectivity (Facchinetti et al., 1993; Ramoa et al., 2001). NMDA-dependent synaptic plasticity in the mammalian hippocampus appears to be essential for learning and memory. Systemic administration of a noncompetitive NMDA receptor antagonist, MK-801, produces an impairment in a variety of learning and memory paradigms (Castellano et al., 2001) such as passive avoidance (Ohno and Watanabe, 1996), spatial learning in the radial maze, and water-maze navigation task (Heale and Harley, 1990; Wozniak et al., 1990).

NMDA receptor channels are heterogeneous in their pharmacological properties and functions depending on the brain region and the developmental stage. It was shown that the adult mature subunit configuration of the rat NMDA receptor replaces the immature receptors during the third postnatal week (Wenzel et al., 1997; Sircar, 2000). It was also shown that chronic NMDA receptor blockade during the critical period of neurodevelopment leads to structural, neurochemical, and functional alterations of the brain. When exposed to MK-801 (0.25 mg/kg twice daily) at postnatal days (PDs) 8–19, rat pups displayed weight loss compared to control

Abbreviations: MK-801, dizocilpine; NMDA, *N*-methyl-D-aspartate; PD, postnatal day.

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littermates and spatial learning deficits (Gorter and de Bruin, 1992). Nonspatial learning (Griesbach and Amsel, 1998) and motor behavior (Facchinetti et al., 1993) were also altered after neonatal regimen of NMDA antagonist treatment.

A specific interest in NMDA receptor physiology arises from a potential involvement of glutamatergic system in schizophrenia. Nowadays, schizophrenia is considered to be a result of abnormalities in brain development and glutamatergic system maturation, particularly (Weinberger, 1995; Carlsson et al., 1997; Parsons et al., 1998). Although definitive evidence supporting the glutamatergic theory of schizophrenia is still lacking, several groups reported abnormalities in NMDA-mediated glutamatergic transmission in schizophrenia patients. As an example, a decrease in the obligatory NMDA receptor subunit NR1 was found to correlate with cognitive impairments in schizophrenic patients (Humphries et al., 1996). The density of glutamate uptake sites assessed with [^{3}H]-D-aspartate binding is decreased in the caudate nucleus, putamen, and nucleus accumbens, indicating impaired glutamatergic innervation of these subcortical regions (Aparicio-Legarza et al., 1997). Additionally, in schizophrenic brains, Arbarian et al. (1996) found a relative increase in NR2D subunit expression that shows relatively slow deactivation kinetics. Furthermore, mice with decreased expression of NR1 subunit of NMDA receptor display striking behavioral abnormalities that are characteristics of pharmacological rodent models of schizophrenia (Mohn et al., 1999).

Therefore, it seems reasonable to suggest that a model of cognitive and social interaction deficits might be developed via chronic neonatal NMDA receptor blockade. In the present study, male Wistar rat pups were treated with noncompetitive NMDA receptor antagonist MK-801 (dizocilpine) on PDs 7–49 at the low dose shown as nontoxic for adult animal (Wozniak et al., 1990). The aim of this work was to examine whether a mild chronic NMDA blockade in a critical period of the glutamate system maturation results in long-term behavioral and cognitive abnormalities.

2. Materials and methods

2.1. Animals

The experiments were performed on male Wistar rats reproduced in the laboratory vivarium. The parent animals were kept with food and water available ad lib at 12 h light/12 h dark cycle and all experiments were performed between 9:00 a.m. and 4:00 p.m. Day of pup birth was counted as PD 0. Every litter was restricted to 10 pups on PD 1.

2.2. Drugs

Male pups were injected at 7–49 postnatal days with (+)ÌK-801-maleate [(5R,10*S*)-(+)-5-methyl-10,11-dihydro-5Í-dibenzo[à,*b*]cyclohepten-5,10-imine] (dizocilpine, Sigma) at a dose of 0.05 mg/kg and a volume of 0.042 ml/10 g body weight, once per day, between 11:00 and 12:00 a.m., subcutaneously. Control animals received saline of an equal volume. Protocols of experiments were approved by the Russian Academy of Medical Sciences (RAMS) Guide for Care and Use of Laboratory Animals (Russian Federation).

2.3. Behavioral tests schema

Body weight was monitored daily beginning on PD 7. To determine an effect of the chronic MK-801 treatment on spontaneous locomotor activity in rat pups, "open-field" testing was performed on PDs 16, 17, 27, and 28, 23 h after previous injection. Moreover, MK-801-treated animals were tested in the "open field" in 15 and 60 min after injection on the same days to estimate an acute response to MK-801 challenge. Control rats were tested simultaneously to get an equal individual experience. In order to evaluate a possible contribution of altered emotionality in rat behavior, testing in the elevated plus-maze and stress version of the "open field" was performed prior injection on PD 22 and PD 40, respectively. Learning and memory capacity was tested in food rewarded spatial task (PDs 50–54: acquisition; PD 61: retention test; PDs 62–63: reversal spatial task performance). Spontaneous locomotor activity was registered on PD 55 in the "open field" to dissociate putative motor and learning disturbances. At the age of 16 weeks, rats of the both groups were exposed to social interaction test.

2.4. "Open-field" testing

Animals were tested in a circular "open field" 40 cm in diameter divided into sectors with eight diameters and two circles equidistant from each other, arena center and wall. White (100 W) and red light (40 W) lamps were settled 80 cm above the arena. During 3 min behavioral parameters were registered visually: (1) horizontal locomotor activity; (2) rearing; (3) number of outer circle crossings (an animal leaves near-wall zone); (4) number of inner circle crossing (arena center visiting); (5) grooming; (6) defecation (bolus number).

2.5. Elevated plus-maze

The plus-maze consisted of two open arms (9×44 cm) and two walled arms ($9 \times 44 \times 50$ cm) with an open roof, arranged around the central platform (9×9 cm) so that the two arms of each type were opposite to each other. The maze was elevated to a high of 60 cm. Two 25-W lamps were positioned 30 cm in front of each open arm ends. The rat was placed on the central platform facing one of the open arms and was observed for 3 min. During testing the latency of central platform leaving, time spent in the open arms, number of entries into the open/closed arm, scanning (protruding the head over the edge of an open arm and fanning

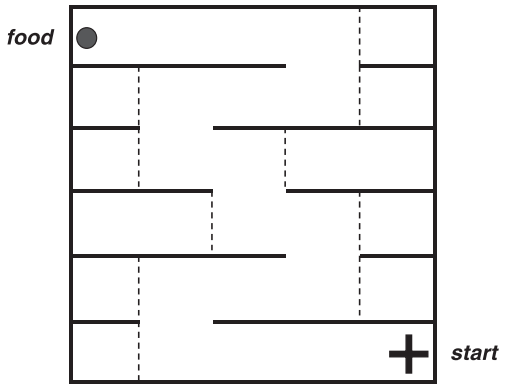

Fig. 1. An apparatus used for spatial learning task. Start point designated as a cross, goal point (food reward) as a circle. Dotted line designates imaginary sections of a maze. Crossing the line was considered an error.

with the vibrissae in any direction), risk assessment (protruding from an closed arm with the forepaws and head only), grooming, and rearing in the open arms were monitored and registered.

2.6. Spatial food rewarded task

An apparatus used for the spatial training was developed using the concept of the "Hebb–Williams maze" (Hoplight et al., 1996) and represented a box of $60 \times 60 \times 25$ cm divided with five partitions with holes 6×7 cm (Fig. 1). Front wall and partitions were made of Plexiglas. The experiment consisted of an acquisition (4 days, 5 trials/day), a retention (1 day/5 trials), and a reversal phase (2 days, 5 trials/day). A day before the first learning session rats were placed (for 30 min) into the maze with food pellets (2 g) randomly scattered throughout for adaptation. Animals were food-deprived 24 h heretofore. At the beginning of each trial, food pellet was placed in the goal point. A rat was placed into the maze facing the wall. The trial ended when a rat obtained the food reward or when 3 min expired, whichever came first. Latencies to reach the food and reference memory errors were monitored and recorded at the end of each trial. Animals received some amount of food in the home cage daily after training.

Retention was measured 7 days after the last acquisition trial (one training day, 24-h food deprivation thereafter). Next 2 days reversal phase testing was performed. Partitions were replaced in a way to change the position of every hole. Thus, "new" maze was a mirror copy of the maze used for acquisition.

2.7. Resident–intruder assay (Corbett et al., 1993; Dixon et al., 1994)

Male animals of both groups were housed individually or in groups of nine for at least 10 days prior to testing and cage bedding was changed 36 h prior to testing. In this assay, a group-housed male (intruder) was placed in the home cage of the individually housed male (resident). For 6 min their behavior was recorded: time spent in social investigation (approaching, sniffing, grooming other rat), in escape or fighting behavior.

2.8. Statistical analysis

Data are presented as means (±S.E.M.) and analyzed using Student's t test for independent samples, two-way repeated-measures ANOVA, and post hoc comparison tests where appropriate (Statistica 5.0 software). The level of significance was set at $P < .05$.

3. Results

The dose of MK-801 used in this study (0.05 mg/kg/day sc) was nonlethal and did not result in any visible morphological abnormalities or delay of sensorimotor reflexes maturation (estimated according to Marshall and Teitelbaum, 1974). The MK-801-treated animals gained weight at a slower rate compared with controls. Although two-way repeated-measures ANOVA showed nonsignificant effect of treatment time on body weight [$F(1,11) = 4.31$, $P < .06$], time [$F(38,418) = 448.76$, $P < .00001$], interaction [$F(38,418) = 2.96$, $P < .00001$], post hoc comparison revealed significant difference between groups beginning from PD 29 (Newman–Keuls test, $P < .05$).

3.1. Open field

Locomotor activity (horizontal component) in MK-801-treated rat was found to be lower than in controls at PDs 17, 27, and 28 when measured prior to daily injection (23 h after previous one) (Fig. 2). Two-way repeated-measures ANOVA shows significant main effect of treatment [$F(1,25) = 12.10$,

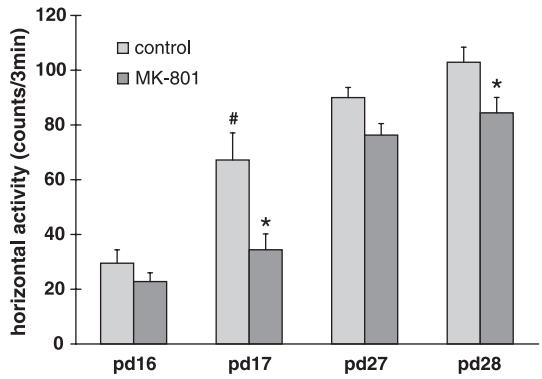

Fig. 2. Effects of chronic MK-801 treatment on locomotor activity in the "open-field" test at PDs 16, 17, 27, and 28 as measured prior to injection. Rats of MK-801-treated group (filled bars, $n = 18$) received subcutaneous injections of 0.05 mg/kg MK-801 on PDs 7–49 daily. Control littermates (open bars, $n = 17$) received an equal volume of saline. Data are plotted as means ± S.E.M. Newman–Keuls test: $^{*}P < .05$, versus control group, $^{\#}P < .05$, PD 17 versus PD 16.

$P<.002$] and time [$F(3,75)=83.04$, $P<.0001$]. Post hoc comparison revealed significant increase of locomotor activity at PD 17 compared to PD 16 in the control group only (Newman–Keuls test, $P<.01$).

As shown in Fig. 3, locomotor response on MK-801 challenge in chronic MK-801-treated rats changed with time. Two-way repeated-measures ANOVA shows significant main effect of time [$F(7,175)=2.48$, $P<.02$] and treatment [$F(1,25)=6.44$, $P<.02$] when percentage of basal level of horizontal activity was estimated. Post hoc analysis showed that in response to the MK-801 challenge at PDs 16 and 17 in 15 min postinjection, chronic treated rats traveled more distance and left near-wall zone of the "open field" more often than control littermates (Newman–Keuls test, $P<.03$). Rearing behavior analysis showed significant effect of time [$F(7,175)=18.07$, $P<.01$], treatment [$F(1,25)=7.57$, $P<.01$], and their interaction [$F(7,175)=3.25$, $P<.003$]

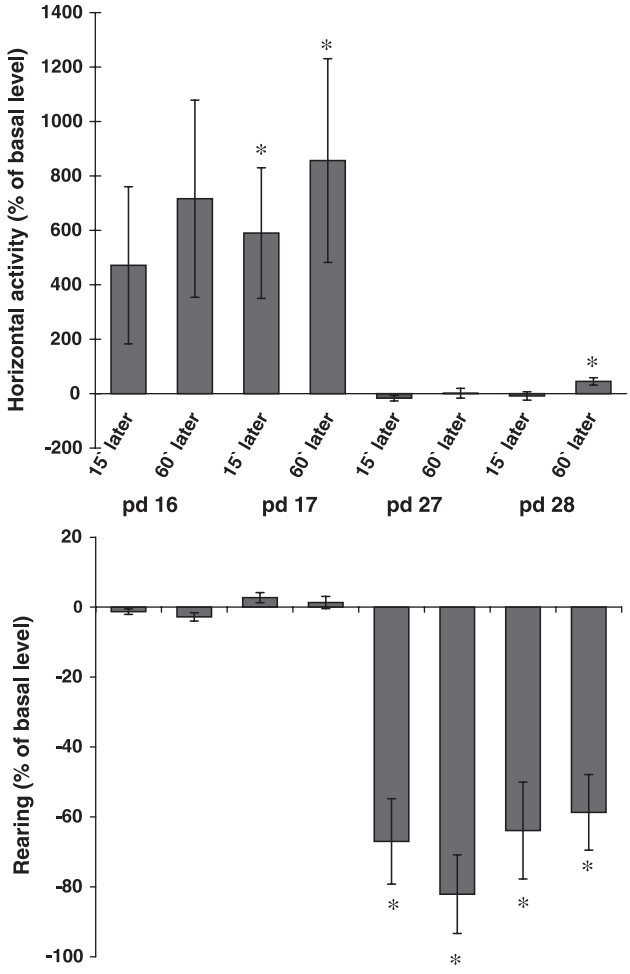

Fig. 3. Effects of acute MK-801 challenge on locomotor activity of chronic MK-801-treated rats in the "open-field" test and 15 and 60 min after injection at PDs 16, 17, 27, and 28. Rats ($n=18$) received subcutaneous injections of 0.05 mg/kg MK-801 on PDs 7–49 daily. Data are presented as a percentage of the basal level (prior injection): above-horizontal activity, below-rearing. Data are plotted as means ± S.E.M. Duncan test: $*P<.05$ versus control.

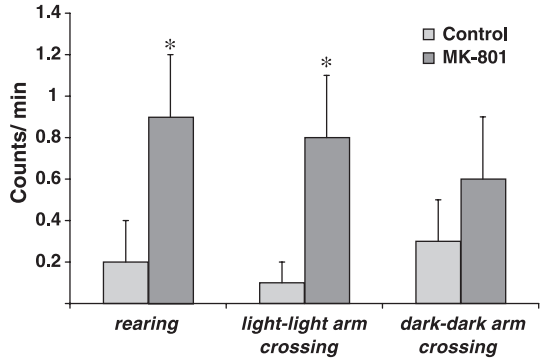

Fig. 4. Effects of chronic MK-801 on behavior in elevated plus-maze at PD 40 prior to the daily injection. Rats of the MK-801-treated group ($n=18$) received subcutaneous injections of 0.05 mg/kg MK-801 on PDs 7–49 daily. Control littermates ($n=17$) received an equal volume of saline. Data are plotted as means ± S.E.M. Student's t test for independent samples: $*P<.05$ versus control group.

(Fig. 3). At PDs 27 and 28, rearing of chronic MK-801-treated rats was decreased in the "open field" 15 min as well as 60 min after injection (Duncan test, $P<.02$). At PD 28 in 60 min after injection, the number of inner and outer circle crossings was significantly increased in chronic treated group as compared to control (Newman–Keuls test, $P<.01$), while traveled distance was the same in both groups.

There was no difference (neither hypo- nor hyperactivity) between MK-801-treated and control pups in the stress version of "open field" at PD 22 tested 23 h after previous injection. At PD 55 (the day before learning was started), animals of the MK-801-treated group did not differ in "open field" from control littermates.

3.2. Elevated plus-maze

Compared with control rats, MK-801-treated animals entered the open arms more often (though spending the same time on them) and explored them more actively as indicated by increased rearing score (Fig. 4). Additionally, MK-801-treated rats in contrast to controls preferred to visit open arms first after placement in the maze.

3.3. Spatial learning

To analyze reference memory of rats in spatial food rewarded task, the ratio of time to reach food (T) at the last trial of training day to T of the first trial next day was calculated. Two-way repeated-measures ANOVA shows significant effect of treatment [$F(1,23)=6.63$, $P<.02$]. Mean increase of T in control rats is 1.49 ± 0.11 times, while that of treated animals is 2.23 ± 0.25 times. During every training day, MK-801-treated rats improved gradually in performance and reached the level of controls to the last trial of training day. In retention and reversal phases, no differences between groups were detected. In Fig. 5, learn-

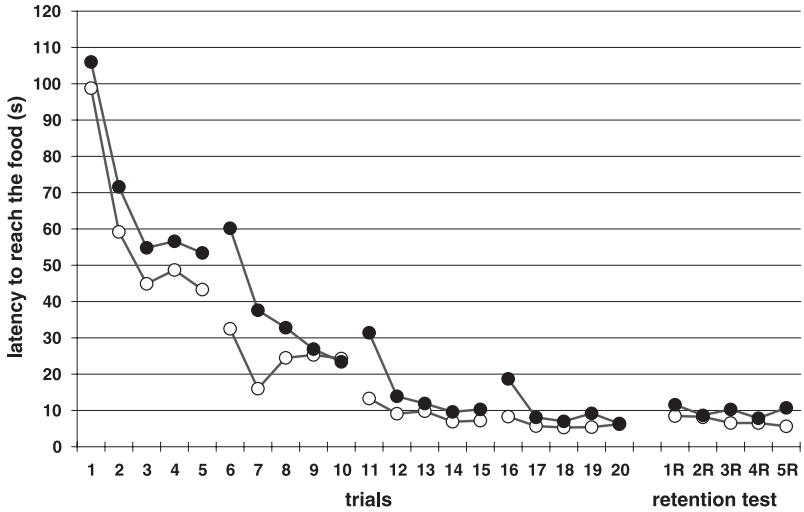

Fig. 5. Effect of chronic MK-801 on the acquisition and retention (7 days later) phases in the spatial learning task with food reward at PDs 50–55: latency to reach the food. Rats of the MK-801-treated group (filled circles, $n=18$) received subcutaneous injections of 0.05 mg/kg MK-801 on PDs 7–49 daily. Control littermates (open circles, $n=17$) received an equal volume of saline.

ing time-course during the acquisition and retention phases is presented with regard to latency to reach the food reward.

3.4. Resident–intruder assay

There was no difference in social behavior after 10 daylong isolation between rats of MK-801-treated and control groups (16 weeks). MK-treated rats behaved very friendly, had a lot of contacts during testing (active exploration), and sometimes were sleeping nestled close to other rats up to the end of the test (data not shown).

4. Discussion

In the present study, chronic neonatal NMDA receptor blockade by a low dose (0.05 mg/kg) of MK-801 was found to induce hypoactivity, decreased exploratory behavior, and slower spatial learning (food rewarded task) in young male rats. To estimate development of abnormal behavioral pattern the spontaneous locomotor activity of rat was monitored at different ages and testing conditions. When tested in open field for two consecutive days (PDs 16–17 prior to daily injection, i.e. 23 h after previous one), rats of control group were more active at PD 17 versus PD 16 ($P<.01$, Newman–Keuls test) perhaps due to the decrease of novelty-induced anxiety. At the same time, placement of MK-801-treated rats into the same environment for two consecutive days did not change their level of locomotor activity, suggesting that MK-801-treated animals may differ from control littermates in perception of testing context. Since the adaptation process can be considered as a kind of learning, it seems reasonable to speculate that learning/memory capacity of treated pups may be significantly affected (Apelqvist et al., 1999). Moreover, relative hypoactivity MK-801-treated rat displayed on PD 17 (vs. controls) persisted to PDs 27 and 28. This fact could also be interpreted as an evidence for increased anxiety in the MK-801 group, but no freezing behavior was noted during the "open-field" test. In fact, a direct measurement of anxiety-related behaviors in the plus-maze test revealed a decreased anxiety level in the MK-801-treated group, that is consistent with reported anxiolytic potential of MK-801 (Dunn et al., 1989; Fraser et al., 1996). It is important to note also that in the stressful conditions ("open field" with bright light), the behavior of MK-801-treated animals was similar to control rats, indicating that their ability to respond to environmental stimuli was not affected.

It is well known that treatment with MK-801 results in a robust stimulation of locomotor activity. A hyperlocomotion in adult rats as well as in pups was described in numerous studies after either acute or chronically administered NMDA antagonist (see, for review, Schmidt and Kretschmer, 1997; Åhlander et al., 1999). In our study, injection of low dose of MK-801 (0.05 mg/kg), known to be ineffective in normal adult rodents (Martin et al., 1998; Gainetdinov et al., 2001), resulted in a significant hyperlocomotion in chronically treated animals on PDs 16–17. However, this stimulating effect of MK-801 was not found on PDs 27–28 in treated pups. The lack of response in older pups might be explained by a chronic treatment-induced receptor desensitization or age-dependent alterations in receptor sensitivity to this treatment. However, MK-801-treated rats displayed decreased rearing behavior as compared to controls and prior-to-injection level (at PD 27 as well as PD 28). Furthermore, at PD 28, treated animals visited the arena center significantly more often than the same animals prior to MK-801 challenge and controls. The behavioral pattern described here might also be interpreted as reflection of decreased anxiety level in MK-801-treated rats. Thus, it

seems that acute MK-801 administration to chronically treated rats at PDs 27–28 results in specific behavioral alterations. While this behavioral pattern is quite unusual, it is interesting to note that mice lacking the NMDA receptor ε4 subunit also showed a reduced spontaneous locomotor activity (both horizontal activity and rearing) in a novel environment (Miyamoto et al., 2002). These mice have a particular type of NMDA receptor dysfunction resulting in secondary changes in monoaminergic transmission and alterations in emotional behavior. Thus, it is possible that pharmacological blockade of NMDA receptor in the development may lead to the abnormal behavioral pattern setting. There is also possibility that MK-801 acted in a different way at the various stages of ontogenesis with regard to different aspects of brain system maturation (motor functions alteration at the early stage and emotional shift later in the development).

However, it is also possible that this abnormal pattern could be a result of competition of two behavioral reactions: rearing and arena center visiting. In the control group, there was strong positive correlation (r=.69, P<.02) of these two parameters, while in the MK-801-treated group, no significant correlation was found (r=−.39, P=.24). At the age of 16 weeks, MK-801-treated rats did not display any abnormalities in spontaneous activity when placed in the open field. Thus, behavioral consequences of mild neonatal NMDA receptor blockade are not long lasting. Similarly, chronic treatment with a higher dose of MK-801 0.25 mg/kg twice a day (PDs 8–19) did not lead to any differences in the open-field behavior at PD 118 (Gorter and de Bruin, 1992).

No difference in open-field behavior at PD 55 (a day after acquisition phase ended) was found between groups, suggesting that the worse performance of the MK-treated rats in spatial-maze task may be due to specific spatial learning impairment and was not a sequel to locomotor or sensory deficits. However, since MK-801 affects a large number of NMDA synapses in both sensory and motor systems (Meoni et al., 1998), it is unlikely that there is a dose range of MK-801 that could "selectively" affect learning and memory (Åhlander et al., 1999).

Numerous studies reported a learning deficit in acquisition of spatial (Butelman, 1988; Whishaw and Auer, 1989; Gorter and de Bruin, 1992; Kesenberg and Schmidt, 1995; White and Best, 1998; Åhlander et al., 1999; Kretschmer and Fink, 1999; Smith-Roe et al., 1999; Nemeth et al., 2002) and nonspatial tasks (Facchinetti et al., 1993; Griesbach and Amsel, 1998) induced by MK-801. A dose of MK-801 (0.05 mg/kg) used in our study was shown to impair acquisition in the water-maze task and fails to affect performance during recall task while exerting no influence on motor activity or general reactivity in adult rats (McLamb et al., 1990). In the present series of experiments, chronic neonatal NMDA receptor blockade disrupted spatial learning in 50-day-old rats in the same manner. Although, initially, learning was slowed in the MK-801-treated group, after four training days the performance was similar. Noteworthy, during any training day MK-801-treated animals did improve in acquisition of performance reaching the level of controls at the last trial. Our observation supports previous reports describing MK-801 failure to impair within-session spatial learning (White and Best, 1998) and its ability to exacerbate specifically the lesion-induced reference memory deficit, i.e., the spatial component of the radial-maze task (Kesenberg and Schmidt, 1995).

In agreement with previous reports, MK-801-treated and control groups were found to be similar in retention performance (7 days later) in the spatial-maze test (Heale and Harley, 1990; McLamb et al., 1990; Wozniak et al., 1990; Gorter and de Bruin, 1992; Brosnan-Watters et al., 1999). Thus, bearing in mind the hypothesis that NMDA receptors are crucial for the initiation of synaptic modification underlying place learning, but are not essential for retrieval of place information (Heale and Harley, 1990; Cain et al., 1997; Cain, 1998), our data demonstrate that chronic NMDA receptor blockade by a low dose of MK-801 disturbs predominantly a proper place learning processing.

Acquisition of reversal version of the task required 2 days similarly in both MK-801-treated and control groups. Caramanos and Shapiro (1994) reported that in rats treated with MK-801 working and reference memory in the radial-maze learning test were normal in the familiar environment but were impaired in an unfamiliar environment. Authors concluded that the mnemonic effect of NMDA antagonists depends on environmental familiarity. Similarly in another study on mice, Brosnan-Watters et al. (1999) interpreted nonassociative effects of MK-801 as not prominent because the same dose did not impair holeboard performance at 5 h posttreatment when the task was well learned. It might be suggested that in our study MK-801-treated young rats displayed the same learning capacity in the reversal phase of learning as controls due to well-known context and similar task used. Thus, it can be speculated that attention deficit, first of all, caused slower learning in chronic MK-801-treated rats.

According to the hypothesis of Carlsson and Carlsson (1990), which suggests one of the main functions of striatum is to open thalamic filter for sensory input involved in the control of the degree of arousal using the striato-pallido-thalamic projections, neural abnormalities after long-lasting NMDA blockade could appear at the thalamic as well as at the striatal and/or cortex levels of sensory informational processing. While it is not totally clear what brain region would be primarily affected by chronic neonatal treatment with a low dose of MK-801, a significant alteration of neural circuits that are being developed during this period could be expected. Thus, the major finding of this study that a specific (although not long lasting) pattern of cognitive abnormalities can be induced even by mild treatment with NMDA antagonist gives further support to the contention that NMDA antagonists provide a comprehensive pharmacological model of behavioral abnormalities related to schizophrenia (Greene, 2001; Andiné et al., 1999).

5. Conclusions

In conclusion, chronic NMDA receptor blockade during the critical period of maturation of the glutamatergic brain system produces locomotor hypoactivity, decreased anxiety level and learning impairment in young rat. At the same time, MK-801-treated animals were able to learn and respond to stressful stimuli in an adequate manner. Behavioral irregularities appear to be transient, as they cannot be detected at the age of 16 weeks. Disturbances in sensory information processing on cortical level, as well as on basal ganglia level, might be suggested as a putative substrate for behavioral abnormalities observed.

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