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Research report

Different MK-801 administration schedules induce mild to severe learning impairments in an operant conditioning task: Role of buspirone and risperidone in ameliorating these cognitive deficits

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HIGHLIGHTS

- Posnatal Mk-801 injection induce severe learning deficits in an operant task.
- Buspirone and risperidone failed to ameliorate these deficits.
- Mk-801 injection disrupts basic mechanism of PFC mediated learning.

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ABSTRACT

Blockade of *N*-methyl-D-aspartate receptor (NMDA) by the noncompetitive NMDA receptor (NMDAR) antagonist MK-801 produces behavioral abnormalities and alterations in prefrontal cortex (PFC) functioning. Due to the critical role of the PFC in operant conditioning task learning, we evaluated the effects of acute, repeated postnatal injections of MK-801 (0.1 mg/kg) on learning performance.

We injected Long-Evans rats i.p. with MK-801 (0.1 mg/kg) using three different administration schedules: injection 40 min before beginning the task (during) ($n = 12$); injection twice daily for six consecutive days prior to beginning the experimental procedures (prior) ($n = 12$); or twice daily subcutaneous injections from postnatal day 7 to 11 (postnatal) ($n = 12$). Next, we orally administered risperidone (serotonin receptor 2A and dopamine receptor 2 antagonist, 1 mg/kg) or buspirone (serotonin receptor 1A partial agonist, 10 mg/kg) to animals treated with the MK-801 schedule described above.

The postnatal and prior administration schedules produced severe learning deficits, whereas injection of MK-801 just before training sessions had only mild effects on acquisition of an operant conditioning. Risperidone was able to reverse the detrimental effect of MK-801 in the animals that were treated with MK-801 during and prior training sessions. In contrast, buspirone was only effective at mitigating the cognitive deficits induced by MK-801 when administered during the training procedures.

The data demonstrates that NMDA antagonism disrupts basic mechanisms of learning in a simple PFC-mediated operant conditioning task, and that buspirone and risperidone failed to attenuate the learning deficits when NMDA neurotransmission was blocked in the early stages of the postnatal period.

1. Introduction

Disruption of N-methyl-D-aspartate (NMDA) neurotransmission by administration of antagonists produces abnormalities in the circuitry of the prefrontal cortex (PFC), and deficits in PFC-mediated behaviors [1–6]. The PFC has a critical role in processes that include behavioral flexibility, working memory, and decision-making and goal-directed behaviors [7–9]. Recently, we have shown that in rats trained in an operant conditioning that involves a lever press to obtain food as a reward, the PFC undergoes a series of molecular, biochemical, and cellular modifications [10–12]. Neuronal loss in the medial PFC by local injection of ibotenic acid has been showed

Abbreviations: PFC, prefrontal cortex; NMDA, *N*-methyl-D-aspartate; NMDAR, *N*-methyl-D-aspartate receptor; 5-HT2A, serotonin receptor 2A; 5-HT1A, serotonin receptor 1A; D2R, dopamine receptor 2; P7, postnatal day 7; P11, postnatal day 11.

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to impair learning of operant behaviors [13]. These observations were confirmed by electrophysiological recording within the PFC that showed a key role for this area during the acquisition of an operant conditioning task [14].

Prior or post-training administration of the NMDAR antagonist MK-801 impaired visual memory and retrieval [15,16]. Similarly, use of MK-801 in reversal learning protocols resulted in detrimental effects on behavioral flexibility [17–19]. In this sense, systemic and local administration of MK-801 in a 5-choice serial reaction time task resulted in a reduction of choice accuracy and negatively influenced behavioral flexibility [20,21]. The effects of postnatal blockade of NMDAR during a critical period of neurodevelopment in PFC circuitry produced long-lasting cognitive deficits in visual recognition and flexibility tasks [17]. Additionally, acute injections of NMDA antagonist have been shown to have deleterious effects on the extinction of the instrumental response [22,23]. Interestingly, healthy volunteers injected with the NMDA antagonist ketamine recapitulate part of the cognitive impairment observed in schizophrenia patients [2]. Based on clinical evidence and considering that NMDA antagonist administration in rodents mimics various aspects of cognitive impairments in schizophrenia [24], it is critical for future translational studies to unravel the underlying mechanisms of these cognitive impairments, and to test potential pharmacological agents to improve cognitive deficits.

Numerous studies suggest that serotonin receptor 1A (5-HT1A) and 2A (5-HT2A) are potential targets to ameliorate the learning deficits produced by NMDA antagonism due to their direct effects on PFC neurons [25,26]. Supporting this hypothesis, risperidone [a 5-HT2A antagonist and a dopamine receptor 2 (D2R) antagonist] and buspirone (a 5-HT1A partial agonist) successfully reversed the deleterious effects of NMDA antagonism in a place-avoidance task and reversal learning protocols [27,28]. However, cognitive abilities in patients with schizophrenia were improved when risperidone was administered; this is still controversial since there are studies that suggest that the effects of risperidone on cognition of patients are very modest [29–33]. Therefore, the aim of this study was to determine whether MK-801 administration in adulthood and postnatally induce different degrees of learning deficits in an operant conditioning task, and to test if 5-HT1A stimulation and 5-HT2A/D2R blockade could ameliorate these deficits. This study provides additional insight into the understanding of the mechanisms of NMDA neurotransmission modulation in a simple reward-dependent task.

2. Material and methods

All experimental procedures were approved by the Ethics Committee of the IByME-CONICET (A2008) and were conducted according to the NIH Guide for Care and Use of Laboratory Animals.

2.1. Animals

Two-month-old male Long-Evans rats (250–300 g, IBYME-CONICET) were used for behavioral testing. Pups on postnatal day 7 were assigned to an experimental group and then after being weaned, littermates were kept together until the beginning of experimental procedures. Animals were housed in stainless-steel cages (40 cm × 22 cm × 20 cm, L × W × H) with sawdust as bedding, and metal lids. The room temperature was maintained at 21 ± 2 °C with a 12/12 h light/dark cycle (lights on at 8 a.m.). Rats were handled for at least 10 min every day and then weighed as a habituation routine to the operator. Handling of experimental animals started 15 days before beginning of behavioral procedures when animal's age was ~45 days old. Animals were assigned randomly but with the restrictive criteria of having only one animal of each litter into an experimental group to avoid a cage effect. Experimental subjects were singly housed to maintain a precise control of their body weight during behavioral testing, and to avoid male dominance issues that could lead to reduced food ingestion and fluctuations in body weight.

2.2. Drugs

Rats were injected intraperitoneally with 0.1 mg/kg (per injection, working dilution 0.1 mg/ml) of MK-801 (Sigma–Aldrich) in saline solution. To evaluate the effects of MK-801 administration during the training sessions of the operant conditioning task, a group of animals was injected 40 min prior to training sessions ($MK\text{-}801_{during}$). Control animals were injected with 1 ml/kg of saline solution. For studying the effects of prior administration of MK-801 on learning performance, rats were injected daily for 6 consecutive days (between 9 and 11 a.m.) prior to the beginning of training sessions ($MK\text{-}801_{prior}$) [34,35]. Control animals were injected with 1 ml/kg of saline solution.

Postnatal administration of MK-801 was performed as previously described by Stefani and Moggadham (2005) [1]. Pups received two daily subcutaneous injections (9 a.m. and 4 p.m.) from postnatal day 7 (P7) to 11 (P11) ($MK\text{-}801_{postnatal}$), whereas control animals were subcutaneously injected with 1 ml/kg of saline solution. Rats with this administration schedule were tested when 2 months old. There were no differences in body weight or food intake between controls and treated groups at the time that the experiments were performed. MK-801 doses and administration schedules were based on previous evidence demonstrating that they induced abnormalities in PFC functioning and deleterious effects on learning [5,6,34–37]. Risperidone (Gador, Argentina) and buspirone (Raffo, Argentina) were orally administered (1 mg/kg and 10 mg/kg, respectively) in PBS (pH 7.5) 1 h prior to training sessions. The working solution of risperidone was 33 mg/ml, whereas, buspirone working solution was 3 mg/ml.

2.3. Operant conditioning task

All behavioral procedures were performed during the light cycle, and the operant conditioning task training was performed in a standard operant chamber (MED associates Inc., St. Albans, Vermont, USA) equipped with an input (DIG 710/711) and output (DIG 720/721/722) card for data acquisition and processing, one automated retractable lever, white house light, context red light, white noise (random signal with a flat power spectral density), and an automated feeder. All animals were housed singly and handled every day for at least 12 days before the beginning of the habituation process. Rats were then food restricted to maintain ~80% of their *ad libitum* body weight for 3 days before training and throughout the experiments. During the period of food restriction and experimental procedures, animals were fed with rat chow plus the same pellets used during training sessions to avoid palatability issues during the experiments.

Before habituation and the training sessions, animals were first placed in the training room for 10 min followed by 20 min of habituation in the operant chamber to acclimate to the new environment for 2 days. In the habituation session, rats in the operant chamber were only exposed to context red light and white noise, and fed with 25 pellets (45 mg, BioServe) given randomly by the automated feeder. Only one habituation session was performed.

Before starting the training procedure, the operant chamber remained with the lever retracted, the house light on, white noise, and a red context light that remained on at all times. An operant conditioning task training session with a fixed ratio of 1 consisted of 25 trials. Each trial began with the delivery of the lever (60 s) and the house light was turned off. If the animal pressed the lever

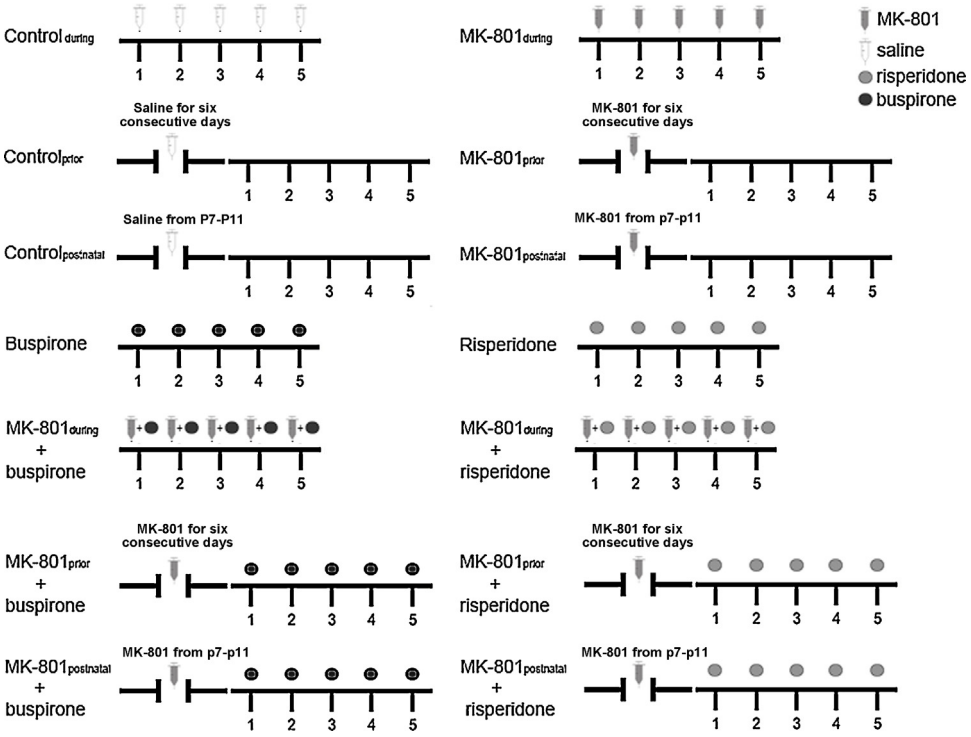

Fig. 1. Experimental design and administration schedule.
White syringes indicate saline injection, whereas grey syringes indicate a MK-801 injection (0.1 mg/kg). MK-801 was injected 40 min prior to the start of the training session ($MK\text{-}801_{during}$), daily for 6 consecutive days prior to the beginning of the training sessions ($MK\text{-}801_{prior}$), or between postnatal days 7 and 11 ($MK\text{-}801_{postnatal}$). Saline injections were performed with the same schedule used for MK-801 administration. Buspirone and risperidone were orally administered 1 h before training session. All animals had five training sessions as can be observed in the figure. Experimental groups were as follows: $Control_{during}$ ($n = 12$), $Control_{prior}$ ($n = 12$), $Control_{postnatal}$ ($n = 12$), $MK\text{-}801_{during}$ ($n = 12$), $MK\text{-}801_{during}$ + buspirone ($n = 12$), $MK\text{-}801_{during}$ + risperidone ($n = 12$), $MK\text{-}801_{prior}$ ($n = 12$), $MK\text{-}801_{prior}$ + buspirone ($n = 12$), $MK\text{-}801_{prior}$ + risperidone ($n = 12$), $MK\text{-}801_{postnatal}$ ($n = 12$), $MK\text{-}801_{postnatal}$ + buspirone ($n = 12$), $MK\text{-}801_{postnatal}$ + risperidone ($n = 12$), buspirone ($n = 12$), and risperidone ($n = 12$).

within 60 s, the lever retracted, and it received a pellet of 45 mg as a reward. A pellet was delivered 1 s after pressing the lever; this was coupled to the activation of a white light inside the feeder for 2 s. When the trial finished, the white house light turned on and the lever remained retracted for 20 s. The action of pressing the lever was considered as a correct response. It is important to remark that the animals were able to press the lever only once in a trial. When the animals did not respond during the trial, no reward was given. The percentage of responses in a session was calculated by counting the total number of lever presses in a training session and then divided by 25 (maximum number of lever presses that can be performed in a session) and then multiplied by 100. The latency time of a response represents the time between the lever delivery and the lever press of the animal. If no response was performed during the trial, the latency time of response was considered as 60 s. Experimental groups and administration schedules are described in Fig. 1.

2.4. Statistical analysis

Statistical analysis was performed using SPSS 19.0 (IBM-SPSS, USA). Values are expressed as means ± SEM and compared using Friedman's non-parametric two-way ANOVA by ranks followed by the Wilcoxon signed test with a Bonferroni correction. Data analysis was performed with different sub-sets of groups. First, we compared $MK\text{-}801_{during}$, $MK\text{-}801_{prior}$ and $MK\text{-}801_{postnatal}$ with their respective control groups. Second, we compare the different MK-801 administration schedules between each other. Finally, we contrasted $MK\text{-}801_{during}$, $MK\text{-}801_{prior}$ and $MK\text{-}801_{postnatal}$ treated with buspirone or risperidone with the animals that received this MK-801 administration schedule without treatment and with their respective control groups. Differences among experimental conditions were considered statistically significant if $p < 0.05$.

3. Results

3.1. Effects of MK-801administration schedules on learning an operant conditioning task

MK-801 administration schedule and buspirone or risperidone treatment is summarized in Fig. 1. Statistical analysis of the percentage of responses by Friedman's test showed an overall effect [$\chi^2 = 201.359$, $p < 0.001$]. For the latency time analysis, Friedman's test showed an overall significant effect [$\chi^2 = 227.092$, $p < 0.001$].

First, we studied the effects of an acute injection of MK-801 40 min before starting the experimental procedures. Animals treated with MK-801 showed a decreased percentage of responses in the second session compared to the control group ($p < 0.05$; Fig. 2A). Latency times were higher in the group treated with MK-801. We found that in the second ($p < 0.01$), third ($p < 0.01$), and fourth ($p < 0.05$) sessions, there was a significant increment in the latency time of $MK\text{-}801_{during}$ group compared to the control group (Fig. 2B).

Next, we evaluated how MK-801 administration prior ($MK\text{-}801_{prior}$ group) to experimental procedures affected learning performance. In the first training session of the operant conditioning task, the $MK\text{-}801_{prior}$ group had a lower percentage of responses relative to the control group ($p < 0.05$; Fig. 3A). Likewise, the percentage of responses in the second ($p < 0.01$) and third ($p < 0.01$) session animals from the $MK\text{-}801_{pior}$ group was lower than that of the control animals ($p < 0.001$; Fig. 3A). Latency times were significantly increased by this administration schedule. We found that

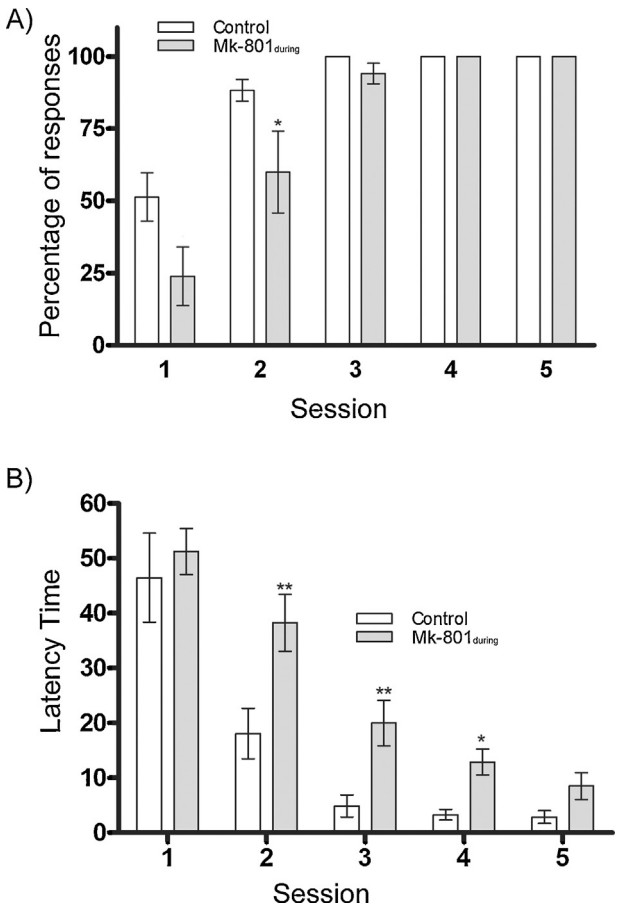

Fig. 2. MK-801$_{during}$ administration (40 minutes before starting training sessions) produces mild deficits on learning of an operant conditioning task.
Percentage of responses is expressed as the mean ± SEM of the total of lever pressings in a training session of 25 trials. Latency time is expressed as the mean ± SEM as the time that elapses between presentation of the conditioned stimulus and occurrence of the lever pressing (panel B). $^{*}p < 0.05$, $^{***}p < 0.001$. Friedman's non-parametric two-way ANOVA by ranks followed by Wilcoxon signed test with a Bonferroni correction.

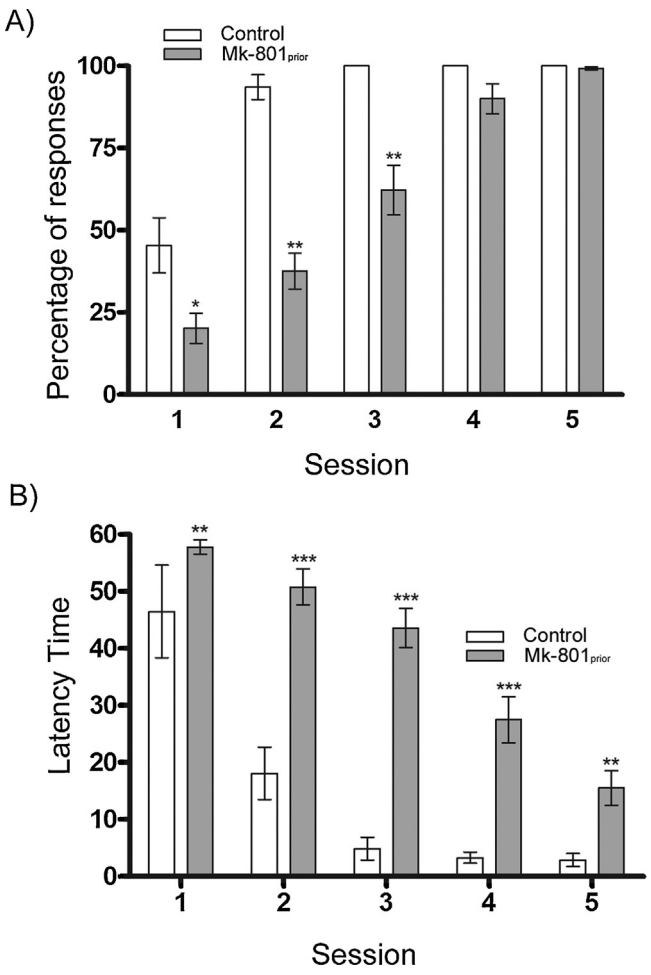

Fig. 3. Six days of consecutive injections of MK-801 prior to training (MK-801$_{prior}$) generate moderate learning impairments.
Effects of MK-801prior administration schedule on the percentage of responses (panel A) and latency time (panel B). Percentage of responses is expressed as the mean ± SEM of the total of lever pressings in a training session of 25 trials. Latency time is expressed as the mean ± SEM. as the time that elapses between presentation of the conditioned stimulus and occurrence of the lever pressing (panel B). $^{**}p < 0.01$, $^{***}p < 0.001$. Friedman's non-parametric two-way ANOVA by ranks followed by Wilcoxon signed test with a Bonferroni correction.

in animals from MK-801$_{prior}$ the latency times were significantly increased compared to animals from the control group across all training sessions (Fig. 3B).

Postnatal injection of MK-801 also induced severe learning deficits. Animals in this group showed a decrease in the percentage of responses in the first ($p < 0.05$) and second ($p < 0.001$) sessions compared to control animals (Fig. 4A). In the third session, a decrement in the percentage of responses by MK-801$_{postnatal}$ relative to controls was detected ($p < 0.001$; Fig. 4A). Then, in the fourth session, there was a decrease in the MK-801$_{postnatal}$ group compared to the control group ($p < 0.01$; Fig. 4A). Compared with the control group, the MK-801 postnatal group had a higher response latency in all but the first session (Fig. 4A). MK-801 injections did not affect the body weight (data not shown).

Analysis of the MK-801$_{during}$ percentage of responses showed an increment in the third session compared to the MK-801$_{prior}$ group ($p < 0.001$; Fig. 5A). Additionally, we found that MK-801$_{during}$ had a higher percentage of responses compared to the MK-801$_{posnatal}$ group in the third ($p < 0.01$) and fourth ($p < 0.01$) sessions (Fig. 5A). Further, we found that the MK-801$_{postnatal}$ group had an increase in the percentage of responses in the third ($p < 0.05$) and fourth sessions ($p < 0.05$) compared with the MK-801 group (Fig. 5A). Next, MK-801$_{during}$ mean latency time was shown to be higher in the third ($p < 0.05$) and fourth ($p < 0.01$) sessions compared to MK-801$_{prior}$ (Fig. 5B). Latency times were lower in the second ($p < 0.05$), third ($p < 0.01$), and fourth ($p < 0.01$) sessions of the MK-801$_{during}$ group compared with the MK-801$_{postnatal}$ group (Fig. 5B).

3.2. *Effects of buspirone on MK-801 learning induced deficits*

Buspirone administration *per se* did not produce effects in the percentage of responses (Fig. 6A). However, there was a significantly increased average latency time in the second ($p < 0.01$), third ($p < 0.01$), fourth ($p < 0.01$), and fifth ($p < 0.05$) sessions compared to the control group (Fig. 6B).

There were no significant differences in the percentage of responses across training sessions among the control and MK-801$_{during}$ + buspirone groups (Fig. 6C). Additionally, no differences were found between MK-801$_{during}$ and MK-801$_{during}$ + buspirone groups (6 C). Statistical analysis of the latency time showed that there was an increment in the MK-801$_{during}$ + buspirone group compared to control group in the second ($p < 0.05$), third ($p < 0.05$), and fourth ($p < 0.01$) sessions (Fig. 6D).

We found that the MK-801$_{prior}$ group had a lower percentage of responses in the first ($p < 0.05$), second ($p < 0.05$), and third ($p < 0.05$) sessions with respect to the MK-801$_{prior}$ + buspirone group (Fig. 6E). Analysis of the latency time showed a difference in the second

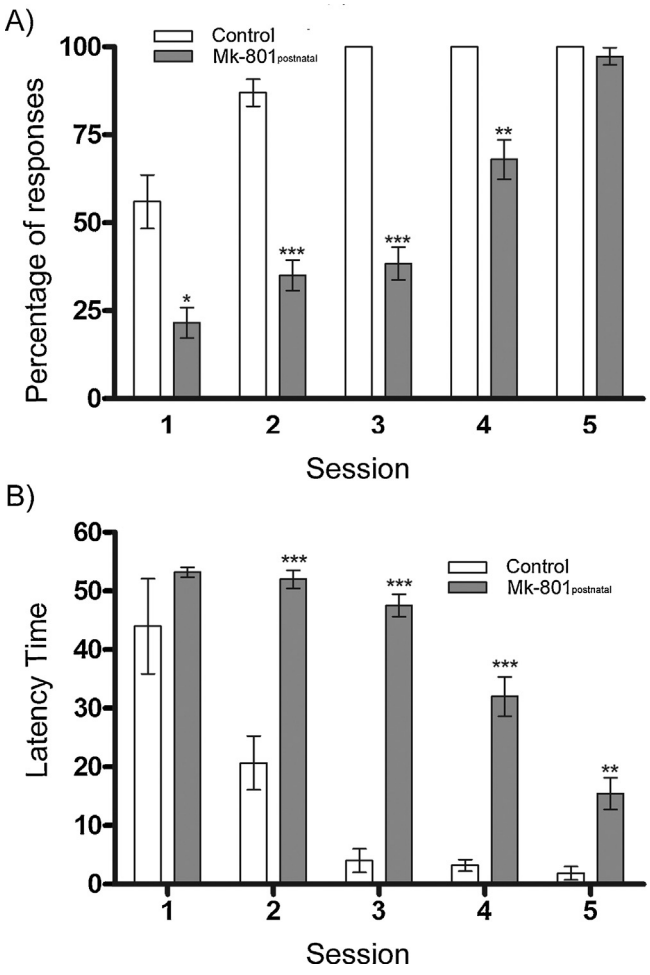

Fig. 4. Postnatal administration of MK-801 (MK-801$_{postnatal}$) results in severe learning deficits.
A strong decrease in the percentage of responses was found in the MK-801$_{postnatal}$ group during all training sessions (panel A) and an increase in the latency time (panel B). Percentage of responses is expressed as the mean ± SEM of the total of lever pressings in a training session of 25 trials. Latency time is expressed as the mean ± SEM as the time that elapses between presentation of the conditioned stimulus and occurrence of the lever pressing (panel B). $^{***}p < 0.001$. Friedman's non-parametric two-way ANOVA by ranks followed by Wilcoxon signed test with a Bonferroni correction.

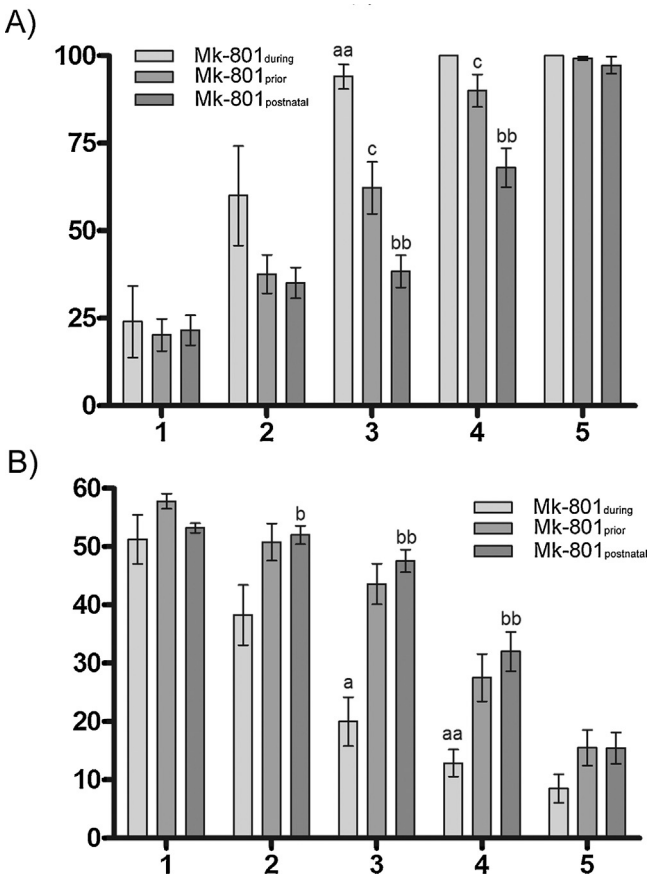

Fig. 5. Postnatal administration of MK-801 generates the most severe learning impairments.
Comparison between the average learning performance (panel A) and latency time (panel B) of MK-801$_{during}$, MK-801$_{prior}$, and MK-801$_{postnatal}$ groups. $^{a}p < 0.05$, $^{aa}p < 0.01$, $^{aaa}p < 0.001$ (MK-801$_{during}$ vs. MK-801$_{prior}$). $^{b}p < 0.05$, $^{bb}p < 0.01$, $^{bbb}p < 0.001$ (MK-801$_{during}$ vs. MK-801$_{postnatal}$). $^{c}p < 0.05$, $^{cc}p < 0.01$, $^{ccc}p < 0.001$ (MK-801$_{prior}$ vs. MK-801$_{postnatal}$). Friedman's non-parametric two-way ANOVA by ranks followed by Wilcoxon signed test with a Bonferroni correction.

($p < 0.01$), third ($p < 0.01$), fourth ($p < 0.01$), and fifth ($p < 0.01$) sessions between the MK-801$_{prior}$ + buspirone and MK-801$_{prior}$ groups (Fig. 6F).

Next, we observed that MK-801$_{postnatal}$ + buspirone had an increase in the percentage of responses across the second ($p < 0.05$), third ($p < 0.05$), and fourth ($p < 0.05$) sessions compared to MK-801$_{postnatal}$ (Fig. 6G). A comparison carried out between control and MK-801$_{postnatal}$ + buspirone showed a significant increase in the second session ($p < 0.05$) compared control group (Fig. 6G). A significant decrease of the control group's latency time was found in the second ($p < 0.01$), third ($p < 0.01$), and fourth ($p < 0.05$) sessions when it was contrasted with MK-801$_{postnatal}$ + buspirone group (Fig. 6H). Additionally, we found that MK-801$_{postnatal}$ + buspirone latency time was lower than in the MK-801$_{postnatal}$ group across the second ($p < 0.05$), third ($p < 0.01$), fourth ($p < 0.01$), and fifth sessions ($p < 0.05$; Fig. 6H).

3.3. Effects of risperidone on MK-801 learning induced deficits

Risperidone *per se* totally impaired learning of the task as treated animals were not able to surpass 20% of responses across all training sessions (Fig. 7A). Latency times of the risperidone group across training sessions were strongly increased compared to the control group (Fig. 7B).

There was no significant difference in the percentage of responses in MK-801$_{during}$ and MK-801$_{during}$ + risperidone (Fig. 7C). Also, no difference was detected between the control and MK-801$_{during}$ + risperidone groups (Fig. 7C). Then, we found that the MK-801$_{during}$ + risperidone group had an increment in the latency time compared to the MK-801$_{during}$ group across the second ($p < 0.05$), third ($p < 0.01$), and fourth ($p < 0.01$) sessions (Fig. 7D).

An increment in the MK-801$_{prior}$ percentage of responses was found in the second ($p < 0.05$) session compared to the MK-801$_{prior}$ + risperidone group (Fig. 7E). The control group showed a higher percentage of responses in the second and third session compared to the MK-801$_{prior}$ + risperidone group (Fig. 7E). The average latency time of the MK-801$_{prior}$ + risperidone group was higher than the control group in the second ($p < 0.05$) and third ($p < 0.05$) sessions (Fig. 7F). The MK-801$_{prior}$ group had an increment with respect to the MK-801$_{prior}$ + risperidone group across the second ($p < 0.05$), third ($p < 0.05$), fourth ($p < 0.05$), and fifth ($p < 0.05$) sessions (Fig. 7F).

There was a decrement in the MK-801$_{postnatal}$ group compared to the MK-801$_{postnatal}$ + risperidone group in the second ($p < 0.05$) and third ($p < 0.05$) session (Fig. 7G). Besides, we found a decrement in the MK-801$_{postnatal}$ group in the second ($p < 0.05$) and third ($p < 0.05$) sessions when compared to the

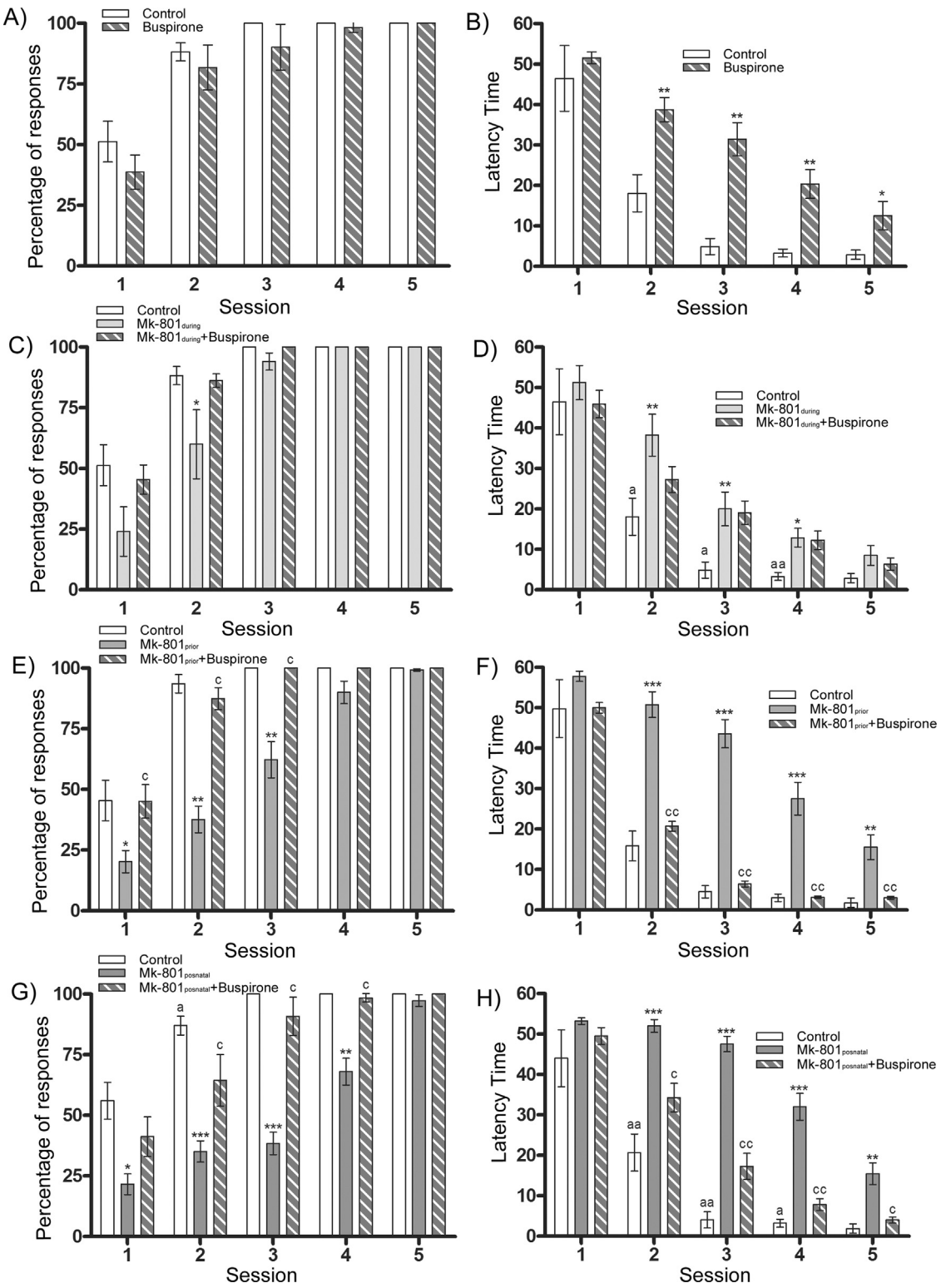

Fig. 6. Buspirone fails to revert the deleterious effects of the three MK-801 administration schedules.
Behavioral consequences of buspirone administration (panels A and B). Learning performance and latency time of MK-801$_{during}$ (panels C and D), MK-801$_{prior}$ (panels E and F) and MK-801$_{postnatal}$ (panels G and H) groups treated with buspirone. $^{*}p<0.05$, $^{***}p<0.001$ (control vs. MK-801$_{during}$, MK-801$_{prior}$ or MK-801$_{postnatal}$). $^{c}p<0.05$, $^{ccc}p<0.001$ (comparisons between MK-801$_{during}$ vs. MK-801$_{during}$ + buspirone, MK-801$_{prior}$ vs. MK-801$_{prior}$ + buspirone, MK-801$_{postnatal}$ vs. MK-801$_{postnatal}$ + buspirone). $^{a}p<0.05$, $^{aa}p<0.01$, $^{aaa}p<0.001$ (control vs. MK-801$_{during}$ + buspirone, MK-801$_{prior}$ + buspirone or MK-801$_{postnatal}$ + buspirone). Friedman's non-parametric two-way ANOVA by ranks followed by Wilcoxon signed test with a Bonferroni correction.

MK-801$_{postnatal}$ + risperidone group (Fig. 7G). The average latency time of the MK-801$_{postnatal}$ group was higher than the MK-801$_{postnatal}$ + risperidone group across the second ($p<0.05$), third ($p<0.05$), fourth ($p<0.05$), and fifth ($p<0.05$) sessions (Fig. 7H). Afterwards, we observed that the latency time of the MK-801$_{postnatal}$ + risperidone group compared to the control group was decreased in the second ($p<0.05$), third ($p<0.01$), and fourth ($p<0.01$) sessions (Fig. 7H).

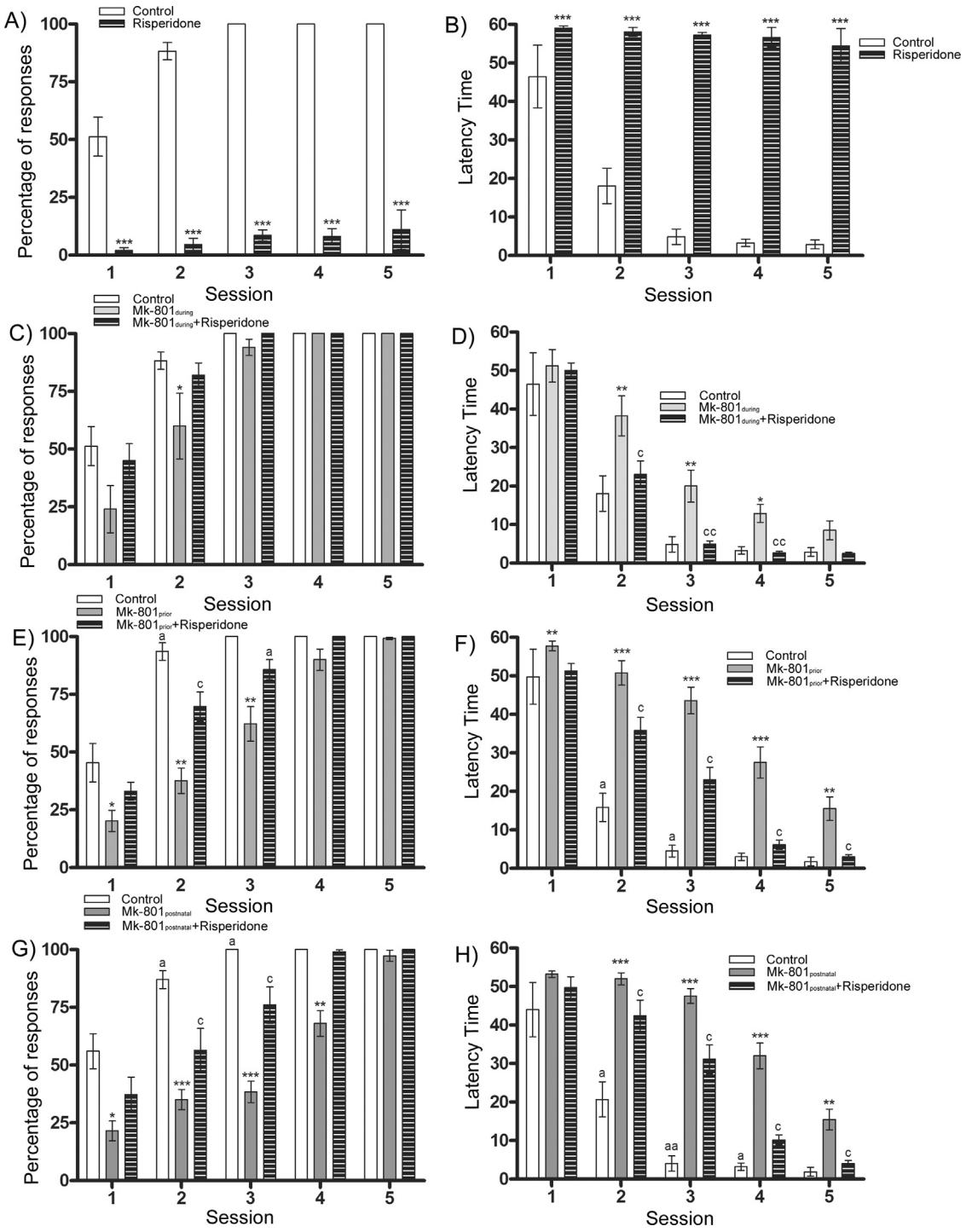

Fig. 7. Risperidone ameliorates cognitive deficits in MK-801$_{during}$ and MK-801$_{prior}$ groups.
Negative effects of risperidone administration on learning performance (panels A and B). Risperidone abolishes the effects of NMDA antagonist administration in MK-801$_{during}$ (panels C and D) and MK-801$_{prior}$ groups (panels E and F). In contrast, risperidone was not able to improve cognition in animals with MK-801 postnatal (panels G and H). $^{*}p < 0.05$, $^{***}p < 0.001$ (control vs. MK-801$_{during}$, MK-801$_{prior}$ or MK-801$_{postnatal}$). $^{ccc}p < 0.001$ (comparisons between MK-801$_{during}$ vs. MK-801$_{during}$ + risperidone, MK-801$_{prior}$ vs. MK-801$_{prior}$ + risperidone, MK-801$_{postnatal}$ vs. MK-801$_{postnatal}$ + risperidone). $^{a}p < 0.05$, $^{aa}p < 0.01$, $^{aaa}p < 0.001$ (control vs. MK-801$_{during}$ + risperidone, MK-801$_{prior}$ + risperidone or MK-801$_{postnatal}$ + risperidone). Friedman's non-parametric two-way ANOVA by ranks followed by Wilcoxon signed test with a Bonferroni correction.

4. Discussion

The results presented here show that NMDA antagonism under different administration schedules induces mild to severe learning deficits in an operant conditioning task. These learning deficits were more severe when MK-801 was administered during the postnatal period, a critical phase of PFC development. We propose that the reduction on the percentage of responses observed are related to a learning deficit, since acute or postnatal MK-801 injections did not produce significant changes in motor activity [1,38] and that only a slight increase in activity was observed with the repeated administration schedule [35]. Additionally, we also discard an effect of MK-801 in motivation for food reward based on the evidence that showed no reduction in the motivation for obtaining food,

where in fact motivation for food was increased only when animals were food deprived [39,40]. The results presented here suggest that NMDA antagonism is generating a disruption in basic mechanisms of cognition. These deleterious effects on cognition were observed in more complex tasks [17,41].

The protocol that consisted of giving a daily injection of MK-801 prior to the training sessions resulted in mild deficits in the acquisition of the operant conditioning task. The learning performance of these animals was reduced only in the second session, but even when these animals reached a high percentage of responses in the last three sessions, the latency times were substantially higher than in the control group. The findings in this manuscript are in agreement with those observed with a reduced operant response after acute MK-801 in different tasks [42]. However, our results are not in accordance with previous studies that showed that there were no effects of MK-801 administration in the acquisition of an operant conditioning [22,23]. However, we conclude that this discrepancy relies on the fact that the protocol used here has a fixed number of trials per session whereas in these previous manuscripts animals had to reach more than one hundred lever presses to pass to the test phase.

Next, we studied whether risperidone and buspirone could lead to a reduction in the learning deficits produced by abnormal NMDA neurotransmission. Firstly, we found that buspirone administration was partially effective in ameliorating the learning deficits induced by MK-801. Although animals treated with buspirone showed no difference from controls with respect to the percentage of responses, the latency times remained increased in the treated group. This weak effect of buspirone to attenuate the effects of acute MK-801 was also observed in animals trained in a radial arm maze task [43]. In addition we found, as previously reported, that in non-reward-dependent tasks buspirone *per se* had deleterious effect on learning [44,45]. The *per se* deleterious effects of risperidone on the operant conditioning task performance are not related to reduced motor activity, since it has been proved that the dose used in this study did not reduce locomotor activity [46]. However, it is difficult to dissociate the effects of risperidone on 5-HT2A and D2R receptors in this task because blockade of these receptors results in decreased operant responding [47–49].

Next, we found that oral administration of risperidone totally abolished the effects produced by acute MK-801 injection and that animals from this group showed no difference in the percentage of responses and latency with respect to controls. The effectiveness of risperidone at reversing the learning deficits produced by NMDA antagonism was observed in the Morris water maze, elevated plus maze, and avoidance task [27,50]. The fact that our results are in agreement with these previous reports suggests that risperidone ameliorates the deleterious effects of MK-801 acute administration.

It is well documented that repeated injections of NMDA antagonists interfere with the functioning of PFC circuitry and produce a series a series of biochemical and molecular alterations [36,51–53]. In the behavioral data presented here, repeated injections of MK-801 produced a learning deficit. Animals belonging to this group were able to reach 100% only in the last session, and latency times were substantially increased throughout the experimental procedure. In our operant conditioning task, the repeated injections of MK-801 resulted in a decreased learning performance compared to the acute administration schedule. Previously, it has been shown that a repeated injections schedule produces lasting alterations in the fast-spiking interneurons and reduces the density of these cells [51,52]. For this schedule of administration, buspirone was efficient in reverting the effects of MK-801 in rats. Also, it has been reported that buspirone, at a lower dose than the one used here, significantly attenuated the deleterious effect in a novel object recognition and a reversal operant conditioning task of repeated phencyclidine (injections [41,54]). Surprisingly, risperidone only partially attenuated the learning deficits found in animals repeatedly injected with MK-801. In this sense, previous reports showed that risperidone was able to revert the MK-801 deleterious effects in an avoidance task, but not disruption of the prepulse inhibition by this NMDA antagonist [27,55].

Postnatal administration of MK-801 between P7 and P11 resulted in severe learning deficits. Disruption of NMDA neurotransmission during this critical period of neurodevelopment in the PFC generated long-lasting effects. The percentage of responses of these animals between the first and fourth sessions was remarkably lower compared to controls, and the mean latency time remained significantly higher in all training sessions. Blockade of NMDAR during this period generates loss of the expression of parvalbumin in the fast-spiking neurons [56] and disrupts prepulse inhibition and learning of a set-shifting task [17,57,58]. Animals treated with these doses of buspirone or risperidone showed a small amount of improvement from the postnatal injections of MK-801; nevertheless, animals continued to show learning deficits compared to the controls. This is the first evidence that risperidone and buspirone fails to reverse the neurodevelopmental abnormalities in the PFC produced by exposure to MK-801 between P7 and P11. There is a general consensus that chronic, postnatal, and peri-adolescent injections of NMDA antagonist mimic some of the cognitive deficits observed in schizophrenia [24,59]. As discussed previously, the results obtained for buspirone and risperidone ameliorating the effects of NMDA antagonism in animal models are not consistent since they depend on the task and the administration schedule and dose of the NMDA antagonist. Buspirone and risperidone has been used to improve cognition in schizophrenic patients [60]. Nevertheless, schizophrenia patients showed no improvement in their cognitive abilities, even when buspirone was concomitantly administered with other atypical antipsychotics [60,61]. Although, clinical studies showed that risperidone enhanced cognition in schizophrenia and early psychosis, the effectiveness to improve cognition is still under debate due to the modest effects produced in patients [62–64].

5. Conclusions

The behavioral evidence presented here showed that NMDA antagonism disrupted basic mechanisms of learning an operant conditioning task. Specifically, we found that postnatal injections of MK-801 produced severe learning deficits that neither buspirone nor risperidone were able to attenuate. The results show that buspirone and risperidone are still useful pharmacological agents to ameliorate PFC abnormal function due to NMDA antagonism. However, further studies will be necessary to determine if these deficits can be attenuated by using other pharmacological agents that improve cognition concomitantly with buspirone or risperidone.

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