EFFECTS OF THE NITRIC OXIDE SYNTHASE INHIBITOR L-NAME ON RECOGNITION AND SPATIAL MEMORY DEFICITS PRODUCED BY DIFFERENT NMDA RECEPTOR ANTAGONIST IN THE RAT

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Running title: effects of L-NAME on NMDA blockade

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Abstract

There is consistent experimental evidence that non-competitive antagonists of the NMDA receptor, such as ketamine, MK-801, and phencyclidine (PCP) impair cognition and produce psychotomimetic effects in rodents. Nitric oxide (NO) is considered as an intracellular messenger in the brain. The implication of NO in learning and memory is well documented. The present study was designed to investigate the ability of the NO synthase (NOS) inhibitor L-NAME to antagonize recognition and spatial memory deficits produced by the NMDA receptor antagonists, MK-801 and ketamine in the rat. L-NAME (1-3 mg/kg) counteracted MK-801 (0.1 mg/kg) and ketamine (3 mg/kg)-induced performance impairments in the novel object recognition task. L-NAME (10 mg/kg) attenuated ketamine (15 mg/kg)-induced spatial working memory and retention deficits in the radial water maze paradigm. L-NAME, applied at 3 mg/kg however, disrupted rodents' performance in this spatial memory task. The present findings indicate a) that L-NAME is sensitive to glutamate hypofunction produced by other than PCP NMDA antagonists such as MK-801 and ketamine and b) that L-NAME alone differentially affects rodents' spatial memory.

Key words: L-NAME, MK-801, ketamine, recognition memory, spatial memory, rat

INTRODUCTION

Schizophrenia is a serious mental disorder that affects up to 1% of the population worldwide. Cognitive deficits in schizophrenic patients are core features of the illness, and predict vocational and social disabilities for patients (Freedman, 2003). Numerous studies have indicated that the function of the glutamatergic system, in particular N-methyl-D-aspartate (NMDA) receptors, might be compromised in schizophrenia. Exposure to non-competitive NMDA receptor antagonists like phencyclidine (PCP) or ketamine produces behavioral symptoms in healthy individuals that resemble both positive and negative symptoms of schizophrenia (Javitt and Zukin, 1991; Krystal *et al,* 1994) and exacerbate symptoms in schizophrenic patients (Malhotra *et al,* 1997; Lahti *et al,* 2001**)**. In addition, ketamine, PCP or MK-801 induce schizophrenia-like symptoms, including cognitive deficits, in rodents (Tricklebank *et al,* 1989; Verma and Moghaddam, 1996; de Lima *et al,* 2005). Due to these psychotomimetic properties, NMDA receptor antagonists are widely used as models of schizophrenia in animals (Geyer and Markou, 1994).

Nitric oxide (NO), a soluble, short-lived and freely diffusible gas, is an important intracellular messenger in the brain (Garthwaite, 1991**)**. Reportedly, NO is involved in the mechanisms of synaptic plasticity in the hippocampus (O'Dell *et al,* 1991; Haley *et al,* 1992) and plays an important role in cognition (Prast and Philippu, 2001).

A plethora of experimental data indicates the implication of NO in schizophrenia (Bernstein *et al,* 2005). In this context, it has been observed an abnormal distribution of nitrergic neurons in the frontal and temporal lobes of schizophrenic patients, which may reflect that the normal pattern of neuronal migration during development of the cerebral cortex may be affected in these patients (Akbarian et *al,* 1993**)**. In addition, experimental evidence suggests that polymorphisms in the neuronal nitric oxide synthase (nNOS) gene are associated with schizophrenia and prefrontal cortex (PFC) function in schizophrenic patients (Reif *et al,*

2006). Contradictory results were reported however, concerning the levels of NO metabolites in the serum of schizophrenic patients. Either high (Taneli *et al,* 2004; Yilmaz *et al,* 2007) or low (Ramirez *et al,* 2004**)** concentrations of NO metabolites have been reported.

Since both alterations in the synaptic organization of the brain (Roberts *et al,* 2005) and neurotransmitter deficits (Costa *et al,* 2004) are key features of schizophrenia, excess NO might contribute to disturbed neurocircuitry in this disease. Reportedly, exceeding NO concentrations are associated with neuronal damage (Dawson *et al,* 1991), mitochondrial dysfunction (Das *et al,* 1998) and impairment of the NMDA-receptor mediated neurotransmission (Clinton *et al,* 2003). As a whole, these data suggest that reduced NOS activity in schizophrenia may be neuroprotective. Therefore, efforts are made to develop NOS inhibitors as possible therapeutic tools for schizophrenia (Bernstein *et al,* 2005).

A number of animal studies have demonstrated that diverse NOS inhibitors antagonized PCP-induced psychotomimetic effects (Johansson *et al,* 1997; Klamer *et al,* 2001; Klamer *et al,* 2004; Klamer *et al,* 2005a; Klamer *et al,* 2005b**)**. In this context, it has been proposed that NOS inhibition selectively amends the behavioral effects of PCP and not those of MK-801 (Klamer *et al,* 2005a). In addition, that application of the non-selective NOS inhibitor L-NAME counteracted PCP-induced acquisition deficits in a spatial learning task in the rat has also been reported (Wass *et al,* 2006a; Wass *et al,* 2006b).

Under our experimental conditions, we have observed that L-NAME (1-3 mg/kg) antagonized delay-dependent deficits in the novel object recognition memory task in the rat (Boultadakis *et al,* 2010). At the moment, it is not clear if and how L-NAME is able to counteract recognition memory deficits produced by NMDA hypofunction. In this context, we have observed that two NMDA receptor blockers with different profile in terms of potency or pharmacokinetic properties, MK-801 and ketamine, disrupted animals' performance in the novel object recognition paradigm (Pitsikas *et al,* 2006; Pitsikas *et al,* 2008).

Taken the above evidences into account, the first aim of our study was to evaluate the efficacy of L-NAME in antagonizing MK-801 and ketamine induced-performance deficits in a recognition memory task in the rat. For this purpose, the novel object recognition task was selected. This test is a non-rewarded paradigm based on the spontaneous exploratory behavior of rats (Ennaceur, 2010). Subsequently, the efficiency of L-NAME to counteract ketamine-induced detrimental effects on spatial memory was assessed by using a radial water maze task (Pitsikas *et al,* 2007).

MATERIALS AND METHODS

Animals

Different groups of male (3-month-old) Wistar rats (Hellenic Pasteur Institute, Athens, Greece), weighing 250-300 g, were used in this study. The animals were housed in Makrolon cages (47.5 x 20.5 x 27 cm), three per cage, in a regulated environment (21±1° C, 50-55% relative humidity, 12-h/12-h light/dark cycle, white lights on from 0700 to 1900 hours), with free access to food and water. Experiments were conducted in the room where only these animals where housed, and took place during the light cycle between 1000 and 1400 hours.

Procedures involving animals and their care were conducted in conformity with the international guidelines in compliance with National and International laws and policies. (EEC Council Directive 86/609, JL 358, 1, December 12, 1987; *NIH Guide for Care and Use of Laboratory Animals,* NIH publication no. 85-23, 1985.

Novel object recognition test

The test apparatus and procedure have been extensively described earlier (Boultadakis *et al,* 2010). Before testing, for 3 consecutive days, rats were allowed to explore the apparatus for 2 min. Testing consisted of a session of two 2-min trials. During the “sample” trial (T1), two

identical samples (objects) were placed in two opposite corners of the apparatus in a random fashion. A rat was placed in the middle of the apparatus and was left to explore these two identical objects. After T1, the rat was put back in its home cage and an intertrial interval (ITI) was given. Subsequently, the "choice" trial (T2) was performed. During T2, a new object (N) replaced one of the samples presented in T1, therefore, the rats were re-exposed to two objects: the familiar (F) and the new (N).

The times spent by rats in exploring each object during T1 and T2 were recorded manually by using a stopwatch. From this measure a series of variables was then calculated: the total time spent in exploring the two identical objects in T1, and that spent in exploring the two different objects, (F) and (N) in T2. The discrimination between (F) and the (N) during T2 was measured by comparing the time spent in exploring the (F) with that spent in exploring the (N). As this time may be biased by differences in overall levels of exploration a discrimination index (D) was then calculated; D=N-F/N+F. D is the discrimination ratio and represents the difference in exploration time expressed as a proportion of the total time spent exploring the two objects in T2 (Cavoy and Delacour, 1993). In addition, motor activity of each animal expressed as total number of steps during each trial was also recorded.

Radial water maze test

Spatial reference and spatial working memories were studied using an eight-arm radial water maze procedure modified from Jarrard *et al,* (1984) and Pitsikas and Algeri (1992). The test apparatus has been described before (Pitsikas *et al,* 2007).

Each trial involved placing the rat in the water, facing the wall, at the end of one of the seven unbaited arms (start positions). Each rat was allowed 90 s to escape onto the platform. If it failed to escape within this time, it was guided to the platform. When the rat found the escape platform it was allowed to stay there for 30 s. Then the rat was placed again into the maze for

its next trial, which used the same platform location (e.g., channel 1) and a different starting position as the previous trial. This was repeated until the rat found the platform or 90 s had elapsed whichever occurred first. Each animal received a daily session of 2 trials for 4 consecutive days.

Choices of arms (defined by whole body entry) were recorded manually for each trial. The order of entry into the maze arms was recorded so that the total number of errors could be calculated. Errors were quantified in terms of reference and working memory incorrect errors (Jarrard *et al,* 1984). Reference memory errors were the number of first entries into a channel which had never contained a platform. Working memory incorrect errors were the number of repeated entries into an arm which had never contained a platform (repeated entries into a reference memory arm thus, reflecting the combined effects of failures of both working and reference memory) (Okaichi *et al,* 1989). These parameters were averaged for each daily block of trials and for each rat, whose daily performance was thus characterized.

On day 5, the rats performed a single spatial probe trial (Gage *et al,* 1984). This trial consisted of removing the platform from the baited arm of the radial water maze and allowing the rat to swim for 60 s in search of it. The time spent in each of the eight channels of the pool was calculated as a percentage over 60 s.

Drugs

[L-NAME, (+)-MK-801 maleate (Sigma, St. Louis, MO, U.S.A.)] and ketamine hydrochloride were dissolved in saline (NaCl 0.9%) and administered intraperitoneally (i.p.) in a volume of 1 ml/kg. Doses of L-NAME were chosen on the basis of a study in which they were effective against learning impairments and did not produce adverse side effects (Boultadakis *et al,* 2010). The doses of MK-801 (0.1 mg/kg) and ketamine (3 mg/kg) were selected based on previous findings in which were found to impair rat performance in the

novel object recognition task without producing side effects (Pitsikas *et al,* 2006; Pitsikas *et al,* 2008). For the radial water maze test, the dose of ketamine (15 mg/kg) was chosen based on a previous report in which was found to impair place learning in a water maze task (Wesierska *et al,* 1990). For all studies, control animals received isovolumetric amounts of the vehicle (NaCl 0.9%).

Experimental protocol

Experiment 1: effects of L-NAME in antagonizing MK-801-induced performance deficits in the novel object recognition task

Rats were randomly divided into eight experimental groups (10 rats per group) as follows: vehicle+vehicle; vehicle+L-NAME 1 mg/kg; vehicle+L-NAME 3 mg/kg; vehicle+L-NAME 10 mg/kg; MK-801 0.1 mg/kg+vehicle; MK-801 0.1 mg/kg+L-NAME 1 mg/kg; MK-801 0.1 mg/kg+L-NAME 3 mg/kg; MK-801 0.1 mg/kg+L-NAME 10 mg/kg. To study the effects of compounds on post-training memory components (storage and/or retrieval) compounds were administered immediately after T1. It has been reported that treatment with MK-801 (0.1 mg/kg) induce hypermotility (Verma and Moghaddam, 1996; Homayoun *et al*, 2004) and produce a sustained increase in stereotypy that peaked 40 min after injection and returned to baseline 2 h later (Homayoun *et al,* 2004). Therefore, it is quite possible that these factors may interfere with learning performance. There is also experimental evidence that recognition memory abilities in the young rat remained intact when a delay condition up to 6 h was used (Bartolini *et al,* 1996). In an attempt to avoid the possibility that rats' performance is influenced by these already mentioned side effects due to the treatment with MK-801, the 3-h ITI was selected.

Experiment 2: effects of L-NAME in antagonizing ketamine-induced performance deficits in the novel object recognition task

Rats were randomly divided into eight experimental groups (10 rats per group) as follows: vehicle+vehicle; vehicle+L-NAME 1 mg/kg; vehicle+L-NAME 3 mg/kg; vehicle+L-NAME 10 mg/kg; ketamine 3 mg/kg+vehicle; ketamine 3 mg/kg+L-NAME 1 mg/kg; ketamine 3 mg/kg+L-NAME 3 mg/kg; ketamine 3 mg/kg+L-NAME 10 mg/kg. To study the effects of compounds on post-training memory components (storage and/or retrieval) compounds were administered just after T1. For this study, the 1-h ITI has been selected since at this delay condition recognition memory is still intact in the vehicle-treated rat (Bartolini *et al*, 1996) and impairments associated with treatment with ketamine (hypermotility, stereotypies, ataxia) (Verma and Moghaddam, 1996) were not observed (Pitsikas *et al*, 2008).

Radial water maze task

Preliminary results have shown that rats treated with L-NAME 3 mg/kg displayed a lower performance with respect to the vehicle-treated animals. Therefore, the effects exerted by 3 mg/kg L-NAME on rats' spatial memory and the ability of L-NAME to antagonize ketamine-induced performance deficits in the radial water maze were subsequently assessed in two separate studies.

Experiment 3: effects of L-NAME (3 mg/kg) on animals' performance in the radial water maze task

Rats were randomly divided into two experimental groups (10 rats per group) as follows: vehicle; and L-NAME 3 mg/kg. L-NAME and vehicle were injected every day 60 min before starting testing.

Experiment 4: effects of L-NAME in antagonizing ketamine-induced performance deficits in the radial water maze task

Animals were randomly divided into six experimental groups (10 rats per group) as follows: vehicle+vehicle; vehicle+L-NAME 1 mg/kg; vehicle+L-NAME 10 mg/kg; ketamine 15 mg/kg+vehicle; ketamine 15 mg/kg+L-NAME 1 mg/kg; ketamine 15 mg/kg+L-NAME 10 mg/kg. Control rats were treated with the vehicle 60 and 40 min, respectively, before starting the daily testing. Animals were treated with L-NAME and vehicle 60 and 40 min, respectively before starting testing. Rats received vehicle and ketamine 60 and 40 min, respectively, before starting the daily testing. Rats received L-NAME and ketamine 60 and 40 min, respectively, before starting the daily session. Ketamine administration (10-30 mg/kg) is associated with behavioral symptoms such as hypermotilty and stereotypy which reach the peak effect 15 min after treatment (Wesierska *et al*, 1990; Verma and Moghaddam, 1996). Based on these evidences, the daily administration of ketamine was performed 40 min before testing since at this condition ketamine did not induce adverse effects.

Statistical analysis

Data are expressed as mean±SEM. In experiments 1 and 2, results were analyzed using the two-way analysis of variance (ANOVA). Post-hoc comparisons were made by the Tukey's t-test. In experiment 3, reference and working memory incorrect errors were evaluated by the two-way ANOVA with a split-plot design (between-within subjects). Post-hoc comparisons were made by the Tukey's t-test. Spatial probe trial data are calculated as median and interquartile ranges. When comparing among groups the percentage of time spent in the previously reinforced arm, the non-parametric test of Wilcoxon was used.

In experiment 4, the effects of drugs on daily performance (reference and working memory) were analyzed by the three-way ANOVA (two between, one within subjects). Significant

interactions between ketamine, L-NAME and trials were further analyzed by comparing ketamine- and L-NAME-treated and non-treated rats at each level separately for the 4 days periods, using the two-way ANOVA with a split-plot design. Post-hoc comparisons were made by the Tukey's t-test. Spatial probe data were analyzed by the non-parametric test of Kruskall-Wallis followed by the Newman-Keuls *post-hoc* test. A p value <0.05 was considered significant (Kirk, 1968).

RESULTS

Experiment 1: effects of L-NAME in antagonizing MK-801-induced performance deficits in the novel object recognition task

A two-way ANOVA analysis of motor activity results demonstrated a main effect either of MK-801 ($F(1,72)=18.4$, $p<0.01$) or of L-NAME ($F(3,72)=2.93$, $p<0.05$) indicating that rats that received both these compounds displayed higher motility levels as compared to the other groups (Table 1). Total exploration levels were not different among the various experimental groups (Table 1).

Data for index *D* (Fig. 1) revealed a significant main effect of MK-801 ($F(1,72)=24.2$, $p<0.01$), of L-NAME ($F(3,72)=4.8$, $p<0.01$) and a significant MK-801 x L-NAME interaction ($F(3,72)=3.8$, $p=0.014$). *Post-hoc* comparisons have shown that the vehicle+MK-801 and the MK-801+L-NAME 10 mg/kg-treated animals were unable to discriminate between the familiar and the novel object as compared to the rest of groups ($p<0.05$).

Experiment 2: effects of L-NAME in antagonizing ketamine-induced performance deficits in the novel object recognition task

A two-way ANOVA analysis of motility results showed only a significant main effect of ketamine ($F(1,72)=29.8$, $p<0.01$) suggesting that animals treated with ketamine exhibited

higher locomotor activity levels than the other experimental groups (Table 2). Total exploration levels were not different among the various experimental groups (Table 2).

Concerning D data, a significant main effect of ketamine ($F(1,72)=15.8$, $p<0.01$), of L-NAME ($F(3,72)=3.4$, $p<0.05$) and a significant interaction between ketamine and L-NAME ($F(3,72)=3$, $p<0.05$) was evidenced. *Post-hoc* analysis has shown that the vehicle+ketamine and the ketamine+L-NAME 10 mg/kg-treated animals were unable to discriminate between the familiar and the novel object as compared with the other groups ($p<0.05$, Fig. 2).

Experiment 3: effects of L-NAME (3 mg/kg) on animals' performance in the radial water maze task

Reference memory

Data are presented in Fig. 3A. An overall ANOVA showed that both the vehicle and the L-NAME-treated animals made progressively fewer reference memory errors over days ($F(3,54)=5.47$, $p<0.01$). In addition, the L-NAME 3 mg/kg-treated animals committed significantly more reference memory errors than their vehicle-treated cohorts ($F(1,18)=8.48$, $p<0.01$).

Working memory

Results are illustrated in Fig. 3B. Statistical analysis of the working memory data evidenced that either the vehicle or the L-NAME-treated animals made progressively fewer working memory incorrect errors over days ($F(3,54)=9.87$, $p<0.01$). Moreover, the L-NAME 3 mg/kg-treated rats made a significantly higher number of working memory incorrect errors than their vehicle-treated cohorts ($F(1,18)=4.4$, $p=0.05$).

Spatial probe trial

Probe analysis comparing percentage of time spent in the previously reinforced quadrant showed an effect of treatment. The 3 mg/kg L-NAME group spent significantly less time in the previously baited channel than the control group ($p<0.05$, Fig. 3C).

Experiment 4: effects of L-NAME in antagonizing ketamine-induced performance deficits in the radial water maze task

Reference memory

Data are presented in Fig. 4A. Overall ANOVA did not show a significant three-way ketamine x L-NAME x days interaction, neither a significant two-way interaction between ketamine and L-NAME, between ketamine and days and between L-NAME and days. A significant main effect of ketamine ($F(1,216)=25.1$, $p<0.01$), of days ($F(3,216)=11.1$, $p<0.01$) but not of L-NAME however was revealed.

Additional analyses of rats' performance in this spatial reference memory task were carried out by split-plot ANOVAs. The ketamine+vehicle-treated animals committed significantly more reference memory errors than their vehicle+vehicle-treated cohorts ($F(1,18)=40.6$, $p<0.01$). L-NAME, at any dose, failed to counteract this ketamine-induced reference memory impairment.

Animals treated with 1 mg/kg L-NAME+ketamine made more reference memory errors than the vehicle+L-NAME 1 mg/kg-treated rats ($F(1,18)=7.3$, $p=0.014$). Choice accuracy abilities, in terms of number of reference memory errors, of the 10 mg/kg L-NAME+ketamine-treated rats and those animals that received vehicle+L-NAME 10 mg/kg were not different.

Working memory

Results are presented in Fig. 4B. Overall ANOVA did not show a significant three-way ketamine x L-NAME x days interaction, neither a significant two-way interaction between

ketamine and days and between L-NAME and days. Interestingly, a significant two-way interaction between ketamine and L-NAME was evidenced ($F(2,216)=3.64$, $p<0.05$) indicating that the rates of learning were different among the various groups. In addition, a significant main effect of ketamine ($F(1,216)=28.1$, $p<0.01$), of days ($F(3,216)=8$, $p<0.01$) but not of L-NAME was evidenced.

Further analyses of animals' performance in this spatial working memory paradigm were carried out by split-plot ANOVAs. Animals that received ketamine+vehicle commtted significantly more working memory incorrect errors than their vehicle+vehicle counterparts ($F(1,18)=17.9$, $p<0.01$). Rats that received 10 mg/kg L-NAME+ketamine made significantly fewer working memory incorrect errors than their counterparts treated with ketamine+vehicle ($F(1,18)=8.35$, $p<0.01$). In addition, these rats performance, in terms of working memory incorrect errors, was not different than that displayed by their vehicle cohorts (10 mg/kg L-NAME+vehicle-treated rats). Animals treated with 1 mg/kg L-NAME+ketamine made a higher number of working memory incorrect errors than the vehicle+L-NAME 1 mg/kg-treated rats ($F(1,18)=13.8$, $p<0.01$).

Spatial probe trial

Probe analysis comparing percentage of time spent in the previously reinforced quadrant showed an effect of treatment ($H=25.8$, $p<0.01$, Fig. 4C). Ketamine+vehicle and ketamine+L-NAME 1 mg/kg-treated animals spent less time in the previously baited channel as compared to the respective control populations (Newman-Keuls, *post-hoc* test; $p<0.05$). L-NAME, at 10 mg/kg, attenuated this ketamine-induced impairment ($p<0.05$ vs ketamine+vehicle-treated animals).

DISCUSSION

The major findings of the present study have shown that the NMDA receptor antagonists MK-801 and ketamine induced performance deficits in different cognitive tasks in the rat. These cognitive impairments could be antagonized by the NOS inhibitor L-NAME.

Novel object recognition task

Our results are in line with previous studies in which post-training administration of both MK-801 and ketamine disrupted rats' performance in the novel object recognition task (Pitsikas *et al,* 2006; Pitsikas *et al,* 2008**)**. A single post-training injection of L-NAME (1 and 3, but not 10 mg/kg) attenuated either the MK-801 or the ketamine-induced performance deficits in this recognition memory paradigm. MK-801, ketamine and L-NAME influenced rats' performance during retention, seemingly reflecting a modulation of post-training mnemonic processes (storage and/or retrieval of information).

Compounds were administered systemically, thus, it cannot be excluded that non-specific factors (attentional, sensorimotor deficits) might have influenced animals' performance. All animals that received these two NMDA receptor antagonists displayed higher levels of motility, but not of general exploration during T2, with respect to their control cohorts. Treatment with L-NAME did not antagonize this NMDA blockade-induced hyperactivity but successfully counteracted recognition memory deficits. This pattern of results implies that the effects of the compounds on rats' cognitive performance were unrelated to the extent of motility and exploratory behavior.

Present results are in agreement with our previous findings showing that L-NAME did not impair recognition memory but it reversed delay-dependent impairments in the novel object recognition task, especially at lower doses (Boultadakis *et al*, 2010).

Radial water maze task

Data have shown that L-NAME (1 and 10 mg/kg) alone did not disrupt animals' spatial learning abilities. A *per se* effect of L-NAME was observed when this NOS inhibitor was delivered at the dose of 3 mg/kg, however. Those animals' spatial learning abilities were poorer as compared to those displayed by the vehicle+vehicle-treated subjects. Rats that received ketamine performed less efficiently than the vehicle+vehicle and the L-NAME (1 and 10 mg/kg)+vehicle-treated rats suggesting that this NMDA receptor antagonist disrupted both spatial reference and spatial working memory. The higher dose of L-NAME (10 mg/kg) attenuated ketamine-induced working, but not reference, memory deficits. Both ketamine+L-NAME groups expressed a lower performance as compared to the respective cohorts treated with L-NAME+vehicle.

When the ability of rats to use the extramaze cues surrounding the radial water maze was evaluated (spatial probe trial) all control rats persistently swam more in the previously reinforced arm of the maze, while L-NAME 3 mg/kg+vehicle, ketamine+vehicle and ketamine+L-NAME 1 mg/kg-treated animals were unable to do so. L-NAME (10 mg/kg) attenuated this ketamine produced disrupting effect.

The radial water maze test evaluates rodents' spatial memory. The procedure employed in the present study offers the possibility to assess in the same animal simultaneously two different aspects of memory (reference and working) (Jarrard, 1984; Pitsikas and Algeri, 1992). This within-subject and within-task design is advantageous because each dissociation is obtained from the same animal at the same time, minimizing many sources of variability such as task magnitude of reinforcement, motivational, perceptual and motor processes (Olton, 1983).

Our results of the radial water maze paradigm are consistent with previous reports in which the effects of L-NAME (10 mg/kg) on glutamatergic hypofunction were assessed (Wass *et al*, 2006a; Wass *et al*, 2006b). The outcome of these studies was that treatment with this NOS

inhibitor antagonized PCP-induced spatial reference, spatial working memory, but not retention deficits in a modified Morris water maze procedure (Wass *et al,* 2006a; Wass *et al,* 2006b).

A potential issue when using NOS inhibitors relates to its hypertensive properties. It is difficult, therefore, to quantify how and at what extent these cardiovascular effects might have specifically affected animals' cognitive performance. Reportedly, NOS inhibitors injected peripherally induce a nearly maximal hypertensive effect at 10 mg/kg (Rees *et al,* 1990). Under our experimental conditions, L-NAME, administered systemically at 10 mg/kg, attenuated ketamine-induced performance deficits in the radial water maze task. This implies that the effects of this compound on rats' cognitive performance were unrelated to its potential hypertensive action.

NMDA hypofunction and nitric oxide

Pharmacological blockade of NMDA receptors has been proposed as a relevant model of schizophrenia in humans and animals. (Javitt and Zukin, 1991; Geyer and Markou, 1994; Krystal *et al,* 1994). The NMDA antagonist treatment model has excellent face validity for cognitive symptoms of schizophrenia because an exposure to these drugs impairs PFC-dependent cognitive functions in a manner that is similar to schizophrenia (Javitt and Zukin, 1991; Krystal *et al,* 1994). PCP, ketamine and MK-801 produce their psychotomimetic effects by blocking NMDA receptors located on GABA interneurons, resulting in decreased firing of GABAergic inhibitory neurons and, thereby, increased excitability in limbic circuits (Olney *et al,* 1991; Moghaddam *et al,* 1997). This disinhibitory action elicits an increase in terms of neuronal activity and excessive glutamate and dopamine release in the PFC and limbic regions (Moghaddam *et al,* 1997; Lorrain *et al,* 2003; Razoux *et al,* 2007).

NOS inhibitors may exert both excitatory and inhibitory influences on NMDA receptor associated events. NO's dual action as mediator and negative modulator of glutamate neurotransmission at the NMDA receptor complex give this system a unique role in regulating the balance of excitatory and inhibitory influences. It is this balance of excitatory and inhibitory processes that is likely to be disrupted in schizophrenia, as proposed by the glutamatergic hypofunction (Dawson *et al*, 1991).

Our findings indicate that L-NAME, for the first time to our knowledge, antagonized both MK-801 and ketamine-induced recognition memory deficits suggesting that this NOS inhibitor is also effective against NMDA blockade produced by the action of NMDA antagonists other than PCP. Furthermore, L-NAME was found to attenuate spatial memory deficits produced by ketamine in the rat. The latter, support and extend previous results in which spatial memory deficits induced by another NMDA antagonist (PCP) were counteracted by this NOS inhibitor (Wass *et al*, 2006a; Wass *et al*, 2006b). Collectively, the present results further suggest an involvement of NO in the NMDA hypofunction-induced cognitive deficits.

Contrary to the expectations, when L-NAME was administered alone at 3 mg/kg, impaired rats' spatial but not recognition memory abilities. It remains uncertain if this dual effect of L-NAME on memory was related to the task specificity, the type of memory studied and/or the different pharmacological design utilized.

The radial water maze, is a negatively reinforced paradigm assessing spatial memory. The novel object recognition test, is a task that does not involve at all, the learning of a rule since it is based on the spontaneous exploratory behavior of rodents evaluating recognition memory (Ennaceur, 2010). In the novel object recognition task rats received a single injection of compounds just after the "sample" trial T1. Conversely, in the radial water maze paradigm,

treatment was applied once per day 60 min before starting the daily testing, for 5 consecutive days.

Interestingly, this impairing effect of L-NAME on spatial memory, was not seen at the "side" doses of 1 and 10 mg/kg revealing thus, an U-shaped dose-effect curve. At present, the biological bases of U dose-response relationship are unknown, although receptor fatigue or tachyphylaxis (Day, 1979) has been suggested as potential mechanisms (Martinez, 1986). Moreover, L-NAME has also been reported to act as a partial agonist in the NO system, antagonizing or stimulating NO synthesis in different tissues (Archer and Hampi, 1992). Further studies are needed however, in order to clarify this important issue.

The mechanism(s) of action of low doses of NOS inhibitors on learning and memory is not yet clarified and is matter of investigation. It has been demonstrated that the neuroprotective effects of NOS inhibitors were obtained when inhibition of NO production was only mild and transient. Conversely, long-lasting inhibition of NO production by high doses of NOS inhibitors leads to neurotoxicity (Contestabile *et al,* 2003). Small changes in local NO concentration and the time of administration therefore, may be a key factor in determining its biological action (Contestabile *et al,* 2003).

It has previously been reported that a wide range of translational PCP-induced effects including deficits in preattentive information processing, nonassociative learning, selective attention, spatial memory can all be prevented by interfering with the production of NO (Johansson *et al,* 1997; Klamer *et al,* 2001; Klamer *et al,* 2004; Klamer *et al,* 2005a; Klamer *et al,* 2005b**)**. These findings suggest that the schizophrenia-like behavioral effects of PCP in rodents are, at least in part, mediated by an increase in NO activity (Palsson *et al,* 2009). This is supported by a recent study showing a NO-dependent increase in cGMP signaling, a main effector of NO in the brain, in the mouse PFC following PCP administration (Fejgin *et al,* 2008). In this context, it has been found that L-NAME attenuated sensorimotor gating deficits

caused by PCP, by reducing PCP-induced increase in cyclic GMP (cGMP) production in the PFC of the mouse brain (Fejgin *et al*, 2008). This mechanism might be a plausible explanation for the beneficial effects of low doses of L-NAME on memory impairments related to glutamatergic hypofunction. Further studies however, are required in order to elucidate this important issue.

Concluding remarks

In summary, studies here-in presented demonstrate that L-NAME was capable in antagonizing MK-801 and ketamine-induced memory deficits and this beneficial action exerted by L-NAME on cognition was observed at a different dose range. This, in turn, indicates that L-NAME is sensitive to glutamatergic hypofunction produced by other than PCP NMDA receptor antagonists such as MK-801 and ketamine. In addition, the present results show that L-NAME alone differentially affects rodents' spatial memory. The latter suggest a careful consideration of the balance between neurodegenerative and neuroprotective effects produced by this NOS inhibitor since L-NAME might constitute a potential candidate for the treatment of schizophrenia.

DISCLOSURE/CONFLICT OF INTEREST

The authors hereby declare that no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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Legend to figures

Fig. 1. Novel object recognition task. Vehicle, MK-801 and L-NAME were injected i.p., just after T1, respectively. The 3-h ITI was used. Results are expressed as mean±SEM. Discrimination index D performance expressed by different groups of rats during T2. *p<0.05 vs all the other groups (except the MK-801+L-NAME 10 mg/kg group); ^{+}p<0.05 vs all the other groups (except the MK-801+vehicle group).

Fig. 2. Novel object recognition task. Vehicle, ketamine and L-NAME were injected i.p., just after T1, respectively. The 1-h ITI was used. Results are expressed as mean±SEM. Discrimination index D performance expressed by different groups of rats during T2. *p<0.05 vs all the other groups (except the ketamine+L-NAME 10 mg/kg group); ^{+}p<0.05 vs all the other groups (except the ketamine+vehicle group).

Fig. 3. Radial water maze task. Vehicle and L-NAME were injected i.p., every day 60 min before testing. (A) Spatial reference memory. Results are expressed as mean±SEM. Number of errors cumulating over days by different groups of rats. (B) Spatial working memory. Results are expressed as mean±SEM. Number of errors cumulating over days by different groups of rats. (C) Percentage of total time spent in the previously reinforced arm of the radial water maze by different groups of rats. Results are expressed as medians. *p<0.05 vs the vehicle-treated animals.

Fig. 4. Radial water maze task. Vehicle, ketamine and L-NAME were injected i.p., every day 40 and 60 min respectively before testing. (A) Spatial reference memory. Results are expressed as mean±SEM. Number of errors cumulating over days by different groups of rats. (B) Spatial working memory. Results are expressed as mean±SEM. Number of errors cumulating over days by different groups of rats. (C) Percentage of total time spent in the previously reinforced arm of the radial water maze by different groups of rats. Results are

expressed as medians. $^{*}p<0.05$ vs the vehicle+vehicle-treated animals; $^{+}p<0.05$ vs the respective control populations; $^{\#}p<0.05$ vs ketamine+L-NAME 10 mg/kg-treated rats.

Table 1. Effects of MK-801 and L-NAME on rats' performance in the novel object recognition task

Group	N	T2 Motor activity (number of steps) Mean±SEM	T2 Exploration time (s.) Mean±SEM
Vehicle+vehicle	10	47.5±1.8	12.5±0.3
Vehicle+L-NAME (1 mg/kg)	10	41.8±5.2	12.2±0.4
Vehicle+L-NAME (3 mg/kg)	10	35.5±2.1	11.6±1.3
Vehicle+L-NAME (10 mg/kg)	10	44.4±3.9	12.5±0.6
MK-801 (0.1 mg/kg)+vehicle	10	58.6±3.5	11.9±0.5
MK-801+L-NAME (1 mg/kg)	10	55.4±3.7	14.2±0.8
MK-801+L-NAME (3 mg/kg)	10	47.2±5.1	12.4±0.9
MK-801+L-NAME (10 mg/kg)	10	54.1±3.6	12.4±0.4

N=number of rats. Compounds were injected intraperitoneally immediately after T1.

Table 2. Effects of ketamine and L-NAME on rats' performance in the novel object recognition task

Group	N	T2 Motor activity (number of steps) Mean±SEM	T2 Exploration time (s.) Mean±SEM
Vehicle+vehicle	10	45.4±2.6	12.7±0.7
Vehicle+L-NAME (1 mg/kg)	10	46.9±4.5	14.2±1
Vehicle+L-NAME (3 mg/kg)	10	46.4±5.2	13.6±0.7
Vehicle+L-NAME (10 mg/kg)	10	45.1±4.9	14.1±1.3
Ketamine (3 mg/kg)+vehicle	10	67.6±1.7	14.5±0.8
Ketamine+L-NAME (1 mg/kg)	10	57.8±3.3	13.3±0.5
Ketamine+L-NAME (3 mg/kg)	10	58.7±3.3	12.7±0.8
Ketamine+L-NAME (10 mg/kg)	10	59.1±4.3	13.2±0.9

N=number of rats. Compounds were injected intraperitoneally immediately after T1.

Fig. 1

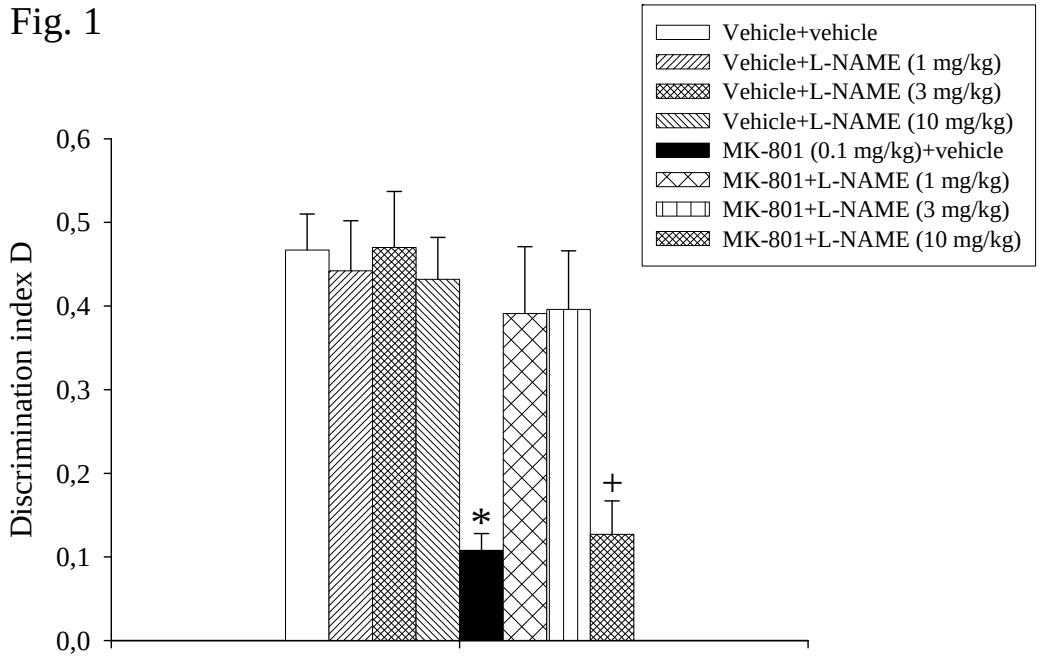

Vehicle+vehicle
Vehicle+L-NAME (1 mg/kg)
Vehicle+L-NAME (3 mg/kg)
Vehicle+L-NAME (10 mg/kg)
MK-801 (0.1 mg/kg)+vehicle
MK-801+L-NAME (1 mg/kg)
MK-801+L-NAME (3 mg/kg)
MK-801+L-NAME (10 mg/kg)
Discrimination index D
0,6
0,5
0,4
0,3
0,2
0,1
0,0
*
+

Fig. 2

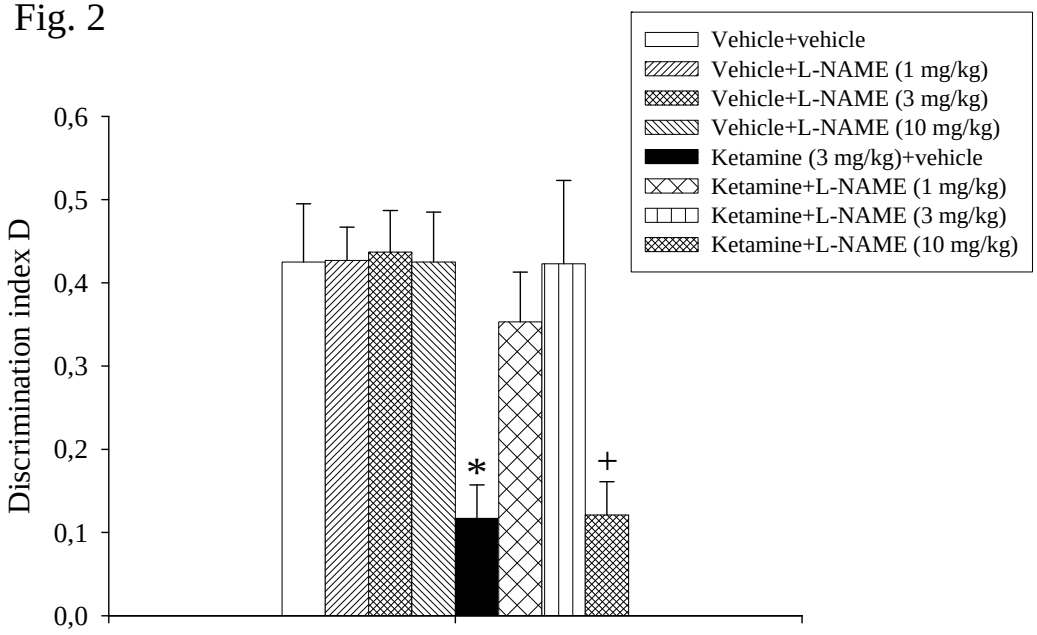
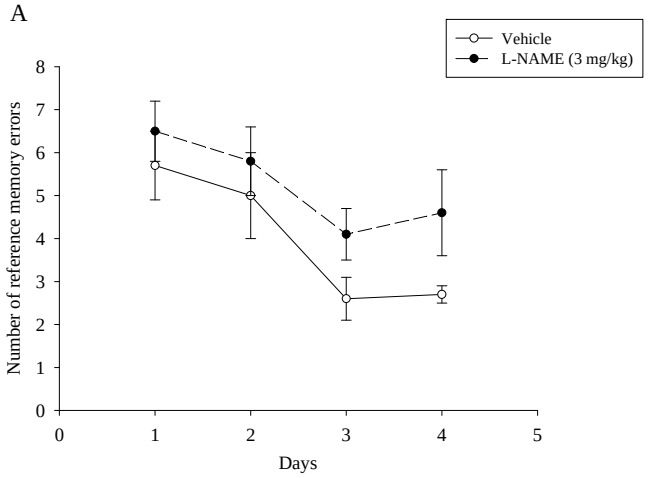
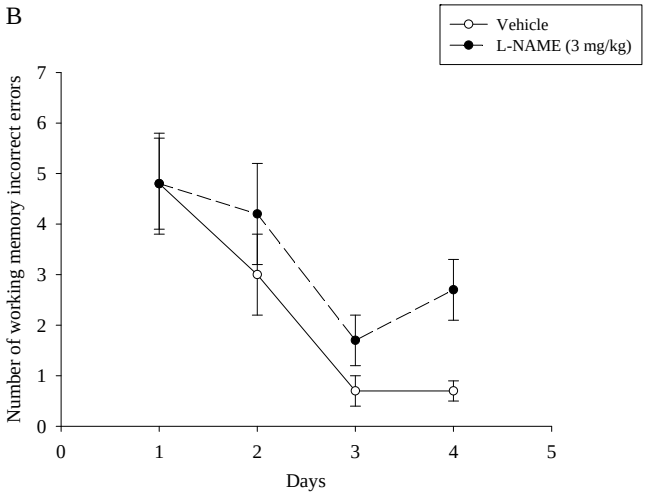

Vehicle+vehicle
Vehicle+L-NAME (1 mg/kg)
Vehicle+L-NAME (3 mg/kg)
Vehicle+L-NAME (10 mg/kg)
Ketamine (3 mg/kg)+vehicle
Ketamine+L-NAME (1 mg/kg)
Ketamine+L-NAME (3 mg/kg)
Ketamine+L-NAME (10 mg/kg)
Discrimination index D
0,6
0,5
0,4
0,3
0,2
0,1
0,0
*
+

Fig. 3

A


Vehicle
L-NAME (3 mg/kg)
Number of reference memory errors
Days

B


Vehicle
L-NAME (3 mg/kg)
Number of working memory incorrect errors
Days

C

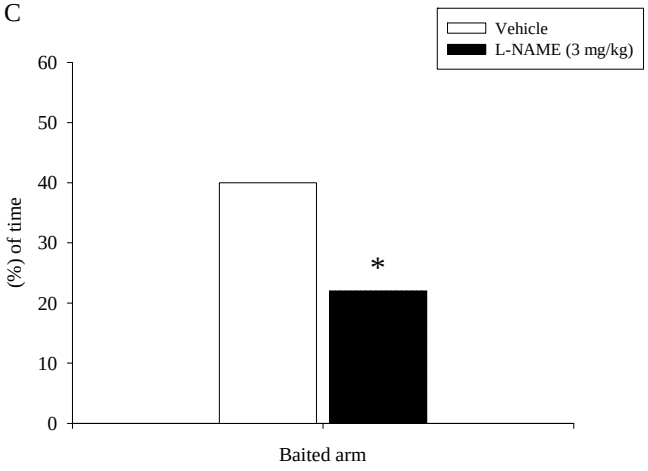
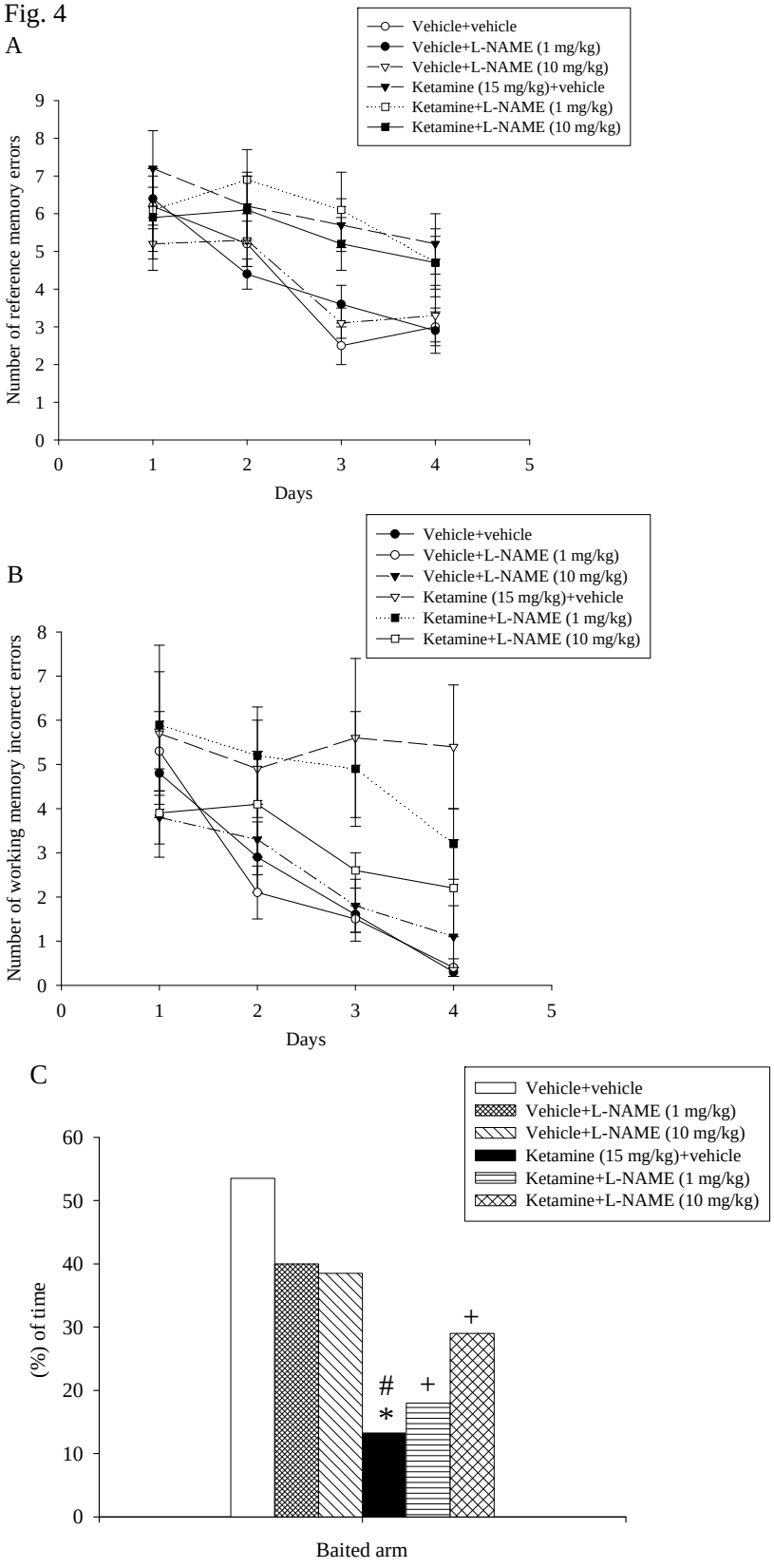

Vehicle
L-NAME (3 mg/kg)
(%) of time
*
Baited arm

Fig. 4


A
Vehicle+vehicle
Vehicle+L-NAME (1 mg/kg)
Vehicle+L-NAME (10 mg/kg)
Ketamine (15 mg/kg)+vehicle
Ketamine+L-NAME (1 mg/kg)
Ketamine+L-NAME (10 mg/kg)
Number of reference memory errors
Days
B
Number of working memory incorrect errors
C
(%) of time
Baited arm
#
*
+