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Interaction between M1-muscarinic and glutamatergic NMDA receptors on an inhibitory avoidance task

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Abstract

It has been demonstrated that MK-801 potentiates the effects of the non-selective muscarinic antagonist scopolamine on memory in rats. In this study, we investigated the role of the M1-muscarinic receptor in this interaction, by administering different doses of dicyclomine (DIC) and MK-801 in combination to male Wistar rats before training on the inhibitory avoidance task. MK-801 and DIC in sub-effective doses were administered in combination. It was observed that MK-801 at a dose of 0.1125 mg/kg with a sub-effective dose of 8 mg/kg of DIC significantly impaired the retention test when compared with saline-treated animals, i.e. MK-801 potentiated the effects of dicyclomine on memory impairment. Our results suggest an important role for the M1-muscarinic receptor in the synergistic interaction between cholinergic muscarinic and glutamatergic NMDA receptors, which is in line with the findings that the interactive modulation between these two neurotransmitters systems constitutes an important mechanism in cognitive functions.

Keywords: Learning; Memory; Dicyclomine; MK-801; Passive avoidance; Rat; Impairment

1. Introduction

It is well documented that mechanisms mediated by glutamatergic NMDA receptors are responsible for the induction of some forms of long-term potentiation (LTP), which is hypothesized to be a neural basis for hippocampal memory formation (for review see refs. [6,26]). The role of glutamatergic transmission on memory may also be verified with the observation that administration of both competitive and non-competitive NMDA receptor antagonists disrupts memory performance in rodents in a variety of behavioral paradigms [5,8,10,18,20,22,31,36,37]. On the other hand, manipulation of the central cholinergic system is also known to affect learning and memory. Lesions or degeneration of cholinergic pathways, such as the basal forebrain, the hippocampal formation, or the basal ganglia or pharmacological antagonism of muscarinic receptors produce cognitive deficits in both experimental animals and humans [2,3,12–14,17,32,33,35]. In addition, an interactive mechanism underlying the deficits observed in manipulations of both glutamatergic NMDA and muscarinic receptors has been suggested [21,25,30].

It has also been shown that the administration of acetylcholine, acting on hippocampal muscarinic receptors, facilitates the "slow" component of excitatory post-synaptic potential (EPSP) mediated by NMDA-receptor activation [27]. Therefore, the action of acetylcholine on the NMDA response may increase the probability of generating NMDA-dependent LTP in the hippocampus [27]. That hypothesis of interaction is strengthened by previous pharmacological studies showing that ineffective doses of MK-801 (a non-competitive NMDA receptor antagonist), when administered in combination with ineffective doses of scopolamine (a non-selective muscarinic receptor antagonist), disrupt performance of animals in the inhibitory avoidance task, the spatial version of the radial maze, and the modified elevated plus-maze test adapted for learning and memory evaluation [21,25,30]. Since scopolamine is a non-selective muscarinic receptor antagonist, it is difficult to determine which of the muscarinic receptor subtypes would be involved in that effect. A variety of studies have used selective muscarinic agents to investigate the specific receptor mechanisms underly-

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ing the scopolamine-induced impairment in learning and memory. These studies focus mainly on the M1 receptor subtype. It was observed that pre- or post-training administration of selective M1 antagonists such as pirenzepine, dicyclomine or biperidine disrupts performance on inhibitory avoidance task showing that pharmacological M1 blockade mimics many of the effects of non-selective muscarinic antagonism blockade and hippocampal lesion [14,15,23,34].

Besides the psychopharmacological evidence described above, autoradiographic studies have shown high densities of M1 or NMDA binding sites in hippocampus, amygdala and cortex, all of which are brain areas where cholinergic and glutamatergic are thought to have an essential role in memory processes [7,29].

The purpose of the present work was to verify whether M1-muscarinic receptor antagonism potentiates proactive amnesic effects of the NMDA antagonist MK-801 on a step-through inhibitory avoidance task, a classical paradigm used to evaluate learning and memory in animals (see refs. [16,24]).

2. Materials and methods

2.1. Subjects

Male Wistar rats, 3–4 months old, bred and raised in the animal facility of the Psychobiology Department of UNIFESP/EPM were used. Animals were maintained under controlled temperature ($23 \pm 2\,^{\circ}C$) and 12-h light:12-h dark cycle (lights on at 7:00 am) conditions. Rat chow and tap water were provided ad libitum. Each experiment was conducted in separate groups of animals.

2.2. Apparatus

The inhibitory avoidance apparatus consisted of two compartments, each measuring 22 cm × 21 cm × 22 cm, connected by a sliding door. The walls of the safe compartment were white whereas the other compartment, where the animals received foot shock, had black walls with visual patterns (two squares measuring 5.5 cm × 5.5 cm and three squares measuring 4.0 cm × 4.0 cm made of white cardboard). The floor consisted of a metal grid (0.4 cm diameter rods placed 1.2 cm apart from each other) connected to a shock generator and control module (Ugo Basile model 7551), through which foot shocks could be delivered.

2.3. Drugs

Dicyclomine chloride (Sigma Chemical Co., USA) was dissolved in 0.9% saline and injected, i.p., in a volume of 1.0 ml/kg. The doses of 8 and 12 mg/kg used were chosen based on our previous work showing that dicyclomine (DIC) impairs inhibitory avoidance task in a dose-dependent manner [13]. MK-801 (Dizocilpine maleate; Sigma Chemical Co., USA) dissolved in 0.9% saline was injected i.p. in a volume of 1.0 ml/kg. The doses used were 0.1, 0.1125, 0.125, 0.15, and 0.3 mg/kg.

2.4. Drug administration

Rats received either a saline (SAL) or DIC (8 or 12 mg/kg) injection followed by a MK-801 or saline administration 5 min later. Training was performed 30 min after the first injection in Experiments 1–3. In Experiment 4, rats received the appropriate injection immediately after the training session.

2.5. Behavioral procedure

After receiving the relevant drug treatment, the animals were individually placed inside the white compartment (safe side) of the avoidance apparatus. Ten seconds later the door was opened and, as soon as the animal entered the black compartment with all four paws, the door was closed and five foot shocks (1 mA, 1 s) were delivered at 30 s intervals. The latency for the animal to enter the black compartment was recorded. Thirty seconds after the last foot shocks the animal was removed from the apparatus. The test was carried out 24 h after training. Each animal was placed again in the white compartment of the avoidance apparatus and 10 s later, the door was opened and the time taken by the animal to cross to the black compartment (four paws in) was recorded (test latency). If the animal did not cross within 300 s, it was removed from the apparatus and a latency of 300 s was attributed. No foot shock was delivered during the test.

2.6. Experimental design

In Experiment 1, we tested the effects of pre-training administration of saline or five different doses of MK-801 on the inhibitory avoidance test (groups of 12–15 animals). In Experiment 2, we tested the effects of pre-training administration of two different doses of DIC on the inhibitory avoidance test (10–13 animals per group). In Experiment 3, the higher sub-effective doses of MK-801 and DIC, as identified in Experiments 1 and 2, were administered in combination in the same behavioral paradigm described above (9–11 animals per group). In Experiment 4, we tested the effects of post-training administration of the higher sub-effective doses of MK-801 and DIC as in Experiment 3 (12 animals per group).

2.7. Statistical analysis

Between group comparisons with latency data from the inhibitory avoidance training and test were analyzed with Kruskal–Wallis followed by Mann–Whitney tests, when necessary ($p < 0.05$). Within group, comparisons between training and test sessions were done with the Wilcoxon's test.

3. Results

3.1. Experiment 1: MK-801

Retention test latencies are shown in Fig. 1(A). Kruskal–Wallis test did not show significant differences between groups ($H = 5.168$; $p = 0.396$) in the training session. In the test session, an effect was observed (Kruskal–Wallis $H = 25.94344$; $p = 0.0001$), and Mann–Whitney *U*-test revealed that rats treated with MK-801 doses ranging from 0.125 to 0.3 mg/kg displayed significantly shorter latency to enter the dark compartment when compared with both SAL and MK-801 0.1 mg/kg groups ($p < 0.05$).

3.2. Experiment 2: dicyclomine

Effects of DIC administration on inhibitory avoidance test are shown in Fig. 1(B). Kruskal–Wallis test did not show significant differences between groups ($H = 0.708$; $p = 0.702$) in the training session. In the test session, an effect was observed ($H = 8.792$; $p = 0.012$), and Mann–Whitney *U*-test revealed that the group DIC 12 mg/kg displayed significant shorter latency to enter the dark compartment when compared with both SAL and DIC 8 mg/kg groups ($p < 0.05$).

3.3. Experiment 3: interaction

Results of the administration of sub-effective doses of MK-801 and DIC, alone or in combination are shown in Fig. 1(C).

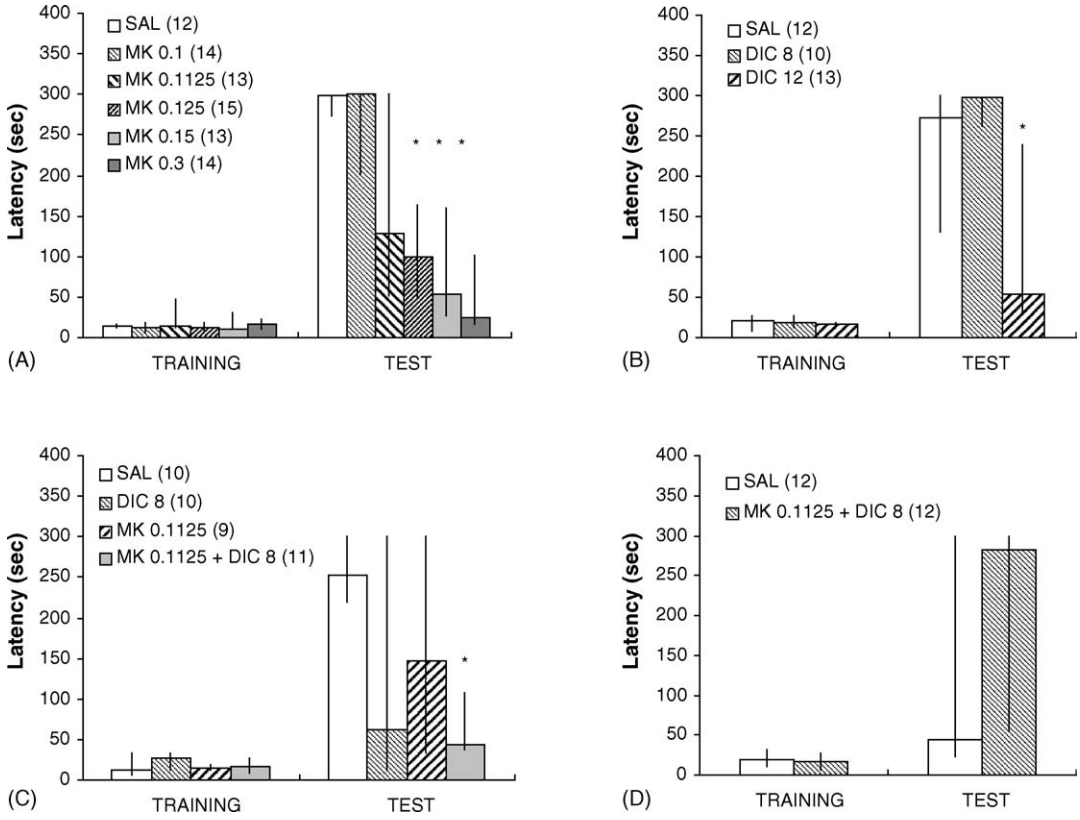

Fig. 1. Effects of MK-801 (A), DIC (B), drug interaction (C) and post-training drug interaction (D) on the latency to enter the dark compartment during retention test of the inhibitory avoidance task. Number of animals in parentheses; $^{*}p < 0.05$ compared with SAL (saline group). Values are expressed as medians ± interquartile intervals (time in seconds).

Kruskal–Wallis test did not show significant differences between groups ($H = 1.880$; $p = 0.598$) in the training session. In the test session, an effect was observed ($H = 10.397$; $p = 0.016$), and Mann–Whitney U-test revealed that only the drug interaction group (MK 0.1125 + DIC 8) was different from the SAL group ($p < 0.05$).

3.4. Experiment 4: post-training interaction

Results of the administration of sub-effective doses of MK-801 and DIC in combination are shown in Fig. 1(D). Mann–Whitney U-test revealed no significant treatment effect, in training ($U = 64$; $p = 0.67$) or in test sessions ($U = 54$; $p = 0.32$).

In all experiments, the Wilcoxon's test revealed significant differences between training and test sessions.

4. Discussion

In this study, MK-801 potentiates dicyclomine-induced disruption of inhibitory avoidance performance. Moreover, both MK-801 and dicyclomine alone also disrupted the performance in the inhibitory avoidance task in a dose dependent manner. The results obtained with dicyclomine are in accordance with previous data from our laboratory, which had shown that 8 mg/kg of this drug was unable to impair the inhibitory avoidance test [14]. In the present work dicyclomine and MK-801, when co-administered at doses that had no behavioral effect when given alone, disrupted the inhibitory avoidance performance, suggesting that NMDA glutamatergic receptors and M1-muscarinic cholinergic receptors interact in the modulation of learning and memory processes.

Muscarinic cholinergic receptors play a fundamental role in memory formation [4]. In particular, the septal cholinergic neurons projecting to the hippocampus are involved in the generation of certain types of memory (for reviews see refs. [11,17]). Antimuscarinic agents, such as scopolamine, disrupt learning and memory processes in a manner similar to that seen with hippocampal damage [2,3]. It is possible, therefore, that the observed effects of anticholinergic drugs on inhibitory avoidance are mediated by the hippocampal cholinergic subpopulation of M1 receptors blocked by dicyclomine. Indeed, several other findings show that the administration of M1 receptor antagonists mimics the effects of hippocampal lesion and scopolamine administration [2,3,14,23,34]. Thus, the administration of these anticholinergic agents produces highly consistent and reproducible data regarding the impairment of memory function.

It is also known that blockade of NMDA receptors disrupts learning and memory in a variety of behavioral tasks [5,8,10,18,20,22,31,37]. Ohno and Watanabe [30], Li et al. [25], and Hlinák and Krejci [21] observed interactive processing between glutamatergic and cholinergic systems through scopolamine administration. Our results confirm this interaction and further suggest that the potentiated deficit could occur through

M1-muscarinic blockade, since we used dicyclomine in combination with MK-801.

It is possible that direct and/or indirect interactions of the central cholinergic and NMDA receptor systems, to a certain extent, influence the cognitive processes [1,9,17]. A direct interaction mechanism is plausible considering that the administration of acetylcholine, acting on hippocampal muscarinic receptors, facilitates the "slow" component of excitatory postsynaptic potential (EPSP) mediated by NMDA-receptor activation [27], therefore, increasing the probability of generating NMDA-dependent LTP. It is not possible, however, to discard a possible relationship between ACh-muscarinic and NMDA receptors in other structures besides the hippocampus in mediating the cognitive impairment observed [1,9,17]. Also, an indirect modulation is observed with intra-septal administration of NMDA and a NMDA receptor-selective agonist that causes an increase in the ACh level in hippocampus [28]. So the blockade of the septal NMDA receptors could result in a decrease of ACh release in hippocampus and a M1 antagonist could potentiate the cholinergic tonus leading to cognitive defects.

Nevertheless, it is not possible to completely rule out any general systemic effect of the antagonists on behavior, since there are several studies in the literature showing the central effects of doses from 0.05 to 1.0 mg/kg MK-801 after peripheral administration affecting cognitive, motor or sensory mechanisms (Brosnan-Watters et al. [8], Castellano et al. [10]).

In the present study, drug treatment was carried out 30 min before the training session; therefore, all animals were trained under the influence of either dicyclomine, MK-801 or both and tested 24 h after training in an "off-drug" state. Therefore, we cannot rule out the possibility of state-dependent learning as an explanation for the impairment on inhibitory avoidance, since effective doses of both MK-801 and anticholinergic drugs such as scopolamine are able to produce state dependent learning [19,32]. It would be important to investigate whether the effect observed after concurrent administration of non-effective doses could be due to state dependent learning.

In Experiment 3, it was not possible to determine whether the effect is on acquisition or consolidation since the drug is probably also present in the first minutes after training. Experiment 4 was conducted in order to clarify this issue. It was observed that the same treatment (drug interaction) that has affected animals performance when administered pre-training did not affect performance when given after training, indicating that that effect is primarily on acquisition rather than on consolidation.

In conclusion, the present results showed evidence that NMDA antagonists exacerbated dicyclomine-induced disruption of inhibitory avoidance and suggested that blockade of both the NMDA receptor and M1-muscarinic receptors interferes with cognition, especially learning and memory processes. Our results suggest an important role for the M1-muscarinic receptor in the synergistic interaction between cholinergic muscarinic and glutamatergic NMDA receptors, which is in line with the findings that the interactive modulation by these two neurotransmitters systems constitutes an important mechanism in cognitive functions.

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References

[1] H. Abe, Y. Ishida, T. Iwasaki, Perirhinal *N*-methyl-D-aspartate and muscarinic systems participate in object recognition in rats, Neurosci. Lett. 19 (2004) 191–194.
[2] S.G. Anagnostaras, S. Maren, M.S. Fanselow, Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination, J. Neurosci. 19 (1999) 1106–1114.
[3] S.G. Anagnostaras, S. Maren, J.R. Sage, S. Goodrich, M.S. Fanselow, Scopolamine and Pavlovian fear conditioning in rats: dose-effect analysis, Neuropsychopharmacology 21 (1999) 731–744.
[4] R.T. Bartus, R.L. Dean, B. Beer, A.S. Lippa, The cholinergic hypothesis of geriatric memory dysfunction, Science 217 (1982) 408–417.
[5] T. Bast, W.N. Zhang, J. Feldon, Dorsal hippocampus and classical fear conditioning to tone and context in rats: effects of local NMDA-receptor blockade and stimulation, Hippocampus 13 (2003) 657–675.
[6] T.V. Bliss, G.L. Collingridge, A synaptic model of memory: long-term potentiation in the hippocampus, Nature 361 (1993) 31–39.
[7] N.G. Bowery, A.L. Wong, E.H.F. Hudson, Quantitative autoradiography of [3H]-MK-801 binding sites in mammalian brain, Br. J. Pharmacol. 93 (1988) 944–954.
[8] G. Bronsanm-Watters, D.F. Wozniak, A. Nardi, J.W. Olney, Acute behavioral effects of MK-801 in the mouse, Pharmacol. Biochem. Behav. 53 (1996) 701–711.
[9] P. Calabresi, D. Centonze, P. Gubellini, A. Pisani, G. Bernardi, Endogenous ACh enhances striatal NMDA-responses via M1-like muscarinic receptors and PKC activation, Eur. J. Neurosci. 10 (1998) 2887–2895.
[10] C. Castellano, V. Cestari, A. Ciamei, NMDA receptors and learning and memory processes, Curr. Drug Targets 2 (2001) 273–283.
[11] P. Dutar, M.H. Bassant, M.C. Senut, Y. Lamour, The septohippocampal pathway: structure and function of a central cholinergic system, Physiol. Rev. 75 (1995) 393–427.
[12] K. Elrod, J.J. Buccafusco, An evaluation of the mechanism of scopolamine-induced impairment in two passive avoidance protocols, Pharmacol. Biochem. Behav. 29 (1988) 15–21.
[13] T.L. Ferreira, K.M. Moreira, D.C. Ikeda, O.F.A. Bueno, M.G.M. Oliveira, Effects of dorsal striatum lesions in tone fear conditioning and contextual fear conditioning, Brain Res. 987 (2003) 17–24.
[14] R.V. Fornari, K.M. Moreira, M.G.M. Oliveira, Effects of the selective M1 muscarinic receptor antagonist dicyclomine on emotional memory, Learn. Mem. 7 (2000) 287–292.
[15] A. Giachetti, E. Giraldo, H. Ladinsky, E. Montagna, Binding and functional profiles of the selective M1 muscarinic receptor antagonists trihexaphenidyl and dicyclomine, Br. J. Pharmachol. 89 (1986) 83–90.
[16] P.E. Gold, The use of avoidance training in studies of modulation of memory storage, Behav. Neural Biol. 46 (1986) 87–98.
[17] P.E. Gold, Acetylcholine modulation of neural systems involved in learning and memory, Neurobiol. Learn. Mem. 80 (2003) 194–210.
[18] T.J. Gould, M.M. McCarthy, R.A. Keith, MK-801 disrupts acquisition of contextual fear conditioning but enhances memory consolidation of cued fear conditioning, Behav. Pharmacol. 13 (2002) 287–294.
[19] S.B. Harrod, R.W. Flint, D.C. Riccio, MK-801 induced retrieval, but not acquisition, deficits for passive avoidance conditioning, Pharmacol. Biochem. Behav. 69 (2001) 585–593.
[20] V. Heale, C. Harley, MK-801 and AP5 impair acquisition, but not retention, of the Morris milk maze, Pharmacol. Biochem. Behav. 36 (1990) 145–149.

[21] Z. Hlinák, I. Krejci, Concurrent administration of subeffective doses of scopolamine and MK-801 produces a short-term amnesia for the elevated plus-maze in mice, Behav. Brain Res. 91 (1998) 83–89.
[22] Z. Hlinák, I. Krejci, MK-801 induced amnesia for the elevated plus-maze in mice, Behav. Brain Res. 131 (2002) 221–225.
[23] A.J. Hunter, F.F. Roberts, The effect of pirenzepine on spatial learning in the Morris water maze, Pharmacol. Biochem. Behav. 30 (1988) 519–523.
[24] I. Izquierdo, J.H. Medina, Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures, Neurobiol. Learn. Mem. 68 (1997) 285–316.
[25] H.B. Li, K. Matsumoto, M. Tohda, M. Yamamoto, H. Watanabe, NMDA antagonists potentiate scopolamine-induced amnesic effect, Behav. Brain Res. 83 (1997) 225–228.
[26] R.C. Malenka, The long-term potential of LTP, Nat. Rev. Neurosci. 4 (2003) 923–926.
[27] H. Markram, M. Segal, Long-lasting facilitation of excitatory postsynaptic potentials in the rat hippocampus by acetylcholine, J. Physiol. 427 (1990) 381–393.
[28] E. Moor, P.D. Boer, H.J.A. Beldhuis, B.H.C. Westerink, A novel approach for studying septo-hippocampal cholinergic neurons in freely moving rats: a microdialysis study with dual-probe design, Brain Res. 648 (1994) 32–38.
[29] K.M. Moreira, D.C. Hipolide, J.N. Nobrega, O.F.A. Bueno, S. Tufik, M.G.M. Oliveira, Deficits in avoidance responding after paradoxical sleep deprivation are not associated with altered [3H] pirenzepine binding to M1 muscarinic receptors in rat brain, Brain Res. 977 (2003) 31–37.
[30] M. Ohno, S. Watanabe, Interactive processing between glutamatergic and cholinergic systems involved in inhibitory avoidance learning of rats, Eur. J. Pharmacol. 312 (1996) 145–147.
[31] M.G. Packard, A.L. Teather, Posttraining injections of MK-801 produce a timedependent impairment of memory in two water maze tasks, Neurobiol. Learn. Mem. 68 (1997) 42–50.
[32] G.L. Quirarte, S.E. Cruz-Morales, A. Cepeda, M. Garcia-Montanez, G. Roldan-Roldan, R.A. Prado-Alcala, Effects of central muscarinic blockade on passive avoidance: Anterograde amnesia, state dependency, or both? Behav. Neural Biol. 62 (1994) 15–20.
[33] S.T. Rouse, M.J. Marino, L.T. Potter, P.J. Conn, A.I. Levey, Muscarinic receptor subtypes involved in hippocampal circuits, Life Sci. 64 (1999) 501–509.
[34] M. Sala, D. Braida, P. Calcaterra, M.P. Leone, F.A. Comotti, S. Gianola, E. Gori, Effect of centrally administered atropine and pirenzepine on radial arm maze performance in the rat, Eur. J. Pharmacol. 194 (1991) 45–49.
[35] G.V. Wallenstein, D.R. Vago, Intrahippocampal scopolamine impairs both aquisition and consolidation of contextual fear conditioning, Neurobiol. Learn. Mem. 75 (2001) 245–252.
[36] L. Ward, S.E. Mason, W.C. Abraham, Effects of the NMDA antagonists CPP and MK-801 on radial arm maze performance in rats, Pharmacol. Biochem. Behav. 35 (1990) 785–790.
[37] A.M. White, P.J. Best, The effects of MK-801 on spatial working memory and within-session spatial learning, Pharmacol. Biochem. Behav. 59 (1998) 613–617.