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Effects of nitric oxide synthase inhibition on spatial discrimination learning and central DA2 and mACh receptors

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Abstract

Cholinergic and dopaminergic systems are involved in spatial memory and are modulated by nitric oxide (NO); NO has well documented effects on place learning in rodents. The aim of the present study was to investigate the effect of NOS inhibition on place learning in the water maze and to evaluate the relationships between NOS inhibition, learning performance, dopamine (DA) D2 and muscarinic acetylcholine (mACh) receptors. Male Sprague–Dawley rats received the NOS inhibitor Nω-Nitro-L-Arginine (L-NA), or saline and were trained in the water maze. Rats that were not trained, but received the same treatments were also included. Following treatments with or without water maze training, [^{3}H]-QNB and [^{3}H]-spiperone binding in cortex, striatum and hippocampus were determined to assess the effects of NOS inhibition and/or learning on DA D2 and mACh receptor regulation. The overall results of the present study showed that: (1) NOS inhibition impairs performance in the MWM; (2) NOS inhibition does not affect specific binding to DA D2 (striatum and hippocampus) and mACh (cortex and hippocampus) receptors; (3) MWM training lowers D2 and mACh receptor binding in cortical regions.

Keywords: Place learning; Rat; Brain; Receptor regulation; Water maze

1. Introduction

Endogenous nitric oxide (NO) plays an important role in transsynaptic regulation. NO involvement in the release of acetylcholine (ACh), catecholamines, excitatory and inhibitory amino acids, serotonin, histamine, and adenosine have been documented (Bowyer et al., 1995; Getting et al., 1996; Hanbauer et al., 1992; Kano et al., 1998; Lees et al., 1997; Lonart et al., 1992, 1993; Lonart and Johnson, 1995; Peterson et al., 1995; Prast and Philippu, 2001; Sandor et al., 1995; Sequeira et al., 1997; Spatz et al., 1995). NO may regulate neurotransmitter release in a Ca^{2+} independent manner (Meffert et al., 1994) and also mediate in volume transmission, spatial signaling, and nonsynaptic conduction among groups of neurons and glia. NO produced by glutamate stimulation induces exocytosis and release of glutamate and DA from nearby neurons, thereby affecting synaptic plasticity (Kline et al., 2002; Schulman, 1997; Vizi, 2000). In addition to modifying release, NO also inhibits neurotransmitter reuptake, specifically as regards glutamatergic and dopaminergic systems (Kiss et al., 1999; Pogun et al., 1994a,b; Pogun and Kuhar, 1994). Both effects, enhancement of neurotransmitter release and inhibition of reuptake, increase the availability of neurotransmitters in the synaptic cleft, which in turn may affect post synaptic receptor modulation and have a role in learning and memory processes.

Interactions between NO and dopamine (DA) actions have been investigated and bidirectional relations have been shown between NO synthesis and DA release (Fujiyama and Masuko, 1996; Hidaka and Totterdell, 2001). Particularly, Liu (1996) emphasized the significance of NO in prolonging the presence and thereby the efficacy of DA in the synapse.

Following dopaminergic lesions induced by intra-nigral 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

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application, DA and its nonconjugated metabolites were lowered in the dorsal striatum and in the prefrontal cortex (PFC), but not in the hippocampus or nucleus accumbens. Although this lesion did not affect the motor performance of the rats or learning of a spatial reference memory task in the water maze, performance in spatial working memory and cued version tasks of the water maze were impaired (Miyoshi et al., 2002). Unilateral striatal injections of 6-Hydroxydopamine (6-OHDA) decreased spontaneous locomotor activity and impaired performance during a spatial navigation task in the water maze in rats (Heim et al., 2001). Controlled cortical impact (CCI) is an in vivo rat model of human traumatic brain injury (TBI), and produces spatial learning acquisition deficits together with initial impairments in motor function and working memory. The D2 receptor agonist bromocriptine applied systemically after CCI did not affect motor function but improved working memory and spatial acquisition deficits. Furthermore, bromocriptine treatment augmented the survival rate of the CA3 neurons of the hippocampus, suggesting cognitive and neural protection by DA following TBI (Kline et al., 2002).

Wilkerson and Levin (1999) infused D1 and D2 receptor agonists and antagonists into ventral hippocampus and used the radial-arm maze to assess working memory performance. While the D1 receptor compounds did not have a profound influence on performance, the D2 agonist quinpirole improved and the D2 antagonist raclopride deteriorated choice accuracy. D2 receptor knockout mice, tested in place-learning tasks, show reduced locomotor activity and slower acquisition (Glickstein et al., 2002; Tran et al., 2002). However Glickstein et al. (2002) showed that the deficit was partly overcome by methamphetamine treatment of D2 and D3 receptor knockout mice, suggesting D1 receptor activation. The stimulation of CCK-B receptors facilitates spatial recognition memory and this can be induced by applying the agonist, BC264, systemically. The facilitating effect induced by BC264 was abolished by the D2 receptor antagonist sulpiride, but not by the D1 receptor antagonist SCH 23390 (Lena et al., 2001) suggesting a key role for D2 but not for D1 receptors. On the other hand, Mele et al. (2004) suggest the involvement of accumbal D1 as well as of D2 receptors in spatial memory as locally applied antagonists (SCH 23390 and sulpiride, respectively) impaired consolidation in mice. On the other hand, Turner and Soliman (2000) have shown that high doses of zinc chloride, which reduces the affinity and increases the Bmax of D1 receptors in relevant regions of rat brain, declines performance in a spatial reference memory task. A recent study by Yang et al. (2004) demonstrates the efficacy of a selective D1/D5 receptor agonist (A68930) in alleviating the cognitive deficits induced by hypoxic encephalopathy.

Reward systems and mesolimbocortical dopaminergic neurotransmission mediate in addiction and learning (Sigala et al., 1997). Both dopamine D1 and D2 receptors in the hippocampus are reported to facilitate acquisition and retention of different working memory tasks (Levin and Rose, 1995; Packard and White, 1991; White and Viaud, 1991; White et al., 1993). Although the D1/D5 receptors are implicated in the facilitation of LTP and in the storage of unpredicted information in the CA1 area of the hippocampus (Li et al., 2003), a substantial number of studies support the involvement of hippocampal D2 activity in spatial working memory.

NO has a modulatory role on cholinergic transmission. While L-Arginine and NO donors decrease ACh release at the inhibitory synapse and increase it at the excitatory synapse, NOS inhibitors have opposite effects (Meulemans et al., 1995; Mothet et al., 1996a,b). Effect of NO on non-quantal ACh release (Mukhtarov et al., 1999, 2000) and calcium-dependent ACh release have been shown as well. NO involvement on cholinergic transmission is independent of choline acetyltransferase (Morot Gaudry-Talarmain et al., 1997). Interactions between cholinergic and dopaminergic systems in mediating spatial memory are plausible (Kim and Levin, 1996; Mattsson et al., 2002).

A large number of studies implicate the role of ACh in cognitive functions, including learning and memory and specifically of hippocampal ACh in cognitive functions including spatial learning (Everitt and Robbins, 1997; Givens and Sarter, 1997; Sarter and Bruno, 1997). On the other hand, studies, using local injections of the selective cholinergic neurotoxin 192IgG-saporin, into the septal area have been reported to cause only small or no impairments of spatial reference memory although hippocampal ACh was reduced (Baxter et al., 1995; Berger-Sweeney et al., 1994; Torres et al., 1994). In contrast, some groups have reported decline in spatial working memory (Lehmann et al., 2002; Walsh et al., 1995) by similar treatments. Recently the simplistic notion that an increase in hippocampal ACh may be facilitatory for learning and memory has been challenged. Elvander et al. (2004) emphasizes the importance of an optimal physiological level of cholinergic function and proposes that complex regulatory mechanisms operating on septal cholinergic and GABAergic neurons will impact hippocampal functions.

There is an impressive amount of evidence suggesting the involvement of NO in hippocampal long-term potentiation (LTP) (Wang et al., 1997; Zorumski and Izumi, 1998), synaptic plasticity and consequently learning and memory. NO has modulatory effects on different learning and memory processes such as motor learning (Yanagihara and Kondo, 1996), avoidance learning (Myslivecek et al., 1996; Qiang et al., 1997; Telegdy and Kokavszky, 1997), olfactory learning (Kendrick et al., 1997; Okere et al., 1996) and spatial learning (Holscher et al., 1996; Kendrick et al., 1997; Okere et al., 1996; Yamada et al., 1996). While some studies report impairment of spatial learning by NO synthase (NOS) inhibition (Bohme et al., 1993; Chapman et al., 1992; Demirgoren and Pogun, 1995), others have diverse interpretations for the behavioral effects of NOS inhibition. For example, considering the confounding effects of systemi-

cally applied NOS inhibitors, Blokland et al. (1999) applied L-NA locally into the dorsal hippocampus and observed impaired performance in L-NA treated rats only during the late phase of acquisition, but no difference between the groups during the memory test (probe trial). Bannerman et al. (1994) observed impairment of spatial learning following NOS inhibition only if the inhibitor is applied before acquisition; NOS inhibition was without effect on retention, reversal learning if applied after acquisition of the task. We have recently shown that NOS inhibition impairs acquisition in Sprague–Dawley rats, especially during the earlier phases (Kanit et al., 2003). These effects are likely to result in plastic changes in related neurotransmission systems and corresponding brain regions (Hawkins et al., 1998; Huang, 1997; Salemme et al., 1996; Wang et al., 1997) which can be observed at the receptor level.

Considering the involvement of the cholinergic and dopaminergic systems in spatial memory, the modulation of cholinergic and dopaminergic activity by NO and the influence of NO on place learning, the aim of the present study was to investigate the effect of NOS inhibition on place learning in the water maze and to evaluate the relationship between NOS inhibition, learning performance, DA D2 and muscarinic acetylcholine (mACh) receptors.

2. Material and methods

2.1. Laboratory animals and experimental design

Three- to four-month-old male Sprague–Dawley rats, kept under standard colony conditions (3–4/cage, 20–22 °C, 12-h light/dark cycle) with ad-lib food and water, were used in experiments.

Thirty-two rats ($n=8$ for each group) were divided into four groups in a 2×2 factorial design: MWM place learning (trained vs. control) × NOS inhibition (drug vs. saline).

Rats were sacrificed 24 h after the last experiment (MWM trained groups) or last injection (control groups) and brains were dissected on ice. Cortex and hippocampus were used for ACh, and corpus striatum and hippocampus were used for DA D2 receptor binding experiments.

The animals were handled under the prescriptions for animal care and experimentation of the pertinent European Communities Council Directive (86/609/EEC), and all the procedures were approved by the Institutional Animal Ethics Committee of Ege University.

2.2. Drug treatment

The NOS inhibitor Nω-Nitro-L-Arginine (L-NA, 50 mg/kg, Sigma 5501) or saline, were administered i.p., 10 min prior to testing, for 7 days. The control groups were not subjected to MWM learning experiments; L-NA and saline subgroups received injections in the same regimen as the MWM learning groups.

2.3. The MWM apparatus

A circular pool (130 cm ∅ and 75 cm high) was filled to a depth of 45 cm with dark yellow opaque water, at 22 °C. The visible platform was constructed of wood (12×12 cm) and protruded 2.5 cm above the surface of the water. The hidden platform was metal (12×12 cm), painted yellow, and submerged 1.5 cm below water level. The maze was located in a 4×3 m room and extramaze (spatial) cues included posters, a window, a cage and two experimenters. The maze was divided into four virtual quadrants, N–S–E–W.

2.4. Morris Water Maze procedure

The rational of the MWM experiments were: (day 1) showing the animal that there is a platform to escape from the water and making sure that the rats do not have sensorimotor deficits that may interfere with the task, (days 2–6) acquisition of place learning using spatial cues and navigational strategy, (Probe trial) test of memory. In a protocol modified from Morris (1984), the platform was in the same position (in the center of quadrant S) throughout the experiment, visible on the first day and hidden (submerged) during days 2–6. There were four trials with an intertrial interval of approximately 20 min for each rat. Testing started 10 min after the injections and the rats were handled before the first trial each day. On the first day of acquisition, the platform was visible and the rat was placed on the platform for 30 s before the trials began, to introduce the platform and show that the platform is a mean of escape from the water.

Throughout the experiment, as stated above, animals were handled before the first trial each day and then were released once from each of the four quadrants facing the center of the pool. The order of the release positions was varied systematically throughout the experiment as follows: day 1: NWES, day 2: WESN, day 3: ESNW, day 4: SNWE, day 5: NWES, day 6: WESN. A trial ended when the rat climbed on the platform. If a rat had not found the platform after 60 s, it was placed on the platform by the experimenter. All the rats were left on the platform for 15 s and then removed to their home cages by the experimenter.

On day 7, the platform was removed and the rats were released from the N starting point. The time spent in the quadrant (S) where the platform had been during acquisition was recorded.

2.5. Receptor binding experiments

2.5.1. Muscarinic receptor binding

Muscarinic receptor binding assays were performed as previously reported (Pogun et al., 1992a; Yamamura and Snyder, 1974). Cortices and hippocampi were dissected on ice after decapitation; tissues were weighed and homogenized using a glass-teflon homogenizer in 10 volumes of cold

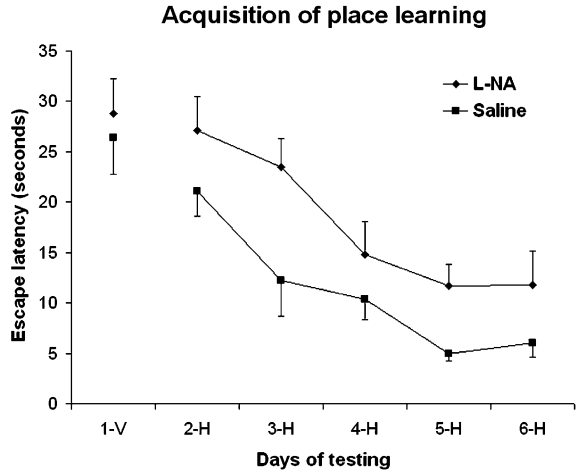

Fig. 1. Average ELs (4 trials/day) during days 1–6; V: visible, H: hidden platform (Mean±S.E.M.). Animals were released from different starting positions for each trial. Significant main effects of days ($p<0.001$) and NOS inhibition ($p=0.002$) during days 2–6 (ANOVA).

0.32 M sucrose. Following centrifugation at $1000\times g$ for 10 min, the pellets were discarded and the supernatants were homogenized again with a polytron in 1:20 volume 0.05 M sodium–potassium phosphate buffer (pH 7.4). Tissue homogenates were incubated in triplicates, with 0.06–0.10 nM L-Quinuclidinyl[*phenyl*-4-^{3}H]Benzilate ([^{3}H]-QNB, Amersham, TRK 604) for 60 min at room temperature; 10^{-6} M atropine was used to define specific activity. Free ligand was rapidly filtered through glass fiber filters (GF/B), and the bound fraction on the filters was counted in a scintillation counter (Packard, Tricarb 2100TR) after the addition of scintillation cocktail.

2.5.2. Dopaminergic receptor binding

Dopaminergic receptor binding assays were performed as previously reported (Pogun et al., 1992b). Corpus striata and hippocampi were dissected on ice, tissues were weighed and homogenized in 100 volume cold 50 mM Tris–HCl (pH 7.7 at 25 °C) with a polytron. Following centrifugation at $50{,}000\times g$ for 10 min, the pellets were re-homogenized and re-centrifuged. Final pellets were resuspended in incubation buffer (96% 50 mM Tris, 4% ion mix [120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% Ascorbate]) and incubated with 0.3–0.5 nM [^{3}H]Spiperone (Amersham, TRK 818) for 40 min at 37 °C; 10^{-3} M butaclamol was used to define specific binding. The bound fraction was obtained and counted as described above.

2.6. Statistical evaluation

Group differences in ELs during the first day of testing with the visible platform was analyzed by *t*-test. The acquisition of place learning was evaluated by repeated measures analysis of variance (ANOVA) with ELs as the dependent variable, and the NOS inhibition groups (L-NA, and saline) and days of testing (2–6) as between- and within-subjects factors, respectively. On the probe trial of day 7, groups were compared (*t*-test) according to the time spent in the quadrant where the platform used to be during acquisition (TS).

For each brain region studied, separate multifactorial ANOVAs were performed with specific [^{3}H]-spiperone or [^{3}H]-QNB binding as the dependent variable, and MWM training (MWM trained and control) and NOS inhibition groups (L-NA and saline) as the factors. Post-hoc tests were performed as required.

Correlation analyses (Spearman's) were performed between behavioral data (acquisition days 1–6 and probe trial) and receptor binding ([^{3}H]-spiperone binding in the striatum and hippocampus and [^{3}H]-QNB binding in the cortex and hippocampus).

SPSS program (version 10.0) was used for all statistical analyses.

3. Results

3.1. Morris Water Maze learning experiments

Acquisition of place learning during days 2–6 of the experiment (Fig. 1) is indicated by a significant decrease in ELs for both groups. Repeated measures ANOVA with days as the within-subjects factor and NOS inhibition (L-NA and saline) as the between-subject factor revealed significant main effects of days [$F(4, 56)=12.842$, $p<0.001$] and NOS inhibition [$F(1,14)=13.628$, $p=0.002$] on performance. Although rats acquired place learning through days 2–6, the performance of the NOS inhibited group (L-NA) was significantly impaired compared to saline treated rats. The groups did not perform differently on day 1 with the visible platform.

On the probe trial (day 7), the NOS inhibited rats spent less time ($p=0.034$) searching for the platform compared to saline treated rats (Fig. 2).

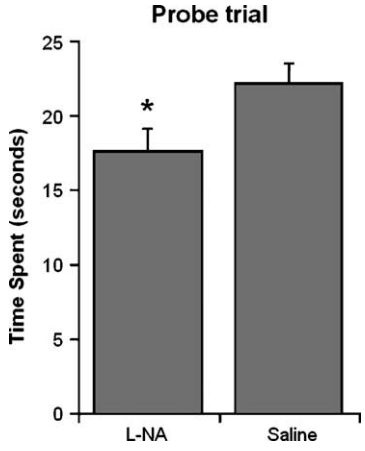

Fig. 2. During the probe trial of day 7 when the platform had been removed, the time spent in the quadrant where the platform had been during acquisition (Mean±S.E.M.). *$p<0.05$ (*t*-test).

3.2. Receptor binding experiments

3.2.1. Dopamine D2 receptors

Multifactorial ANOVA for specific [^{3}H]-spiperone binding with MWM training and NOS inhibition as factors revealed a significant effect of MWM training in hippocampus [$F(1, 31)=5.419$, $p=0.027$]. Rats which were subjected to MWM place learning experiments had decreased DA D2 receptor binding compared to control rats which only received injections. (Fig. 3A). NOS inhibition did not emerge as a significant main effect. Post-hoc analysis showed that saline treated control rats had the highest ^{3}H-spiperone binding of all groups ($p<0.05$). No significant effects were observed in the striatum (Fig. 3B).

3.2.2. Acetylcholine receptors

Multifactorial ANOVA for specific [^{3}H]-QNB binding with MWM training and NOS inhibition as factors revealed a significant effect of MWM training in both cortex [$F(1, 31)=8.558$, $p=0.007$] and hippocampus [$F(1, 31)=13.839$, $p=0.001$]. MWM training resulted in decreased [^{3}H]-QNB binding (Fig. 3C and D). As in DA D2 receptor binding, NOS inhibition did not emerge as a significant effect.

Significant negative correlations were depicted between performance on days 1 and 3 of acquisition and [^{3}H]-QNB binding in the cortex, indicating shorter escape latencies are correlated with higher receptor binding during early acquisition.

4. Discussion

The overall results of the present study can be summarized under three main sections: (1) NOS inhibition impairs performance in the MWM; (2) NOS inhibition does not affect specific binding to DA D2 (striatum and hippocampus) and mACh (cortex and hippocampus) receptors;

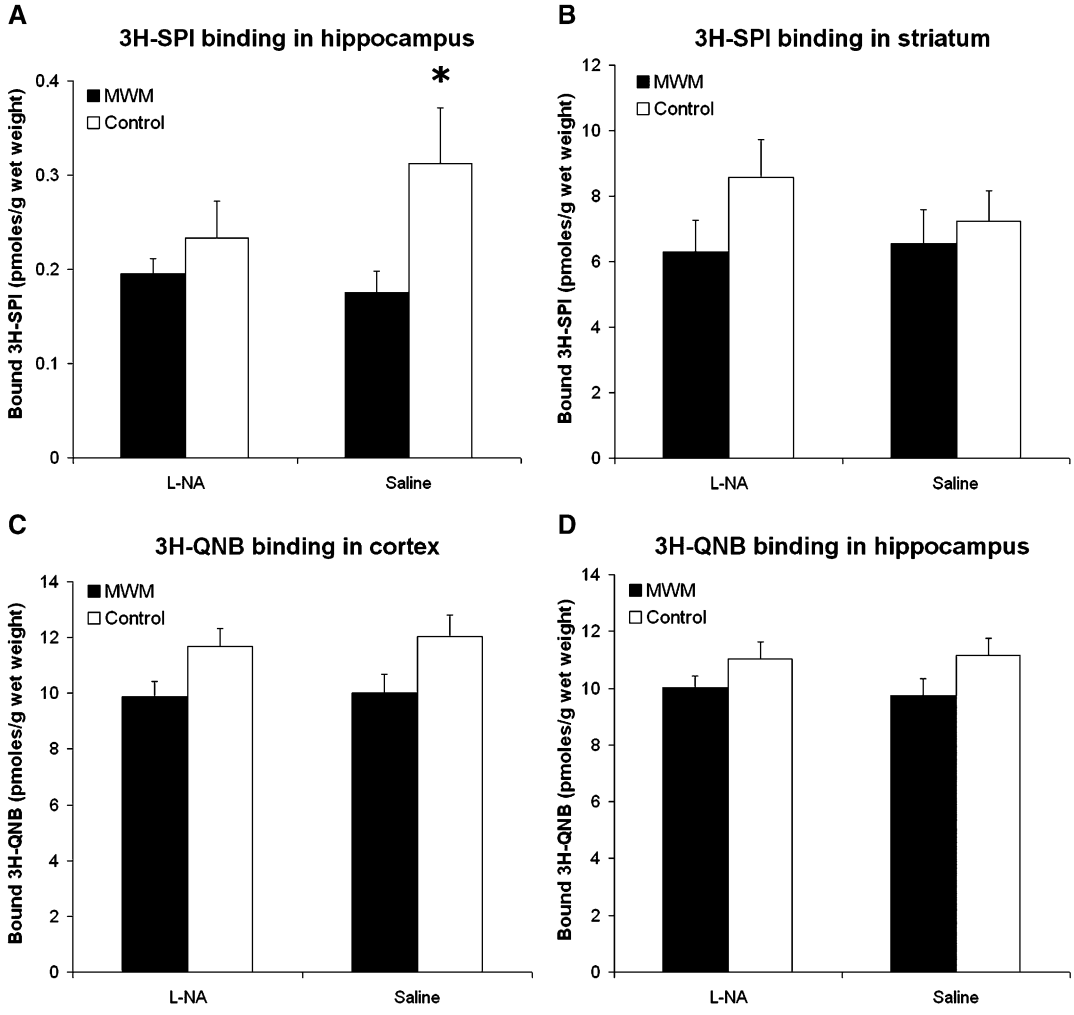

Fig. 3. ^{3}H Spiperone binding in hippocampus (A) and striatum (B); ^{3}H-QNB binding in cortex (C) and hippocampus (D). MWM=rats subjected to learning experiments in Morris Water Maze; Control=rats that were not trained in the water MWM but received only L-NA or saline injections. SPI=spiperone, spiroperidol (Mean±S.E.M.). Significant main effect of MWM training (ANOVA); (A) $p=0.027$, (C) $p=0.007$, (D) $p=0.001$. Different from L-NA treated groups: *$p<0.05$, Duncan's test.

(3) MWM training lowers D2 and mACh receptor binding in cortical regions studied.

We have shown that brain $NO_2^- + NO_3^-$ levels remain depressed up to 24 h following NOS inhibition by L-NA (Kanit et al., 2000) at the dose employed in the current study, suggesting persisting effects on brain NOS. Therefore, $NO_2^- + NO_3^-$ levels can be expected to be approximately 50% of the control values during and 24 h after behavioral testing when the receptor assays were performed. However, since the current study was designed to assess persisting changes, short-term modifications induced by MWM testing may have been missed.

The NO synthase inhibitor L-NA administration deteriorated the acquisition of place learning (Fig. 1). Although the L-NA treated rats did show a decrease in EL throughout acquisition indicating learning, the ELs were higher in L-NA treated rats compared to saline treated controls. This finding confirms reports by our group and other groups who have demonstrated impairment of place learning by NOS inhibition in rats (Demirgoren and Pogun, 1995; Holscher et al., 1996; Kanit et al., 2003; Qiang et al., 1997). Furthermore, NOS inhibition impaired performance during the probe trial, suggesting deficient memory. Our experimental design was similar to the first experiment in the Bannerman et al. (1994) study who have obtained almost identical results during both acquisition and probe trial with NOS inhibition applied before acquisition. In the same paper, combining the results from a series of delicately planned experiments, Bannerman et al. (1994) discuss that systemic NOS inhibition at the doses employed do not induce gross sensorimotor impairments which may interfere with the spatial learning task, although the effect may be causing a behavioral syndrome which may extend beyond the domain of learning. On the other hand, Sandi et al. (1995) have shown that L-NA alters exploratory pattern and reduces locomotion in a novel environment, reduces startle response to either acoustic or electric stimuli and alters cardiovascular measures in rats. The effect of NOS inhibition on motor activity may influence behavior in the WM (Abekawa et al., 1997; Prendergast et al., 1997; Tatchum-Talom et al., 2000). Escape latency was taken as the measure of performance since in another study in our laboratory, using the same rat strain and L-NA dose, we have shown that L-NA does not effect swim speed in male rats but decreases speed in females resulting in an interaction (Kanit et al., 2003).

Although NOS inhibition in adult animals by pharmacologic manipulations cannot be a direct equivalent of genetic modifications, studies in endothelial NOS knockout animals (Dere et al., 2001; Frisch et al., 2000; Reif et al., 2004) also depict behavioral and neurochemical changes which may impact learning and memory performance. For example the knockout mice had higher concentrations of 5-HIAA in the cerebellum, an accelerated serotonin turnover in the frontal cortex, and a higher DA turnover in the ventral striatum (Frisch et al., 2000). In our study, the lack of significant differences between the groups on day 1 suggests that the impaired performance observed in the present study cannot be attributed solely to psychomotor deficit.

In the present study we did not observe an effect of NOS inhibition on D2 and mACh receptor binding. Some NOS inhibitors can alter the binding capacity of neurotransmitter receptors (Bidmon et al., 1999) and increase the binding at NMDA and AMPA receptors. We did not study the involvement of the glutamatergic system in the present study where NOS inhibition may have more profound effects on glutamate receptors.

Our data shows that L-NA did not effect the specific binding of QNB or spiperone to mACh (hippocampus and cortex) and D2 (striatum and hippocampus) receptors. On the other hand, MWM training caused a decline of receptor binding, regardless of treatment, in the cortex and hippocampus for mACh and only in the hippocampus but not in the striatum for D2 receptors. This affect may be due to increased endogenous neurotransmitter levels following a cognitive task, which also has sensorimotor components, and a subsequent receptor down-regulation. Brown et al. (2000) have shown an increase in DA synthesis in the medial prefrontal cortex following a water maze task in rats; however, receptor assays were not employed.

The negative correlation between cortical QNB binding and performance (EL) on days 1and 3 of acquisition suggest that higher binding is correlated with better performance (lower ELs) only during the earlier phases of the study.

The radioligand used in the present study (^{3}H-QNB) was unspecific to depict changes in different subtypes of cholinergic receptors; therefore, more pronounced changes in one subtype (e.g. M1) may have been missed.

Most of the studies that aim to elucidate the role of D2 and ACh receptors in spatial memory treat the animals with agonists and antagonists of the receptor and evaluate performance subsequently (Brown et al., 2000; Heim et al., 2001; Kim and Levin, 1996; Milivojevic et al., 2001; Miyoshi et al., 2002; Setlow and McGaugh, 2000; Wilkerson and Levin, 1999). The protocol in our study was almost the inverse of the aforementioned studies; drugs that exert their actions through receptor binding were not employed. Receptor binding was assessed after water maze training, without any in vivo exposure to compounds acting directly on receptors. Therefore it is hard to make direct comparisons between the present study and those with similar aims employing different approaches.

Water maze is one of the tests to study interference with neurochemical systems and DAergic and AChergic systems rank high in having impact on learning and memory tests. Lower ACh receptor binding in the cortex and hippocampus and lower D2 receptor binding in the hippocampus in MWM trained rats compared to controls suggest that increased neurotransmitter levels resulting from training may be causing a down-regulation of receptors. As suggested by Myhrer (2003) in a recent review based on meta-analyses, the memory systems in the rat brain involve

substantial interactions between neurotransmitter systems, including DA and ACh, rather than being related to a specific system. Glutamate and acetylcholine were the most extensively studied systems and impacted behavior in the MWM. Similarly influencing dopaminergic activity modified performance while noradrenaline and serotonin had weaker associations. Discrepant finding with the same agent was observed and attributed to the design of the experiments. Future studies are needed to elucidate the complex interactions between different neurotransmission systems and behavior.

In conclusion, NOS inhibition does not alter the binding of ^{3}H-QNB to mACh (cortex and hippocampus) and of ^{3}H-Spiperone (hippocampus and striatum) to DA D2 receptors, but impairs place learning in adult male Sprague–Dawley rats. During early acquisition, cortical mACh receptor binding is correlated with better performance. However, later on in the study, water maze training lowers ^{3}H-QNB and DA D2 receptor binding in cortical regions suggesting increased neurotransmitter concentration in regions critical for place learning.

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