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ORIGINAL INVESTIGATION

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NMDA or AMPA/kainate receptor blockade prevents acquisition of conditioned place preference induced by $D_{2/3}$ dopamine receptor stimulation in rats

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Abstract *Rationale:* Recent experiments from this laboratory demonstrated synergistic effects of AMPA/kainate receptor blockade and $D_{2/3}$ dopamine (DA) receptor stimulation on brain stimulation reward and locomotor activity. *Objectives:* Using place conditioning, this study explored further the interaction between DA and glutamate (Glu) using the *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801, the AMPA/kainate receptor antagonist NBQX, and the $D_{2/3}$ DA receptor agonist 7-OH-DPAT. *Methods:* Effects of these compounds, alone and combined, were measured in male Sprague–Dawley rats using an unbiased two-compartment place conditioning procedure. *Results:* 7-OH-DPAT (0.03–5.0 mg kg^{-1}, s.c.) administered immediately prior to conditioning was ineffective; when administered 15 min prior to conditioning, only the highest dose (5.0 mg kg^{-1}, s.c.) induced conditioned place preference (CPP). Acquisition of 7-OH-DPAT-induced CPP was blocked by MK-801 (0.06 or 0.13 mg kg^{-1}, i.p.) or NBQX (0.5 μg) microinjected into the nucleus accumbens (NAS) shell subregion. Intra-NAS shell administration of 7-OH-DPAT (5.0 μg) or NBQX (0.5 μg), alone or combined, failed to induce place conditioning, and this lack of effect was not due to state dependency. Administration of MK-801 or 7-OH-DPAT (5.0 mg kg^{-1}) during the conditioning phase acutely increased horizontal activity, but neither compound, alone or combined, induced conditioned locomotor effects. *Conclusions:* Acquisition of place conditioning induced by systemic administration of 7-OH-DPAT is blocked by systemic NMDA receptor antagonism by MK-801 or by the AMPA/kainate receptor antagonist NBQX microinjected into the NAS shell subregion.

Keywords 7-OH-DPAT · AMPA/kainate · Dopamine · Glutamate · Locomotor activity · MK-801 · NMDA · Place conditioning · Place preference · Reward

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Introduction

Interactions between dopamine (DA) and glutamate (Glu) may be an important component of neural mechanisms of schizophrenia and drug abuse. Dopamine receptor subtypes are subdivided into two subfamilies, D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4). The D_3 DA receptors, preferentially localized within DA terminal areas of the limbic forebrain, are of interest in this context in relation to cognitive, emotional, and endocrine functions (Sokoloff et al. 1990; Levesque et al. 1992). Among these DA terminal areas, the nucleus accumbens (NAS) shell subregion is associated with motivation and reward (Deutch et al. 1993).

To induce place conditioning, a classical conditioning phenomenon, animals are given a drug in one neutral environment and saline in an alternate neutral environment. When later allowed to explore either environment, a preference for the drug-paired environment indicates conditioned reward. Place conditioning is a widely accepted procedure for exploring reward-related neural processes in

vivo. Compounds that enhance DA transmission (e.g., amphetamine or cocaine) consistently induce conditioned place preference (CPP, e.g., Spyraki et al. 1982; Biala and Langwinski 1996; Bardo et al. 1999). More selective DA agonists have implicated a role for $D_{2/3}$ DA receptors in this context. For example, the agonist (±)-7-OH-DPAT, which has >100, >1,000, and >10,000-fold selectivity for D_3 over D_2, D_4, and D_1 DA receptors respectively (Levesque et al. 1992), may induce CPP (Mallet and Beninger 1994; Chaperon and Thiebot 1996; Kling-Petersen et al. 1995).

Glu receptor antagonists may affect CPP induced by DA receptor agonists. MK-801 (dizocilpine), a non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist (Wong et al. 1986), reduces methamphetamine-induced CPP (Kim and Jang 1997) and blocks cocaine-induced CPP (Cervo and Samanin 1995; Kim et al. 1996). The AMPA/kainate receptor antagonist NBQX may prevent the induction of amphetamine-induced CPP in mice (Mead and Stephens 1999). CPP induced by amphetamine or cocaine may also be blocked by intra-NAS administration of the AMPA/kainate receptor antagonist DNQX (Layer et al. 1993b; Kaddis et al. 1995). Such studies indicate the importance of DA–Glu interactions in this context, but have used non-specific or indirect DA agonists.

Using relatively receptor-specific compounds, the present study: (1) examined the effects of AMPA/kainate receptor blockade, NMDA receptor blockade, and $D_{2/3}$ DA receptor stimulation alone on place conditioning; (2) investigated the interaction in place conditioning between NMDA or AMPA/kainate receptor blockade and $D_{2/3}$ DA receptor stimulation, both systemically and in the NAS shell subregion; and (3) controlled for state-dependent learning to place conditioning (e.g., Spyraki et al. 1985) induced by $D_{2/3}$ DA receptor stimulation. As conditioned increases in activity may contribute to the development of CPP (Bozarth and Wise 1981), conditioned locomotor responses were also investigated.

Materials and methods

Animals

Male Sprague–Dawley rats (200–250 g; Health Sciences Laboratory Animal Services, University of Alberta) were housed individually in a temperature-controlled (21±1°C) and humidity-controlled environment with a 12-h light/dark cycle (lights on 0700–1900 h). Food (LabDiet 5001 Rodent Diet, PMI Nutrition International Inc., Brentwood, MO, USA) and water were freely available. Separate groups of animals were used for each experiment, and testing always occurred between 0900 and 1700 h. Care and use of animals complied with the *Guide to the Care and Use of Experimental Animals* (Vol. 1, 2nd Edn., 1993; Vol. 2, 1984) published by the Canadian Council on Animal Care (CCAC).

Drugs

(+)-MK-801 maleate salt [dizocilpine; (5*S*,10*R*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate], 7-OH-DPAT [(±)-7-hydroxy-2-dipropylamino tetralin hydrobromide], and NBQX (2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide disodium) were obtained from Sigma RBI (Oakville, ON, Canada). (+)-Amphetamine sulfate was obtained from SmithKlineBeecham Pharmaceuticals (Mississauga, ON, Canada). MK-801 and 7-OH-DPAT were dissolved in isotonic saline (Fisher Scientific Ltd., Nepean, ON, Canada) while NBQX was dissolved in double distilled water. Artificial cerebrospinal fluid (CSF) was freshly prepared (Lewis and Elliot 1950) and drug solutions were made daily (pH 5.0–7.0). Amphetamine and MK-801 (i.p., 10 or 15 min prior, respectively) and 7-OH-DPAT (s.c., 0 or 15 min prior) were injected in 1.0 mL kg^{-1}. Doses (free base) were determined from prior work in this laboratory (Choi et al. 2000, 2005; unpublished M.Sc. thesis).

Stereotaxic surgery

Animals were anesthetized using Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane; Halocarbon Laboratories, River Edge, NJ, USA) and placed in a Kopf stereotaxic instrument (Kopf Instruments, Tujunga, CA, USA). Animals were implanted bilaterally with 22-gauge stainless steel guide cannulae (Plastics One Inc., Roanoke, VA, USA) in the NAS shell subregion. To minimize damage to blood sinuses and cerebral ventricles, angled stereotaxic coordinates (Greenshaw 1997) were interpolated from the target site (defined by Paxinos and Watson 1986). NAS shell coordinates were: AP +10.1 mm, LM ±0.2 mm, DV +3.7 mm, 16° lateral to the sagittal plane, relative to interaural zero with the incisor bar 2.4 mm below the interaural line. Guide cannula tips were placed 1.0 mm above the target site and fixed to the skull using stainless steel screws (Lomat Watch Co., Montréal, QC, Canada) and dental acrylic (Caulk/Dentsply, Milford, DE, USA). Dummy cannulae (Plastics One Inc.) were left in place between microinjections to prevent occlusion. Testing began at least one week after surgery.

Microinjection procedure

Dummy cannulae were removed and 28-gauge injection cannulae (Plastics One Inc.) were slowly lowered into each guide cannula. The tip of each injection cannula extended 1.0 mm beyond the guide cannula tip. Bilateral microinjections (0.5 µL/side at 0.2 µL/min) were delivered over 2.5 min using 0.03 mL m^{-1} Accu-rated pump tubes (Fisher Scientific Ltd.) and 10-µL glass microsyringes (Hewlett-Packard, Mississauga, ON, Canada) attached to a Bee Hive Controller (Bioanalytical Systems Inc., West Lafayette, IN, USA). Injection cannulae remained in place for one min after infusion to allow for drug absorption and diffusion.

Microinjection was immediately followed by behavioural procedures (see below).

Apparatus—Place conditioning

Plexiglas place conditioning boxes (I. Halvorsen System Design, Phoenix, AZ, USA) were divided into two compartments (each 30 cm *L* × 30 cm *W* × 25 cm *H*) with distinct floor textures (1 cm^2 wire mesh or 14 parallel horizontal bars spaced 1.25 cm apart) that were separated by an opaque plastic tunnel (7.5 cm *L* × 8 cm *W* × 7.5 cm *H*). The test environment was illuminated with light extending into the visible red frequency in accordance with prior studies from this laboratory.

Procedure—Place conditioning

Each experiment had three phases: pre-conditioning (baseline), conditioning, and post-conditioning (retention). Each animal was always exposed to the same apparatus that was cleaned thoroughly between animals using diluted ammonia-based window cleaner (1:6 in water; No Name Glass Cleaner with Ammonia, Loblaw Companies Ltd., Toronto, ON, Canada). **Pre-conditioning**: Each animal accessed the apparatus for 15 min/day for 3 days; a trained observer recorded time spent in each compartment and the tunnel. **Conditioning**: Animals received drug or saline (or CSF) injections on alternating days and were restricted to the respective drug- or vehicle-paired compartment for 30 min (tunnel was inaccessible). Groups were counterbalanced so that equal numbers of animals received drug in each compartment. **Post-conditioning**: Each animal accessed the apparatus in a drug-free state (except for experiment 6—see "Experiment 6: Place-conditioning state dependency of intra-NAS NBQX and 7-OH-DPAT") for 15 min/day for 3 days; a trained observer recorded time spent in each compartment and the tunnel.

Apparatus—Locomotor activity

Spontaneous locomotor activity was measured using computer-monitored Plexiglas photobeam boxes (43 cm *L* × 43 cm *W* × 30 cm *H*; I. Halvorsen System Design). Horizontal and consecutive beam breaks were recorded using a 12×12 array of horizontal infrared beams, while vertical activity was measured using 12 horizontal beams 12 cm above the floor. The test environment was illuminated with light extending into the visible red frequency in accordance with prior studies from this laboratory.

Experimental designs

In experiments 1–6, animals were randomly assigned to an unbiased place conditioning design (see Table 1). In experiment 7, animals (*n*=64) were randomly assigned to eight groups in an unbiased design: MK-801 (0, 0.03, 0.06, and 0.13 mg kg^{-1}), 7-OH-DPAT (5.0 mg kg^{-1}), or combination of MK-801 + 7-OH-DPAT. The procedure was identical to experiment 4, except that animals were placed into a locomotor activity apparatus following drug or home cages following saline.

Table 1 Experimental designs for experiments 1–6

Experiment	Group size	Treatments
1	*N*=49; *n*=7	0.03, 0.06, 0.18, 0.56, 1.67, 5.0 mg kg^{-1} 7-OH-DPAT (no delay), or 5.0 mg kg^{-1} (+)-amphetamine
2	*N*=36; *n*=6	0.03, 0.06, 0.18, 0.56, 1.67, or 5.0 mg kg^{-1} 7-OH-DPAT (15 min prior)
3	*N*=24; *n*=6	NBQX (0.5 μg), 7-OH-DPAT (5.0 mg kg^{-1} 15 min prior), combination of NBQX and 7-OH-DPAT, or 5.0 mg kg^{-1} (+)-amphetamine[a]
4	*N*=56; *n*=8	MK-801 (0.03, 0.06, 0.13 mg kg^{-1}), 7-OH-DPAT (5.0 mg kg^{-1}), or combination of MK-801 + 7-OH-DPAT
5	*N*=26; *n*=6–7	NBQX (0.5 μg), 7-OH-DPAT (5.0 μg), combination of NBQX and 7-OH-DPAT, or (+)-amphetamine (5.0 μg)[a]
6	*N*=26; *n*=6–7	Same as experiment 5, except that animals received conditioning treatment immediately prior to retention testing[a]

[a]Animals received an intra-NAS microinjection immediately prior to conditioning

Statistical analyses

Place conditioning (and extinction) was determined by comparing time spent in the drug-paired compartment on pre-conditioning day 3 (baseline) with each day of post-conditioning (retention) using paired-samples *t*-tests. An increase or decrease in time spent in the drug-paired compartment reflects CPP or conditioned place aversion (CPA), respectively.

Locomotor activity data were analyzed using a two-way (MK-801 × 7-OH-DPAT) analysis of variance (ANOVA) on pre-conditioning (baseline) day 3 and post-conditioning (retention) day 1. Data from conditioning days were analyzed using a three-way ANOVA (MK-801 × 7-OH-DPAT × Days). In the absence of MK-801 × 7-OH-DPAT interaction, the main effect of MK-801 was explored using a one-way ANOVA followed by Dunnett's *t*-tests. Analyses were completed using SPSS 11.5 (SPSS Inc., Chicago, IL, USA).

Histology

Implanted animals were deeply anesthetized with sodium pentobarbital (Bimeda-MTC Animal Health Inc., Cambridge, ON, Canada; Somnotol or Euthanyl) and perfused intracardially with ice-cold isotonic saline followed by 10% w/v buffered formalin phosphate (Fisher Scientific Ltd.). Brains were removed and stored in 10% w/v buffered formalin phosphate for 4–6 h, then stored in 30% sucrose/10 mmol PBS buffer for 24 h before freezing. Cannula placements were verified by inspection of 40-μm coronal brain sections; only animals with correct cannula placements were included in data analyses.

Results

Experiments 1 and 2: Place conditioning with 7-OH-DPAT (no delay/15-min delay)

7-OH-DPAT (no delay) did not induce place conditioning (Fig. 1A), while (+)-amphetamine (5.0 mg kg^{-1}) induced a CPP [$t(6)=2.641$, $P<0.05$] that extinguished the next day. In experiment 2, 7-OH-DPAT (5.0 mg kg^{-1}, 15 min prior) induced a CPP [$t(5)=3.154$, $P<0.05$] that extinguished the next day (Fig. 1B).

Experiment 3: Place conditioning with systemic 7-OH-DPAT and intra-NAS shell NBQX

7-OH-DPAT (5.0 mg kg^{-1}, 15 min prior) induced a CPP [$t(4)=5.471$, $P<0.05$] that extinguished the next day (Fig. 2A). Intra-NAS shell NBQX (0.5 μg), or 7-OH-DPAT with intra-NAS NBQX, was ineffective. (+)-Amphetamine (5.0 mg kg^{-1}) induced a CPP [$t(5)=6.733$, $P<0.05$] that extinguished the next day. A representative photomicrograph of intra-NAS shell infusion sites is shown in Fig. 3A, and cannula placements are illustrated in Fig. 3B.

Experiment 4: Place conditioning with systemic 7-OH-DPAT and MK-801

MK-801 did not induce place conditioning, while 7-OH-DPAT induced a CPP [$t(6)=3.719$, $P<0.05$] (Fig. 2B). 7-OH-DPAT with 0.03 mg kg^{-1} MK-801 also induced CPP

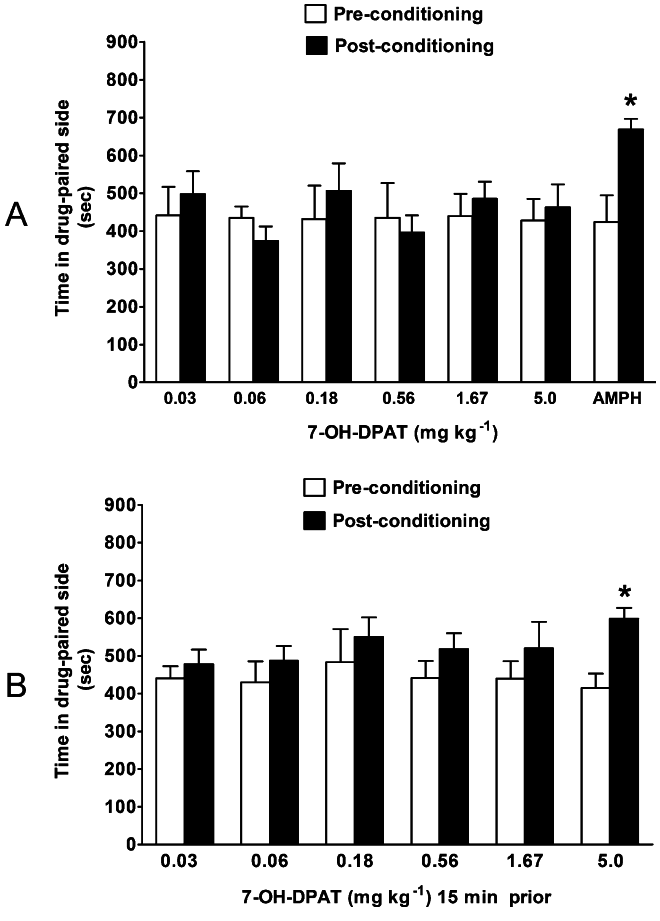

Fig. 1 Place conditioning by 7-OH-DPAT administered immediately prior to conditioning (**A**) or 15 min prior to conditioning (**B**). Data are shown as mean±SEM. Post-conditioning data are from the first day of retention testing. *Significant at $P<0.05$

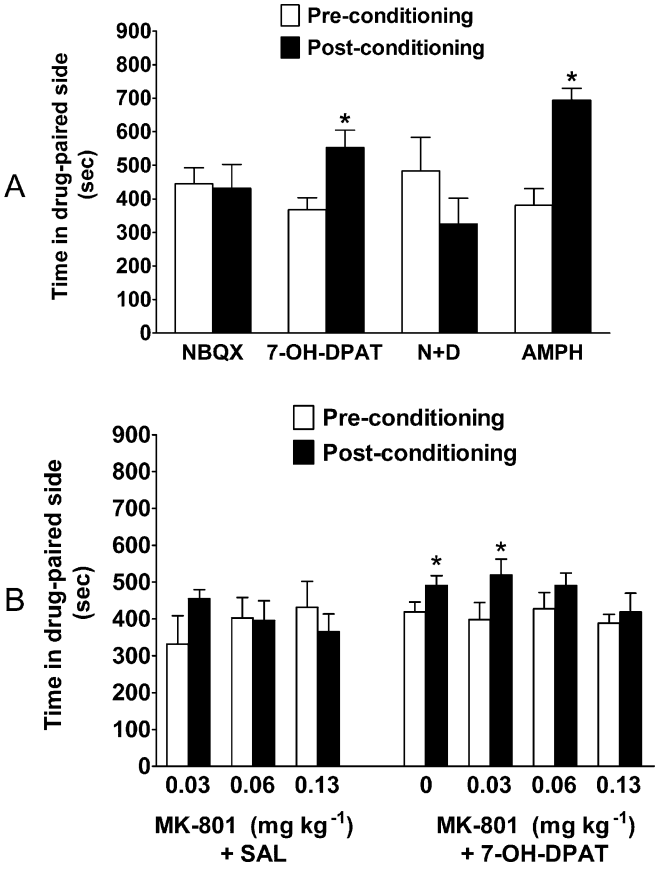

Fig. 2 Conditioned place preference induced by systemic 7-OH-DPAT (5.0 mg kg^{-1}, 15 min prior) is blocked by intra-NAS shell NBQX (**A**) or systemic MK-801 (**B**). Data are shown as mean± SEM. Post-conditioning data are from the first day of retention testing. *Significant at $P<0.05$

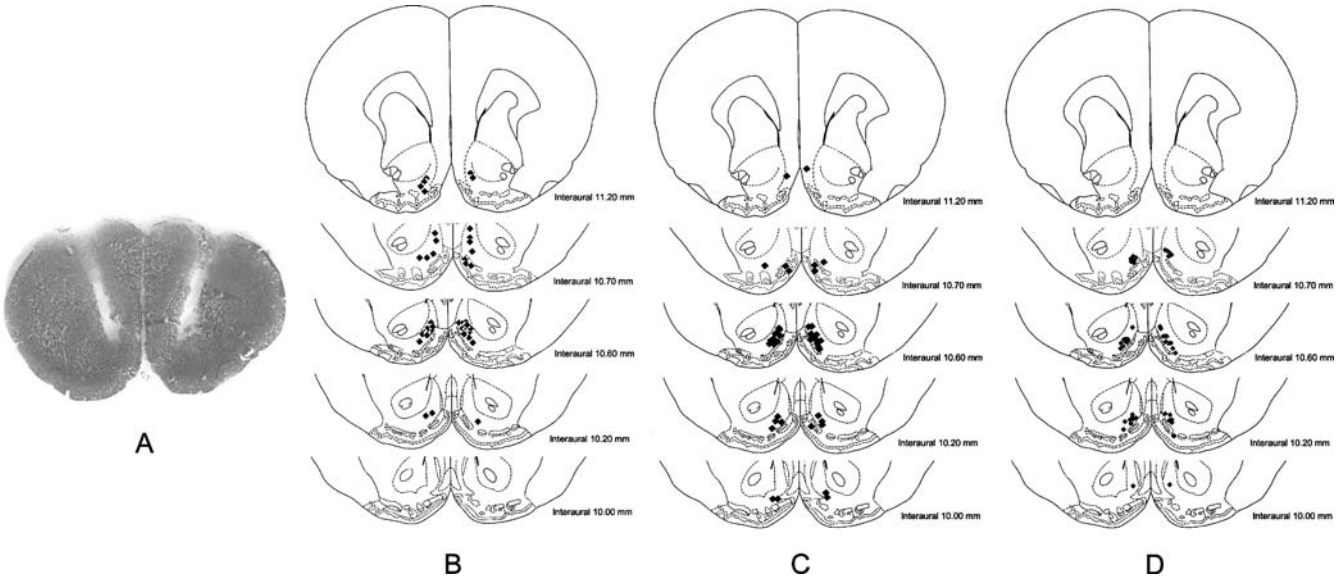

Fig. 3 Representative photomicrograph (**A**) and histological verification of microinjection sites in the NAS shell for experiment 3 (**B**), experiment 5 (**C**), and experiment 6 (**D**). *Numbers* represent distances in the coronal plane from interaural zero according to the modified atlas of Paxinos and Watson (1986)

[$t(7)=3.453$, $P<0.05$]. These preferences extinguished the next day. 7-OH-DPAT with higher doses of MK-801 did not induce place conditioning.

Experiment 5: Place conditioning with intra-NAS shell NBQX and 7-OH-DPAT

Intra-NAS shell 7-OH-DPAT (5.0 μg) and NBQX (0.5 μg), alone or combined, was ineffective (Fig. 4A). Intra-NAS shell (+)-amphetamine induced a CPP [$t(5)=3.231$, $P<0.05$] that extinguished the next day. Cannula placements are illustrated in Fig. 3C.

Experiment 6: Place conditioning state dependency of intra-NAS NBQX and 7-OH-DPAT

Intra-NAS shell 7-OH-DPAT (5.0 μg) and NBQX (0.5 μg), alone or combined, did not induce place conditioning when animals were tested in the drugged state (Fig. 4B). Intra-NAS shell (+)-amphetamine induced a CPP [$t(5)=2.849$, $P<0.05$] that extinguished the next day. Cannula placements are illustrated in Fig. 3D.

Experiment 7: Conditioned locomotor effects of systemic 7-OH-DPAT and MK-801

All groups showed equivalent activity counts on pre-conditioning day 3. Analysis of conditioning days revealed neither MK-801 × 7-OH-DPAT interaction nor MK-801 × 7-OH-DPAT × Days interaction, but significant main effects of MK-801 and 7-OH-DPAT [$F(3,56)=12.771$, $P< 0.05$ and $F(1,56)=61.532$, $P<0.05$, respectively]. In the absence of an MK-801 × Days interaction (i.e., lack of sensitization to MK-801), post hoc tests revealed that MK-801 (0.13 mg kg^{-1}) significantly increased activity during conditioning. All groups showed equivalent activity counts on post-conditioning day 1 (retention).

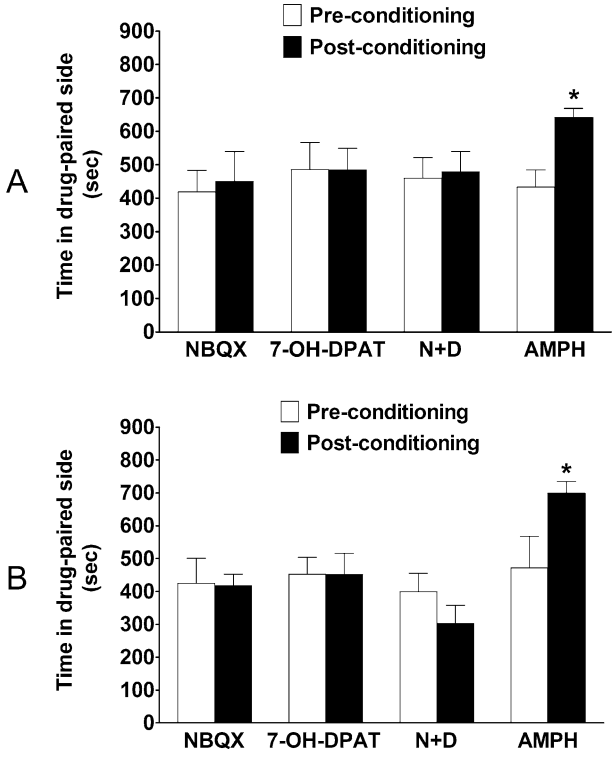

Fig. 4 Intra-NAS shell 7-OH-DPAT (5.0 μg), NBQX (0.5 μg), or NBQX + 7-OH-DPAT does not induce place conditioning when tested for retention in a drug-free state (**A**) or in a drugged state (**B**). Under these conditions, AMPH induced a CPP. Data are shown as mean±SEM. Post-conditioning data are from the first day of retention testing. *Significant at $P<0.05$

Discussion

The highest dose of 7-OH-DPAT (5.0 mg kg^{-1}) induced a CPP when administered 15 min prior to conditioning, in accord with prior studies that used a delay (5.0 mg kg^{-1} 30 min prior, Mallet and Beninger 1994; 4.0 mg kg^{-1} 30 min prior, Chaperon and Thiebot 1996; 0.25 mg kg^{-1} 10 min prior, Kling-Petersen et al. 1995). Based on receptor subtype affinity (Levesque et al. 1992), higher doses of 7-OH-DPAT may induce CPP by stimulation of D_2 DA receptors. Similarly, other D_2 DA receptor agonists (bromocriptine and quinpirole) also induce CPP (Hoffman et al. 1988). A relatively high dose of (+)-7-OH-DPAT (1,000 nmol kg^{-1} or 0.328 mg kg^{-1}) also induces sniffing behaviour, a marker of post-synaptic D_2 DA receptor effects, while lower doses do not (Damsma et al. 1993).

Some reports suggest that 7-OH-DPAT may be ineffective in place conditioning (0.003–5.0 mg kg^{-1}, Khroyan et al. 1995; 0.1, 0.25, and 0.5 mg kg^{-1}, Rodriguez De Fonseca et al. 1995). In addition to the present ineffectiveness of low doses, 7-OH-DPAT (0.005 or 0.01 mg kg^{-1}, s.c.) also fails to induce place conditioning (unpublished results). This result contrasts reports of CPA induced by low-dose 7-OH-DPAT (0.004 or 0.008 mg kg^{-1}, Chaperon and Thiebot 1996; 1 or 3 mg kg^{-1} in mice, Kamei and Ohsawa 1996; 0.05 or 0.1 mg kg^{-1}, Gyertyán and Gál 2003). The selective D_3 DA agonist PD-128907 and D_3 DA partial agonist BP-897 may also induce CPA (Gyertyán and Gál 2003). Potentially aversive properties of low doses of 7-OH-DPAT may be attributable to stimulation of D_3 DA receptors; there is evidence of inhibitory post-synaptic D_3 DA receptors (Svensson et al. 1994) and D_3 DA autoreceptors that decrease synaptic DA (Pugsley et al. 1995). Inconsistencies in 7-OH-DPAT effects are likely due to procedural or apparatus differences among laboratories.

The lack of effect of 7-OH-DPAT with zero delay may be related to the time course of drug effects reflected by changes in locomotor activity. Khroyan et al. (1995) reported decreased locomotor activity 10 min after 7-OH-DPAT (5.0 mg kg^{-1}), but hyperactivity from 20 to 40 min. The initial effect was attributed to stimulation of D_3 DA receptors and latter effects to D_2 DA receptor activation. In the present study, the 15-min delay may have allowed conditioning sessions to occur during a period of D_2 DA receptor stimulation. Without the delay, conditioned reinforcing effects of D_2 DA receptor stimulation may be masked by (potentially aversive) stimulation of inhibitory D_3 DA receptors.

CPP induced by intra-NAS shell amphetamine provides an internal validation of the injection site and of our procedures. That intra-NAS shell 7-OH-DPAT was ineffective is surprising and suggests that 7-OH-DPAT in the NAS shell may be necessary but not sufficient for place conditioning, as intra-NAS shell NBQX blocks CPP induced by systemic 7-OH-DPAT. Lower doses of intra-NAS 7-OH-DPAT would likely be ineffective as the dose used is maximally effective in studies of locomotor activity (Choi et al. 2000). Stimulation of D_3 DA receptors in the NAS shell may offset the positively reinforcing actions of D_2 DA receptor stimulation, producing no detectable place conditioning. The contrast between effects of intra-NAS amphetamine and 7-OH-DPAT indicates that the balance of DA receptor subtype stimulation in the NAS may be important for determining the net reinforcing actions of certain drugs; this may be clarified by further investigation into the systemic vs. intra-NAS actions of a variety of agonists for DA receptor subtypes.

Although intra-NAS 7-OH-DPAT may induce sedation (Meyer 1996; Ouagazzal and Creese 2000; Choi et al. 2000), this may not account for the lack of place conditioning. The learned association between drug and environment may be formed without ambulation while in the drugged state; Animals unable to explore the environment during conditioning still demonstrate conditioned locomotor responses to amphetamine (Swerdlow et al. 1989). Similarly, at doses that induce sedation, a GABA receptor ligand (zolpidem) fails to disrupt CPP induced by cocaine or amphetamine (Meririnne et al. 1999).

The lack of effect of MK-801 in place conditioning agrees with prior reports (Steinpreis et al. 1995; Sufka 1994; Hoffman 1994; Tzschentke and Schmidt 1998, 1995, Sukhotina et al. 1998; Kim et al. 1996; Kim and Jang 1997). However, some studies show that MK-801 may induce a CPP (Layer et al. 1993a; Papp and Moryl 1994; Sufka 1994; Suzuki et al. 1999; Panos et al. 1999; Steinpreis et al. 1995; Hoffman 1994; Biala and Kotlinska 1999; Papp et al. 1996; Sukhotina et al. 1998). Procedural and apparatus differences may account for these inconsistencies. The lack of effect of intra-NAS NBQX in place conditioning is consistent with studies using other AMPA/kainate receptor ligands (Cervo and Samanin 1995; Gong et al. 1997; Layer et al. 1993b) and with reports of the ineffectiveness of AMPA/kainate receptor antagonists in behavioral tests (Bubser et al. 1995; Danysz et al. 1994; Li et al. 1997). Although NBQX (30 mg kg^{-1}, i.p., 20 min prior) alone may induce CPA in mice (Mead and Stephens 1999), this effect may be non-specific; as Mead et al. (1999) indicate, "such high doses of NBQX exert additional, non-specific effects on behavior (Turski et al. 1992; Jackson et al. 1998)." Brain stimulation reward studies corroborate the view that AMPA/kainate receptor-related compounds may lack rewarding or aversive properties; CNQX or NBQX (0.5 μg) microinjected into the NAS shell or core have no significant effect on frequency thresholds of rats responding for VTA electrical stimulation (Choi et al. 2005).

MK-801 inhibited acquisition of CPP induced by the selective $D_{2/3}$ DA receptor agonist 7-OH-DPAT. Since MK-801 alone was ineffective, attenuation of the CPP induced by systemic 7-OH-DPAT cannot be due to additive effects. Similar doses of MK-801 may also inhibit or attenuate acquisition of CPP induced by cocaine or methamphetamine (Kim et al. 1996; Cervo and Samanin 1995; Kim and Jang 1997). Similar effects have been found with another non-competitive NMDA antagonist memantine (Kotlinska and Biala 2000). In contrast, MK-801 may not affect acquisition of CPP induced by amphetamine (Hoffman 1994); however, in that study, MK-801 alone induced CPP.

In the present study, it is unlikely that MK-801 directly reduced the reinforcing effect of 7-OH-DPAT, as MK-801 may facilitate brain stimulation reward (Corbett 1989; Herberg and Rose 1989; Carlezon and Wise 1993, 1996; Olds 1996; Cabeza de Vaca and Carr 1998; Sukhotina et al. 1999; De Vry et al. 2001; Sundstrom et al. 2002; Kenny et al. 2003; unpublished results from this laboratory). Rather, we suggest that MK-801 may non-specifically impair the process of associative learning necessary for the development of place conditioning; NMDA receptor antagonists may impair both associative and non-associative forms of learning (e.g., Riters and Bingham 1994; Thompson and Disterhoft 1997).

NMDA antagonists like MK-801 induce state-dependent operant learning for food reward (Jackson et al. 1992). However, it is not likely that the blockade of 7-OH-DPAT CPP by MK-801 may be due to state dependency; MK-801 may block morphine-induced CPP, but does not make the retention of this CPP state-dependent (Tzschentke and Schmidt 1997).

MK-801 effects should also be interpreted with caution, as this compound demonstrates some affinity for σ receptors (Rothman et al. 1992) and attenuates, but does not block, behavioural impairments induced by NMDA receptor agonists (e.g., Zajaczkowski et al. 1997).

Acquisition of systemic 7-OH-DPAT-induced CPP is also blocked by NBQX; 7-OH-DPAT CPP was not simply opposed by CPA induced by NBQX, as NBQX alone was ineffective. This finding is consistent with effects of less selective DA agonists: intra-NAS DNQX suppressed or blocked CPP induced by amphetamine (Layer et al. 1993b) or cocaine (Kaddis et al. 1995). Stimulation of AMPA/kainate receptors in the NAS may be necessary for the acquisition of conditioned reinforcement induced by indirect DA agonists and selective $D_{2/3}$ DA receptor agonists.

Psychostimulants (e.g., amphetamine) induce hyperactivity that can be reinstated by the drug-paired environment (Beninger and Hahn 1983). In the present study, 7-OH-DPAT (5.0 mg kg^{-1}) or MK-801 induced hyperactivity, in accord with prior reports (e.g., Daly and Waddington 1993; Ford et al. 1989). That repeated administration of either drug does not induce conditioned locomotor hyperactivity is surprising. This lack of effect is not likely due to latent inhibition, as habituation to the test environment may not affect conditioned locomotor responses to cocaine (Martin-Iverson and Reimer 1996).

Repeated administration of some compounds, such as psychostimulants, may induce progressively greater behavioral effects, a process referred to as behavioral sensitization. In the present study, MK-801 did not induce sensitization; this is generally consistent with prior studies (Oles et al. 1990; Wolf and Khansa 1991; Wolf et al. 1993; Dall'Olio et al. 1992; Tzschentke and Schmidt 1997).

Conclusions

AMPA/kainate receptor blockade in the NAS shell or systemic NMDA receptor blockade by MK-801 inhibited the acquisition of CPP induced by the $D_{2/3}$ DA receptor agonist 7-OH-DPAT. The neural substrate for this interaction is likely based upon the triadic synaptic arrangement of DA and Glu receptors in the NAS (Sesack and Pickel 1992; Goldman-Rakic 1992). As both AMPA/kainate receptors and D_2-like DA receptors have been implicated in the neural circuitry underlying some psychiatric disorders of motivation, behavioural interactions of $D_{2/3}$ DA receptors and the excitatory amino acid system within the midbrain may be a potential target for therapeutic drug development.

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