Psychopharmacology (2010) 212:441–448
DOI 10.1007/s00213-010-2004-7

ORIGINAL INVESTIGATION

Intrahippocampal LSD accelerates learning and desensitizes the 5-HT_{2A} receptor in the rabbit, Romano et al.

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Received: 28 October 2009 / Accepted: 19 July 2010 / Published online: 9 September 2010

Abstract
Rationale Parenteral injections of *d*-lysergic acid diethylamide (LSD), a serotonin 5-HT_{2A} receptor agonist, enhance eyeblink conditioning. Another hallucinogen, (±)-1(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI), was shown to elicit a 5-HT_{2A}-mediated behavior (head bobs) after injection into the hippocampus, a structure known to mediate trace eyeblink conditioning.
Objective This study aims to determine if parenteral injections of the hallucinogens LSD, *d*,*l*-2,5-dimethoxy-4-methylamphetamine, and 5-methoxy-dimethyltryptamine elicit the 5-HT_{2A}-mediated behavior of head bobs and whether intrahippocampal injections of LSD would produce head bobs and enhance trace eyeblink conditioning.
Materials and methods LSD was infused into the dorsal hippocampus just prior to each of eight conditioning sessions. One day after the last infusion of LSD, DOI was infused into the hippocampus to determine whether there had been a desensitization of the 5-HT_{2A} receptor as measured by a decrease in DOI-elicited head bobs.
Results Acute parenteral or intrahippocampal LSD elicited a 5-HT_{2A} but not a 5-HT_{2C}-mediated behavior, and chronic administration enhanced conditioned responding relative to vehicle controls. Rabbits that had been chronically infused with 3 or 10 nmol per side of LSD during Pavlovian conditioning and then infused with DOI demonstrated a smaller increase in head bobs relative to controls.
Conclusions LSD produced its enhancement of Pavlovian conditioning through an effect on 5-HT_{2A} receptors located in the dorsal hippocampus. The slight, short-lived enhancement of learning produced by LSD appears to be due to the development of desensitization of the 5-HT_{2A} receptor within the hippocampus as a result of repeated administration of its agonist (LSD).

Keywords Hallucinogens · LSD · DOI · DOM · 5-MeO-DHT · Learning · Eyeblink conditioning · Rabbit · Serotonin 5-HT_{2A} receptor · Serotonin 5-HT_{2C} receptor · Hippocampus

Introduction

The serotonin 2A (5-HT_{2A}) receptor has been implicated in the production of hallucinations in humans (Nichols 2004) and cognitive effects such as enhanced acquisition during autoshaping in rodents (Meneses 2002), enhanced acquisition of trace eyeblink conditioning in the rabbit (Harvey 1996; Harvey 2003; Aloyo et al. 2009), and enhanced working memory in the non-human primate (Jakab and Goldman-Rakic 1998; Williams et al. 2002). The dose required to produce reliable hallucinations in humans was reported to be 1 μg/kg for *d*-lysergic acid diethylamide (LSD; Abramson et al. 1955; Rothlin 1957; Brawley and Duffield 1972), 50 μg/kg for *d*,*l*-2,5-dimethoxy-4-methylamphetamine (DOM; Snyder et al. 1967), 250 μg/kg for *d*, *l*-methylenedioxymethamphetamine (MDMA; Cole and Sumnall 2003), and 1,000 μg/kg *d*,*l*-methylenedioxyamphetamine (MDA; Marquardt et al. 1978; Shulgin 1978;

This work was supported by NIMH grant MH16841-40 (JAH).

All experiments comply with current laws governing animal experimentation in the USA.

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Braun et al. 1980). These values compare favorably with the dose of hallucinogen required to produce a half maximal enhancement of learning during eyeblink conditioning in the rabbit: 0.8 μg/kg for LSD (Gimpl et al. 1978), 52 μg/kg for DOM (Harvey et al. 1982), 500 μg/kg for MDMA (Romano and Harvey 1994), and 800 μg/kg for MDA (Romano et al. 1991). This correspondence in the efficacy of hallucinogens between humans and rabbits is consistent with the reported correspondence between the pharmacological profiles of the 5-HT_{2A} receptor for these two species (Aloyo and Harvey 2000). Thus, the high degree of correlation obtained between the binding affinities of various ligands for the rabbit and human 5-HT_{2A} receptor (r=0.91) indicates that the rabbit provides an excellent animal model for predicting the effects of 5-HT_{2A} ligands in humans.

The agonist action of hallucinogens at the 5-$HT_{2A/2C}$ receptor can be distinguished and quantified in humans, rats, and rabbits by the elicitation of species-specific motor responses (Pranzatelli 1990; Schreiber et al. 1995; Dave et al. 2002; Nichols 2004). In the rabbit, head bobs (a stereotyped rapid down–up movement of the head) are mediated by the 5-HT_{2A} receptor, while body shakes, similar to wet dog shakes in rodents, are mediated by the 5-HT_{2C} receptor (Dave et al. 2002). For example, parenteral injection of the hallucinogen (±)-1(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI) elicits both head bobs and body shakes. The DOI-elicited head bobs were blocked by the selective 5-HT_{2A} receptor antagonist ketanserin with no effect on body shakes, while body shakes were blocked by the selective 5-HT_{2C} receptor antagonist SB 206553 with no effect on head bobs (Dave et al. 2002). Recently, it was reported that infusion of DOI into the lateral ventricle elicited head bobs but not body shakes. The elicitation of head bobs by DOI was blocked by ketanserin, a selective 5-HT_{2A} receptor antagonist, indicating that DOI was acting at central serotonin 5-HT_{2A} receptors (Dave et al. 2004a). The central effects of DOI were further localized to its agonist action at 5-HT_{2A} receptors located in the dorsal hippocampus (Dave et al. 2004b). Thus, bilateral intrahippocampal infusions of DOI also elicited head bobs but not body shakes, and the head bobs were also blocked by ketanserin.

The hippocampus is known to be critically involved in the acquisition of trace eyeblink conditioning in rabbits (Solomon et al. 1986; Moyer et al. 1990) and humans (Clarke and Squire 1998; LaBar and Disterhoft 1998) and to contain both 5-HT_{2A} and 5-HT_{2C} receptors (Dave et al. 2004b). As noted above, parenteral injection of hallucinogens in the rabbit accelerates acquisition of the rabbit's eyeblink response with LSD being the most efficacious in this regard (Harvey 2003; Aloyo et al. 2009). We therefore examined whether intrahippocampal injections of LSD would enhance the acquisition of the rabbits eyeblink response.

We first determined whether acute parenteral injections of LSD would activate central 5-HT_{2A} and 5-HT_{2C} receptors as measured by head bobs and body shakes, respectively, and compared the effects of LSD with two other hallucinogens, DOM and 5-methoxy-dimethyltryptamine (5-MeO-DMT). We then examined the effects of acute central injections of LSD into the lateral ventricle and dorsal hippocampus on the elicitation of head bobs and body shakes. Finally, we determined whether bilateral infusions of LSD into the dorsal hippocampus just prior to each of eight conditioning sessions would accelerate acquisition of trace eyeblink conditioning. The chronic (8 day) systemic injection of LSD has been shown to produce a desensitization of the 5-HT_{2A} receptor as measured by a decrease in receptor density (Aloyo et al. 2001). Therefore, we also examined whether the chronic intrahippocampal infusion of LSD during eyeblink conditioning would produce desensitization of the 5-HT_{2A} receptor as measured by the elicitation of head bobs. This was accomplished by the infusion of DOI into the dorsal hippocampus 1 day after the last infusion of LSD.

Material and methods

Animals and surgery

New Zealand White rabbits (Covance, Devon, PA) were individually housed with free access to rabbit chow and tap water in an AALAC-approved facility at Drexel University College of Medicine. Rabbits were approximately 90 days old on arrival and weighed 1.8–2.2 kg. The colony room was maintained at 22±1°C under a light–dark cycle of 12 h. One week after arrival, some animals were anesthetized, and guide cannula/obturator assemblies (Welsh and Harvey 1991) were implanted either unilaterally into the lateral ventricle or bilaterally into the dorsal hippocampus using previously described coordinates and procedures (Dave et al. 2004a, b). All animal experiments were approved by the Institutional Animal Care and Use Committee.

Drugs

LSD, DOI, and 5-MeO-DMT were obtained from Sigma-Aldrich, St. Louis, MO, and DOM from NIDA. For parenteral injections, LSD was dissolved in sterile, distilled water and injected intravenously, along with its vehicle control, via the marginal ear vein in a volume of 0.4 ml/kg delivered over a 1-min period (Romano et al. 2005) at doses of 3, 30, and 300 nmol/kg (1.29, 12.9, and 129 μg/kg). DOM (0.3, 1.0, and 3.0 μmol/kg; 0.063, 0.21, 0.63 mg/kg) and 5-MeO-DMT (1.4 μmol/kg; 0.29 mg/kg) were dissolved

in physiological saline and injected subcutaneously along with the vehicle control. For acute or chronic intracerebral injections, LSD was dissolved in artificial cerebrospinal fluid (ACSF) and the pH adjusted to 6.8–7.2 as previously described (Dave et al. 2004a). LSD was infused into the left lateral ventricle at a dose of 3 or 10 nmol (1.29 or 4.3 μg) in a volume of 10 μl delivered over 1 min, and the injector cannula was left in place for an additional 1 min (Dave et al. 2004a). For bilateral intrahippocampal injections, LSD was injected at doses of 1, 3, 10, or 30 nmol per side (0.43, 1.29, 4.3, or 12.9 μg per side) in a volume of 1 μl delivered in 2 min, and the injector cannula was left in place an additional 2 min as previously described (Dave et al. 2004b). Bilateral intrahippocampal injections of DOI (3.0 μmol per side; 1 mg per side) were delivered through the same cannulae and followed the same procedures used for LSD.

Measurement of head bobs and body shakes

On the day of behavioral observations, food and water were removed from the home cage. The incidence of drug-elicited behaviors (head bobs and body shakes) was recorded for 90 min by video cameras for subsequent analysis. Head bobs were defined as rapid vertical "down–up" movements of the head (Dave et al. 2002). Vertical head movements that were followed by sniffing, chewing, and/or gnawing were not counted as head bobs. Body shakes, a 5-HT_{2C} mediated behavior, were defined as a paroxysmal shudder of the head, neck, and trunk combined, similar to wet dog shakes in rodents (Dave et al. 2002).

Eyeblink conditioning

Animals were exposed to Pavlovian conditioning of the eyeblink response as measured by extension of the nictitating membrane (NM). Standard conditioning procedures were employed (Romano et al. 1991). Each animal was placed in a Plexiglas restrainer and fitted with a head mount that supported a potentiometer which was coupled directly to a suture placed in the right NM. Movements of the NM were transduced to DC voltages and digitized every 5 ms with a resolution of 0.03 mm of NM movement per analog-to-digital count. A response was defined as a 0.5 mm or greater extension of the NM, and its onset latency was calculated from the time at which the response first deviated from baseline by at least 0.03 mm. Response topography was also quantified in terms of peak amplitude and latency to peak amplitude. The head mount also supported a 16-gauge metal tube positioned 6±1 mm from the center of the right cornea for delivery of an air puff as the unconditioned stimulus (US). Tailor hooks were used to hold the eyelids open. The animals were trained in illuminated, sound-attenuated chambers with a stimulus and interconnection panel mounted above and in front of the animal. The conditioned stimulus (CS) consisted of a 90-dB (20-μN/m^2 reference), 1-kHz tone of 100-ms duration. The US was a 100-ms corneal air puff exerting a peak pressure of 210 g/cm^2 measured at the end of the delivery tube. Data acquisition and stimulus delivery were controlled by a standard desktop PC and ASYST software interfaced with a Metrabyte DAS-16 data acquisition board and Metrabyte PIO-12 digital I/O board.

One day prior to the first conditioning session, rabbits received one 60-min adaptation session during which no stimuli were presented or drug injected. However, in order to obtain a measure of baseline responding, NM extensions were recorded at observation intervals employed during conditioning. One day after adaptation, all rabbits entered the acquisition phase consisting of eight daily conditioning sessions. Each 60-min session consisted of 60 pairings of the tone CS and corneal air puff US. The CS–US interval, measured from CS onset to US onset was 500 ms, thus yielding a trace interval of 400 ms. A conditioned response (CR) was defined as a 0.5 mm or greater extension of the NM occurring during the 500-ms post-CS-onset interval. Trials were presented at an average intertrial interval of 60 s (range 55–65 s).

Twenty minutes prior to each of the eight conditioning sessions, rabbits received intrahippocampal infusions of LSD or ACSF delivered simultaneously into the left and right dorsal hippocampus at LSD doses of 1, 3, 10, or 30 nmol per side (0.43, 1.3, 4.3, 13 μg per side). Twenty-four hours after the last (eighth) conditioning session, animals were removed from their home cages and received bilateral intrahippocampal infusions of DOI at a dose of 3 μmol per side (1 mg per side) or ACSF using the same injector cannula that had been used for infusion of LSD. This dose of DOI was based on a previously established dose–response curve following intrahippocampal infusions of DOI (Dave et al. 2004b). Animals were then placed back into their home cages, and the number of head bobs and body shakes were measured as described above. At the end of this observation period, the brains of each animal were obtained for verification of cannula site.

Data analysis

The percentages of CRs were analyzed with a repeated-measure analysis of variance (ANOVA) using the SYSTAT statistical package, version 7.0 (SPSS, Inc.; Chicago, IL). Because we expected short-lived effects of LSD on the rate of learning, we focused on the interaction with the repeated measure by testing for group differences on each day of training as a follow up to the overall ANOVA. LSD-elicited head bobs and body shakes were analyzed by *t* tests. Significance was set at $p<0.05$.

Results

Acute parenteral injections of LSD, DOM, and 5-MeO-DMT

The catecholamine hallucinogen DOM produced a significant ($p<0.05$) increase in head bobs and body shakes with a maximum effect for both measures occurring at a dose of 1 μmol/kg (Fig. 1). Another catecholamine hallucinogen, DOI, was previously reported to also increase both head bobs and body shakes with the maximum effect occurring at 1 μmol/kg. However, while both of the two indoleamine hallucinogens, LSD and 5-MeO-DMT, elicited a significant ($p<0.05$) increase in head bobs, they failed to elicit body shakes. The maximum effect of LSD on head bobs occurred at 30 nmol/kg (Fig. 2). The increase in head bobs elicited by the single dose of 5-MeO-DHT (1.4 μmol/kg) was also significant (Fig. 3). Although all of the hallucinogens are known to be relatively selective for 5-HT_2 receptors and to have comparable affinities for the 5-HT_{2A} and 5-HT_{2C} receptor subtype, they had differential actions at these two receptors. As would be expected, the injection of catecholamine hallucinogens (DOI, DOM) activates both 5-HT_{2A} and 5-HT_{2C} receptors, whereas indoleamine hallucinogens (LSD, 5-MeO-DMT) only activate the 5-HT_{2A} receptor. This cannot be explained by differences between LSD and DOI in the densities (Bmax) or affinities (Kd) for the 5-HT_{2A} and 5-HT_{2C} receptors (Aloyo et al. 2001).

Intraventricular or intrahippocampal infusions of LSD elicits head bobs

Intraventricular infusions of LSD produced a significant and dose-dependent increase in head bobs with no change in body shakes (Table 1). Head bobs were significantly increased at both the 3- and 10-nmol doses. The bilateral intrahippocampal infusion of LSD (10 nmol per side) also produced an increase in head bobs but not in body shakes (Table 1). The increase in head bobs produced by LSD after parenteral, intraventricular, or intrahippocampal injections with no change in body shakes suggests that LSD is having an agonist action at the 5-HT_{2A} receptor located in the dorsal hippocampus, but not at the 5-HT_{2C} receptor.

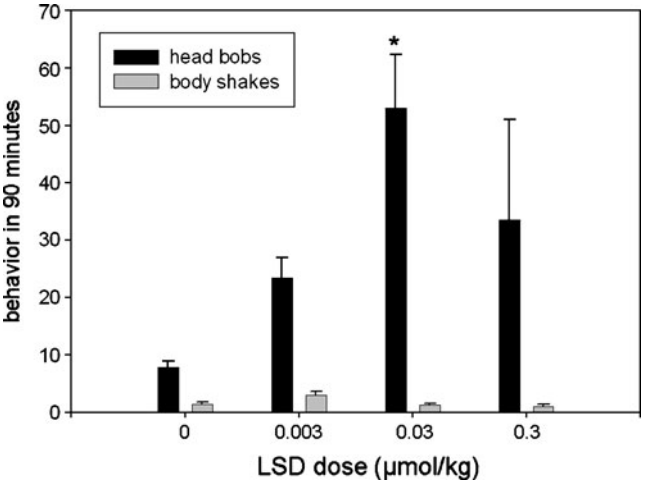

Fig. 2 Effect of parenteral injections of LSD on head bobs and body shakes

Intrahippocampal delivery of LSD enhances CR acquisition

All of the animals receiving intrahippocampal infusions of ACSF or LSD demonstrated significant acquisition of CRs (Fig. 1). There was no significant effect of 1 nmol LSD on CR acquisition relative to ACSF controls (Fig. 4a). LSD doses of 3, 10, and 30 nmol per side (Fig. 4b–d) produced a small but significant acceleration of learning as compared with their ACSF controls. For two of the groups, this effect

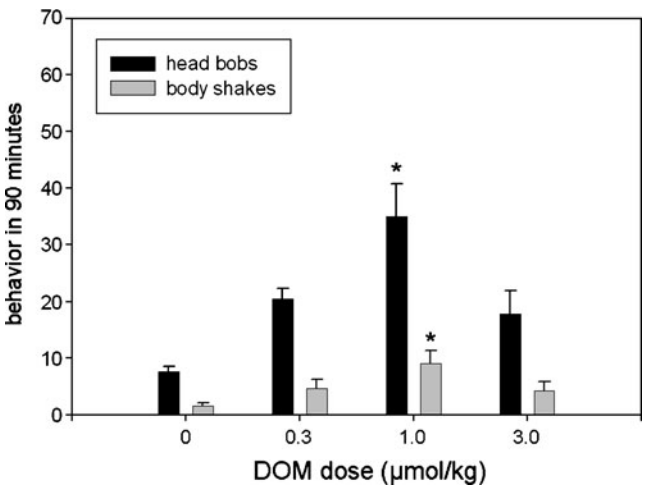

Fig. 1 Effect of parenteral injections of DOM on head bobs and body shakes

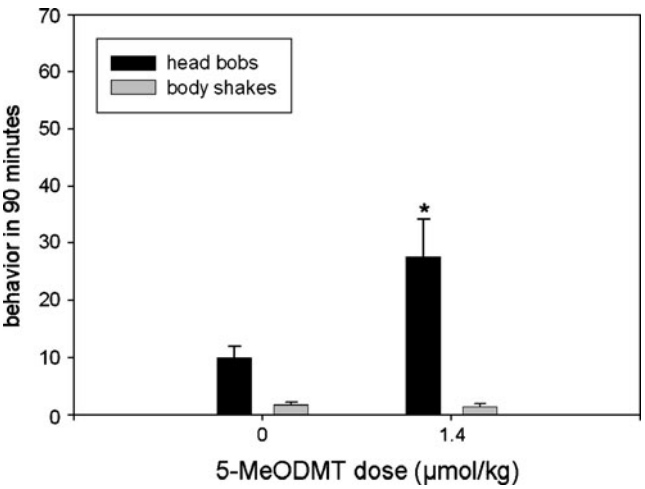

Fig. 3 Effect of parenteral 5-MeO-DMT on head bobs and body shakes

Table 1 Effect of LSD on the elicitation of head bobs and body shakes after unilateral infusion into the lateral ventricle or bilateral infusion into the dorsal hippocampus

	Head bobs (mean ± SE)	Body shakes (mean ± SE)
Acute intraventricular infusion of LSD		
ACSF (n=5)	8.0±2.2	2.0±1.2
LSD 3 nmol (n=3)	28.0±4.6[a]	3.3±0.7
LSD 10 nmol (n-5)	42.0±5.8[a]	3.4±0.9
Acute intrahippocampal infusion of LSD		
ACSF (n=4)	12.2±4.6	5.8±0.9
LSD 10 nmol (n=4)	28.5±2.6[a]	8.2±1.4

[a] Significant mean difference between LSD and ACSF (p<0.05)

occurred in the early portions of conditioning, day 2 for the 3-nmol dose [F(1, 10)=5.42] and day 3 for the 10-nmol dose [F(1, 13) = 5.45]. Thus, at the 3-nmol dose of LSD, animals demonstrated an increase of 24% CRs from days 1 to 2, while ACSF controls showed an increase of only 7% CRs. Similarly, animals receiving the 10-nmol dose of LSD showed an increase of 30% CRs from days 2 to 3 while their ACSF controls showed only an increase of 19% CRs. The 10-nmol dose also produced superior conditioned responding throughout training although the only other

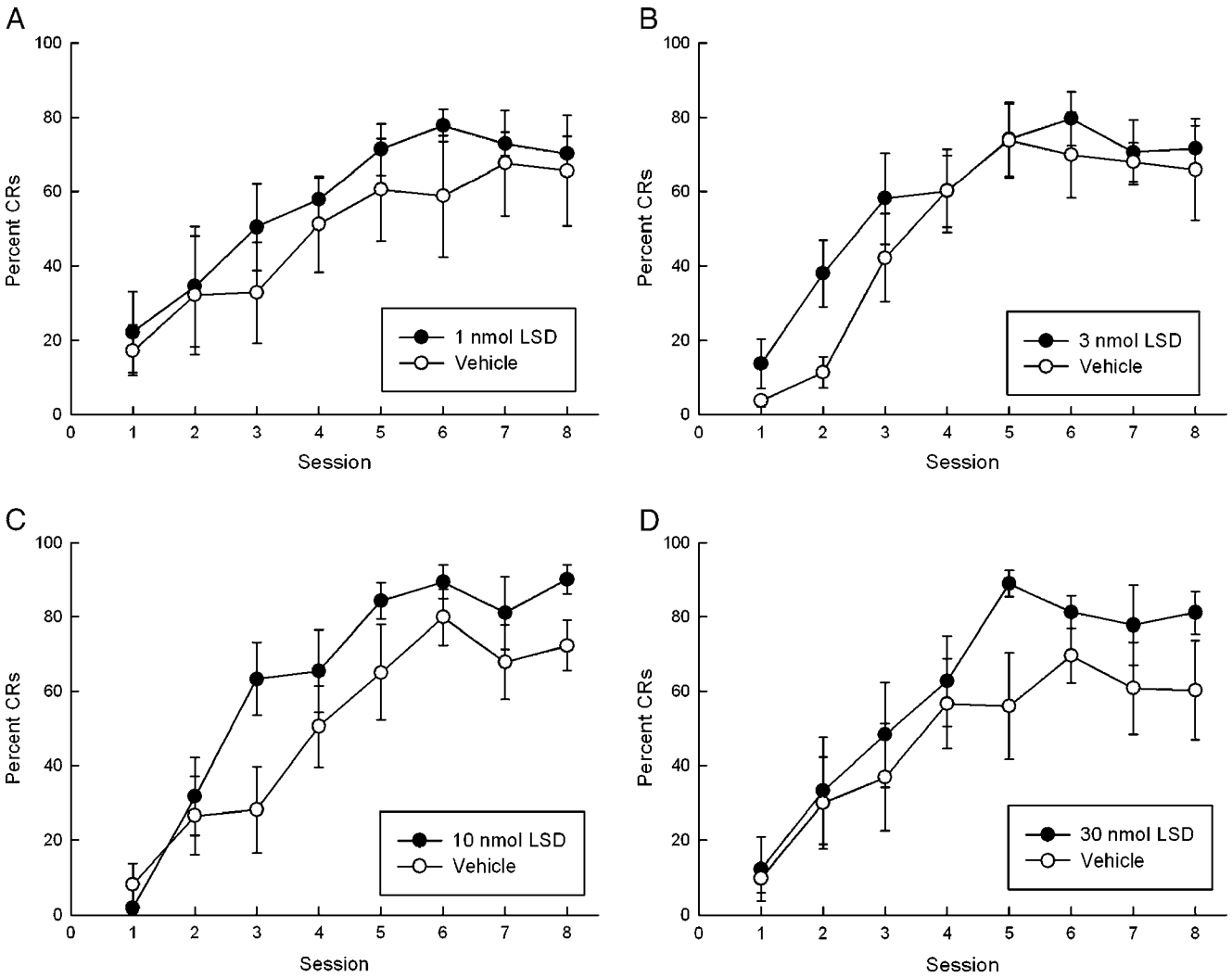

Fig. 4 Effect of intrahippocampal LSD on acquisition of the eyeblink response. The acquisition of trace eyeblink conditioning in rabbits receiving bilateral intrahippocampal infusions of LSD or ACSF just prior to each of eight conditioning days. **a** Infusion of LSD (1 nmol per side, n=4) or their ACSF vehicle controls (n=4), **b** infusion of LSD (3 nmol per side, n=5) or ACSF vehicle controls (n=7), **c** infusion of LSD (10 nmol per side, n=7) or ACSF vehicle controls (n=8), **d** infusion of LSD (30 nmol per side, n=7) or ACSF vehicle controls (n=7)

significant difference occurred on day 8 [$F(1, 13)=5.68$]. The 30-nmol dose of LSD produced an increase in conditioned responding much later in training than either the 3- or 10-nmol doses (Fig. 4d) with a significant increase occurring on day 5 [$F(1, 11)=5.74$]. This was also a short-lived effect, confined to only 1 day.

Chronic intrahippocampal LSD desensitizes the 5-HT_{2A} receptor

Bilateral infusions of DOI (0.3 μmol per side) into the hippocampus were used to determine whether the chronic intrahippocampal infusion of LSD during CR acquisition had altered 5-HT_{2A} or 5-HT_{2C} receptor sensitivity. DOI was infused 24 h after the last dose of LSD, and the occurrence of DOI-elicited head bobs (a 5-HT_{2A}-receptor-mediated behavior) and body shakes (a 5-HT_{2C}-receptor-mediated behavior) was recorded. Infusion of DOI in animals that had received chronic (8 days) exposure to either 1 or 30 nmol of intrahippocampal LSD demonstrated small and non-significant decreases in head bobs of 18.0% and 7.4%, respectively, as compared to their ACSF vehicle controls (Table 2), indicating no change in 5-HT_{2A} receptor sensitivity. Animals receiving the 3-nmol dose of LSD demonstrated a 43.5% decrease in DOI-elicited head bobs, but this was only marginally significant ($p=0.06$). Animals receiving 10 nmol of intrahippocampal LSD did demonstrate a significant decrease (44.4%) in DOI-elicited head bobs as compared with their ACSF controls (Table 2). The mean number of body shakes elicited by DOI in the groups receiving chronic infusion of LSD or ACSF varied from 1.9 to 3.1 with no significant differences between LSD and ACSF groups (data not shown). These results indicate that chronic LSD did produce a selective desensitization of the 5-HT_{2A} receptor.

Cannula placements

Cannula placements were examined by the infusion of methylene blue dye prior to obtaining each animal's brain. Cannulae were judged to be correctly placed if the dye was in the dorsal hippocampus and included the CA1 field. The location of the dye varied in the rostrocaudal position between Plates 3 and 5 of McBride and Klemm (1968). Intrahippocampal cannulae were placed in eight animals per group. Four of the eight rabbits receiving 1 nmol per side infusions of LSD and four of the eight ACSF controls were found to have correct placement into the dorsal hippocampus. Five of the eight animals receiving 3 nmol of LSD and seven of their ACSF vehicle controls were placed correctly. Seven of the eight animals receiving 10 nmol LSD and all eight of the animals receiving ACSF had correct placements. Six of the eight animals receiving 30 nmol LSD and six of the eight animals receiving ACSF were placed correctly. All animals that had a cannula placed into the lateral ventricle demonstrated the presence of the dye in the ventricle. For acute intrahippocampal administration, the four animals receiving10 nmol/side LSD and four animals receiving the ACSF vehicle had correct placements in the dorsal hippocampus.

Discussion

In agreement with their classification as partial agonists at the 5-HT_{2A} and 5-HT_{2C} receptors, the parenteral administration of the two phenethylamine hallucinogens, DOI (Dave et al. 2004a) and DOM, elicited both head bobs, a 5-HT_{2A}-mediated behavior, and body shakes, a 5-HT_{2C}-receptor-mediated behavior. In contrast, the two indoleamine hallucinogens, LSD and 5-MeO-DMT, only elicited head bobs and not body shakes, suggesting the absence of behavioral actions at the 5-HT_{2C} receptor. However, when injected centrally, both DOI and LSD produced head bob but not body shakes. The selective behavioral effects of intracerebral injections of DOI and LSD are puzzling since the hippocampus of the rabbit not only contains both receptors, but also the density of the 5-HT_{2C} receptor is twice that of the 5-HT_{2A} receptor (Dave et al. 2004b).

This study has demonstrated that the hallucinogen LSD can produce a dose-dependent acceleration of trace eyeblink conditioning due to a selective agonist action at serotonin 5-HT_{2A} receptors located in the dorsal hippocampus. In addition, the intrahippocampal infusion of LSD across 8 days of conditioning also produced a dose-dependent desensitization of the 5-HT_{2A} receptor. This desensitization most likely accounts for the small effect of the 3 and 10 nmol LSD on CR acquisition that occurred only during the very early portions of conditioning, days 2 and 3, respectively, presumably before maximal desensiti-

Table 2 Effect of intrahippocampal DOI (3.0 μmol/side) on the elicitation of head bobs

DOI was injected 24 h after the last (eighth) injection of LSD

[a] Significant mean difference between LSD and ACSF ($p<0.05$)

LSD dosage group	Chronic ACSF	Chronic LSD	Percent change (%)
1 nmol group	12.2±6.8 ($n=4$)	10.0±2.8 ($n=4$)	−18
3 nmol group	17.0±3.2 ($n=5$)	9.6±2.0 ($n=7$)[a]	−43
10 nmol group	26.1±3.6 ($n=5$)	14.5±2.3 ($n=7$)[a]	−44
30 nmol group	14.8±2.7 ($n=6$)	13.7±3.7 ($n=6$)	−7

zation had occurred. However, the 30-nmol dose of LSD had an anomalous effect in that it produced a delayed acceleration of learning (day 5) but with no evidence of desensitization. It is possible that at these high doses of intrahippocampal LSD (30 nmol/side) some other receptors were being engaged. These and previous findings also confirm that hallucinogens such as LSD and DOI can elicit 5-HT_{2A}-receptor-mediated behaviors but not 5-HT_{2C}-mediated behaviors when infused into either the lateral ventricle or dorsal hippocampus even though they are considered to be non-selective 5-$HT_{2A/2C}$ receptor agonists. Previous studies employing intravenous or subcutaneous injections of hallucinogens such as LSD, DOM, MDA, and MDMA also accelerate the rate of eyeblink conditioning (Harvey et al. 1982; Romano et al. 1991; Romano and Harvey 1994). Moreover, the threshold doses of these drugs required to produce hallucinations in humans were comparable to the ED50s for these drugs in enhancing eyeblink conditioning (Harvey 2003). Parenteral injections of LSD produce a dose-dependent increase in CR acquisition between 1 and 100 nmol/kg, but no effect at 300 nmol/kg. The absence of an effect on eyeblink conditioning at the 300-nmol/kg intravenous dose of LSD also suggests that high doses of LSD engage a different group of receptors.

The selective action of intrahippocampal LSD to acutely elicit head bobs but not body shakes and to chronically desensitize hippocampal 5-HT_{2A} but not 5-HT_{2C} receptors is in agreement with some previous studies with parenteral LSD injections (Aloyo et al. 2001). Thus, 8 days of intravenous LSD injections (30 nmol/kg) produced a significant and selective decrease in the density (Bmax) of the 5-HT_{2A} receptor with no change in the density of the 5-HT_{2C} receptor. The affinity for these receptors (Kd) was not altered (Aloyo et al. 2001). In addition, it has been shown that downregulation of the 5-HT_{2A} receptor is associated with decreases in the ability of DOI to elicit head bobs (Aloyo et al. 2001; Dave et al. 2007). Similarly, upregulation of the 5-HT_{2A} receptor with no change in the density of the 5-HT_{2C} receptor resulted in an enhancement of trace eyeblink conditioning (Harvey et al. 2004).

Not only do 5-HT_{2A} receptor agonists produce hallucinations, but also antipsychotic drugs are proposed to reduce hallucinations in schizophrenic patients through an inverse agonist action at the 5-HT_{2A} receptor (Weiner et al. 2001). Similarly, while hallucinogens enhance eyeblink conditioning, antipsychotic drugs appear to act as inverse agonists in that they retard acquisition (Welsh et al. 1998; Harvey 2003; Aloyo et al. 2009). The occurrence of inverse agonism at the 5-HT_{2A} receptor suggests that the receptor is constitutively active in vivo, and this has been confirmed in the rabbit (Romano et al. 2005; Aloyo et al. 2009). In conclusion, this study supports the current view that hallucinogens act at the 5-HT_{2A} receptor and suggest an important role for 5-HT_{2A} receptors located in the hippocampus in the regulation of cognitive and motoric behaviors. These results may provide additional clues for the role of these receptors in schizophrenia and in the actions of antipsychotic drugs.

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