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ORIGINAL INVESTIGATION

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Enhanced stimulus-reward learning by intra-amygdala administration of a D_3 dopamine receptor agonist

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Abstract The amygdala is considered to be a critical neural substrate underlying the formation of stimulus-reward associations, and is known to receive substantial innervation from dopaminergic neurons located within the ventral mesencephalon. However, relatively little is known about the function of the mesoamygdaloid dopamine projection in stimulus-reward learning. Recently, we have found post-session intra-amygdala microinjections of *d*-amphetamine to enhance appetitive Pavlovian conditioning as assessed in a discriminative approach task. In the present study, we have examined the effects of dopamine receptor agonists possessing relative selectivity for the D_1, D_2 and D_3 receptor subtypes in order to examine more fully the role of the mesoamygdaloid dopamine projection in stimulus-reward learning. Thus, subjects were trained to associate an initially neutral stimulus (CS^+) with 10% sucrose reward (US). A second, control stimulus (CS^-) was also presented but never paired with sucrose reward. In order to measure specifically the conditioned response to CS^+/CS^- presentation, responding during CS and US presentations was measured separately. Immediately following each training session, subjects received bilateral intra-amygdala infusion of 0.1, 1 or 10 nmol/side of SKF-38393, quinpirole or 7-OH-DPAT. Infusions of SKF-38393 or quinpirole were without effect on CS^+ approach. However, post-session intra-amygdala infusions of 7-OH-DPAT enhanced selectively CS^+ approach in a dose-dependent fashion. No dose of any drug affected CS^-approach, US behaviours, or measures of extraneous behaviour. Subsequent acquisition of a novel conditioned instrumental response was also unaffected. Thus, the present data indicate a selective involvement of the D_3 dopamine receptor subtype in the modulation of stimulus-reward learning by the mesoamygdaloid dopamine projection.

Key words Stimulus-reward learning · Discriminative approach · Dopamine · Amygdala · SKF-38393 · Quinpirole · 7-OH-DPAT · D_3 receptor

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Introduction

Human and non-human subjects with lesions of the amygdala display a range of emotional deficits, including an inability to recognise the affective significance of environmental stimuli (Aggleton 1992; LeDoux 1992). Hence, one type of task in which animals with amygdala damage show impaired performance is one requiring the association of stimuli with reward value (Everitt and Robbins 1992). Such deficits are not readily attributable to impairments of perceptual or motoric function, which have been reported to be unaffected by amygdala lesions (Cador et al. 1989; Burns et al. 1993). In rats, one method by which the effects of lesions of the amygdala on stimulus-reward learning has been assessed is an appetitive Pavlovian conditioning procedure, in which subjects are trained to associate an arbitrary stimulus (the conditioned stimulus; CS) with the presentation of a reward, such as food or water (an unconditioned stimulus; US). Intact rats rapidly acquire a conditioned approach response following presentation of the CS. In contrast, while rats with excitotoxic lesions of the amygdala learn readily an approach response to the unconditioned stimulus, acquisition of the conditioned response is severely retarded (Burns et al. 1993). Such a deficit is typically interpreted as an impairment of stimulus-reward learning, a conclusion that is supported by results from a variety of formally comparable procedures, including second-order schedules of reinforcement (Everitt et al.

1989; Hatfield et al. 1996) and conditioned place preference (Everitt et al. 1991; Brown and Fibiger 1993).

To date, five dopamine receptor subtypes have been cloned and are differentiated as belonging either to the D_1 (D_1 and D_5 subtypes) or D_2 (D_2, D_3 and D_4 subtypes) receptor families (Schwartz et al. 1992; Gingrich and Caron 1993; Seeman and Vantol 1994). The D_3 dopamine receptor is linked specifically with the limbic system (Schwartz et al. 1993), of which the amygdala forms a part. Further, the D_3 dopamine receptor is suggested to be a critical mediator of drug reward (Caine and Koob 1993). Both D_1- and D_2-like dopamine receptors are present within the amygdaloid complex and appear to be unevenly distributed between the various nuclei (Scibilia et al. 1992). In addition, it has been known for some time that the amygdaloid complex is innervated by dopaminergic neurons located primarily within the ventral mesencephalon in the cell body groups designated A8, A9 and A10 (Fallon et al. 1978; Fallon and Ciofi 1992). Despite awareness of the existence of mesoamygdaloid dopamine innervation, little is known concerning the possible functions of this projection. Yet, recent data suggest its potential involvement in the mediation of stimulus-reward learning. First, increased extracellular dopamine levels were observed in the amygdala of rats during, and following performance under a discriminative, but not a non-discriminative operant task (Hori et al. 1993). Second, post-session intra-amygdala administration of *d*-amphetamine enhanced retention both in a spatial and cued version of a water maze task (Packard et al. 1994). Third, the enhanced retention of an inhibitory avoidance response produced by post-session systemic cocaine administration was prevented by lesioning of the amygdala (Cestari et al. 1996). Consistent with these data, we have recently found post-session intra-amygdala administration of *d*-amphetamine to enhance acquisition of a discriminative approach task (Hitchcott et al. 1997). However, the pharmacological action of *d*-amphetamine involves not only the release of dopamine but also the release of noradrenaline (Hernandez et al. 1983; Parada et al. 1988). Therefore, our results do not demonstrate conclusively an involvement of a dopaminergic mechanism within the amygdala in stimulus-reward learning. This fact is of particular significance in view of findings demonstrating that post-session intra-amygdala administration of noradrenergic agonists and antagonists modulate acquisition of aversively motivated conditioning (Gallagher et al. 1981; Liang et al. 1986, 1990; Introini-Collison et al. 1991).

To confirm or refute the involvement of amygdala dopaminergic modulation of stimulus-reward learning, the present study examined the effects of the dopamine receptor agonists SKF-38393, quinpirole and 7-OH-DPAT on acquisition of a discriminative approach response. Respectively, these compounds possess, in varying degrees, selectivity for the D_1, D_2/D_3 and D_3 dopamine receptor subtypes (Levesque et al. 1992; Schwartz et al. 1992; Seeman and Van Tol 1994). Thus, separate groups of male Lister hooded rats received bilateral intra-amygdala infusions of one of various doses of these drugs immediately following training sessions in a discriminative approach procedure. In this procedure, subjects were trained to associate an initially neutral stimulus (CS^+) with 10% sucrose reward (US). Responding (defined as approach behaviour into the recess housing sucrose reward) was measured immediately before each stimulus presentation, and subtracted from that during each stimulus presentation. Approach behaviour was also recorded separately for CS^+ and US periods in order to provide a separate measure of anticipatory responding to the CS^+(which is based on associative learning) from responding to the US (which is not), and thus provide an uncontaminated measure of conditioned responding. In addition, a second, control stimulus (CS^-) was also presented but never paired with sucrose reward. Thus, by comparing responding during the $CS^+$and CS^- it was possible to separate the effects of CS-US pairings from non-associative effects on responding produced by, for example, habituation to a CS, or sensitization resulting from the presence of the reward (for discussion see Rescorla 1967). Following acquisition of the discriminative approach response, the conditioned rewarding properties of the CS^+ stimulus was assessed. In drug-free subjects, tests were carried out in the absence of primary reward and the presentation of the conditioned stimuli was made dependent upon a novel response. The ability of conditioned stimuli to support new learning in the absence of primary reward represents a stringent criterion of conditioned reward (Mackintosh 1974, 1983). If responding on the CS^+ lever exceeded to a significant degree responding on the CS^- lever, the CS^+ was adjudged to have acquired significant rewarding value.

Materials and methods

Subjects

A total of 108 male Lister hooded rats was used (Charles Rivers, Margate, Kent, UK). Immediately prior to surgery animals weighed 334 ± 3 g (275–395 g), and were housed singly under a 12 h:12 h light/dark cycle (lights on 0800 hours) at a constant temperature of 22 ± 2°C, and humidity of 55 ± 10%. Experiments were carried out between 1000 and 1700 hours. Following recovery from surgery, the body weights of animals were reduced to 85% of their free-feeding weight by restricting access to food. Access to water was restricted to the period of food availability (1800–1900 hours).

All experimental procedures used were subject to UK Home Office approval (Project Licence PPL 50/01257).

Apparatus

Testing was carried out in eight operant chambers (28 × 21 × 21 cm; Med Associates, St Albans, Vt., USA). Each chamber was equipped with a dipper (model ENV-202; cup capacity 0.06 ml) located within a small recess in the middle of the front wall and was used for the

presentation of a 10% w/v sucrose solution. Two retractable levers each 5 cm wide, were positioned symmetrically upon this wall 8 cm apart and 5 cm from the grid floor, either side of the dipper recess (6 × 6 × 3 cm). The levers were adjusted such that a force of 0.118 N was required to produce switch closure. During discriminative approach training, the levers remained retracted. The operant chamber could be illuminated by a white 15 W houselight located at the top of the wall opposite. Each chamber was also equipped with two white stimulus lights (15 W), positioned directly above each retractable lever 13 cm above the grid floor, and a 75 dB SonAlert tone generator. The stimulus lights and SonAlert were under computer control and were utilised as the conditioned stimuli in the present experiment. The operant chamber was housed in a sound-attenuating box and external noise was masked by a ventilating fan mounted on the side of the box.

Each chamber was also fitted with four photobeams for the measurement of activity. Two photobeams recorded horizontal activity, and were positioned just 1 cm above the grid floor. They were aligned parallel with the wall containing retractable levers, i.e. from front to back. In order to ensure that only activity not directly associated with dipper-approach or lever-pressing was recorded by the photobeams, they were positioned on the opposite side of the chamber to the retractable levers one beam 4.5 cm from the wall opposite, the second 5.5 cm from the first. A further photobeam recorded vertical activity, and was positioned 11.5 cm above the grid floor, 14 cm from the back wall of the chamber. To avoid placing the beam on the chamber door itself, it was aligned perpendicular to the lower beams, i.e. from left to right. To ensure that only vertical activity not directly associated with dipper-approach or lever-pressing was recorded, only upper beam breaks that occurred concurrently, or within 1 s of a lower beam break were recorded. The final photobeam was located in the side walls of the dipper recess and was used to monitor nose-poking behaviour.

The apparatus was controlled, and the data collected, by a standard IBM compatible 386 PC with appropriate software platform (Med Associates).

Drugs

SKF-38393 hydrochloride, quinpirole hydrochloride and 7-OH-DPAT hydrobromide [Semat Technical (UK) Ltd, St Albans, UK] were dissolved in sterile phosphate buffered saline (PBS), which also served as vehicle, at an initial concentration of 20 μmol/ml. Subsequent concentrations (2 and 0.2 μmol/ml) were prepared by dilution of this stock solution. All concentrations/doses were that of the respective base.

Surgery

Rats were anaesthetised with an injection IP of a solution containing 2,2,2-tribromoethanol in sterile PBS (volume injected: 10 ml/kg, for method of preparation, see Phillips et al. 1994).

Bilateral stainless-steel guide cannulae (22 gauge single cannulae; Plastics One, Roanoke, Va., USA) were implanted to gain access to the amygdala. The stereotaxic coordinates used were: AP −2.8 mm from bregma, L ± 4.5 from the midline, V −6.6 mm from the surface of dura (Paxinos and Watson 1986). Implanted guide cannulae were secured to the skull with a minimum of four stainless steel screws and dental cement. The cannulae were closed by screw-in stainless steel wire obturators (28 gauge dummy cannulae; Plastics One) and the animals returned to their home cages for a period of recovery of no less than 7 days.

Post-session infusions

Intracerebral infusions within the amygdala were made using infusion pumps (Model A, 3.33RPM motor; Razel Scientific Instruments, Stamford, Conn., USA). Rats were hand-held while 28 gauge infusion cannulae (Plastics One) were placed into the surgically implanted guide cannulae. The infusion cannulae were attached to the pump microsyringes (Hamilton 801RNE; Scientific Laboratory Supplies Ltd, Hessle, East Yorkshire, UK) by polyethylene tubing filled with sterile PBS. Drug solutions were backloaded within the cannulae and tubing to prevent contamination of the microsyringes, and separate pump and infusion systems were used for vehicle infusions and each of the drugs to prevent contamination of infusates. Infusion cannulae projected from guide cannulae by 1 mm. The volume infused was 0.5 μl over 25 s, and infusion cannulae remained in place for a further 1-min period.

Procedure

Discriminative approach training

Rats were first trained to consume the sucrose solution from the dipper during four sessions in which sucrose was presented 40 times/session according to a variable time 60 s (VT-60s) schedule (100 possible intervals generated using the progression sequence of Fleshler and Hoffman 1962). During these preliminary sessions, all rats received mock infusions in order to accustom subjects to the infusion procedure. Thus, in all respects mock infusions were identical to the infusion procedure as outlined under "Post-session infusions" above, with the sole exception that no solution was infused.

Following dipper training, rats were trained to associate an initially neutral stimulus, (the conditioned stimulus; CS^+) with delivery of the sucrose reward (the unconditioned stimulus; US). During each training session, subjects were also exposed to a second neutral stimulus (CS^-) which was not paired with sucrose presentation. Thus, each CS trial consisted of either a 10-s stimulus presentation followed immediately by 10-s access to 10% sucrose solution (CS^+ trial), or a 10-s presentation of the control stimulus (CS^- trial). The probability of each type of trial occurring was 0.5, with the proviso that a total of four CS^+ and four CS^- trials were presented during each session. Total entries during appropriate periods were recorded. Trial frequency was set according to a fixed time 240-s schedule (FT-240s). The stimuli used were either Houselight off, wall-lights on; or SonAlert tone on. The designation of stimulus type to the CS^+ or CS^- category was counterbalanced across animals. Approach behaviour during the 10-s period immediately preceding the presentation of the stimuli was recorded as a measure of baseline rates of approach behaviour and subtracted from that occurring during presentation of the stimuli themselves. Thus, the specificity of the approach response in relation to the stimulus-reward association could be assessed. Extraneous behaviour was measured concurrently and was recorded both as horizontal and vertical activity. Immediately following each training session animals were removed from the operant chambers and received bilateral intra-amygdala infusions. Ten groups of rats received either PBS or 0.1, 1 or 10 nmol/side of SKF-38393, quinpirole or 7-OH-DPAT. There were 11 sessions in all, each separated by an interval of at least 72 h.

Conditioned reward testing

Forty-eight hours after the last training session, the ability of the CS^+ and CS^- to act as a conditioned reward (CR) was assessed. During CR testing sucrose reward was not presented and the dipper remained inactive throughout. Two novel levers were introduced into the operant chambers. Depression of one lever (the CS^+ lever) resulted in the brief (0.5 s) presentation of the CS^+, responding on the second lever (the CS^- lever) resulted in the brief (0.5 s) presentation of the CS^-. The position of the CS^+ lever was counterbalanced across animals. The probability of occurrence of the CS after depression of either lever was 0.5. Sessions began

following a response upon either lever, and continued for a total of 30 min. Rates of responding on both levers were recorded.

Histology

At the conclusion of the experiments, animals were killed by pentobarbital overdose and the brain removed for histological examination. Brains were blocked and cut at 25 μm sections (Bright OTF Cryostat, Bright Instrument Company Ltd, Huntington, Cambs, UK). The sections were mounted on glass slides and stained with cresyl violet. The accuracy of cannula placements, and the effects of intracerebral infusions upon brain tissue were then assessed.

Statistical analysis

Data from discriminative approach training and conditioned reward testing were analysed using split-plot parametric analysis of variance, with group as the between subjects variable and training session (discriminative approach training) or lever (conditioned reward testing) as the within subjects variable. The effects of each drug were analysed separately. Statistically significant main effects were analysed further. Within-factor comparisons were analysed initially using simple main effect analyses of variance, and where appropriate were completed post hoc using Dunnett's t(D-t) test.

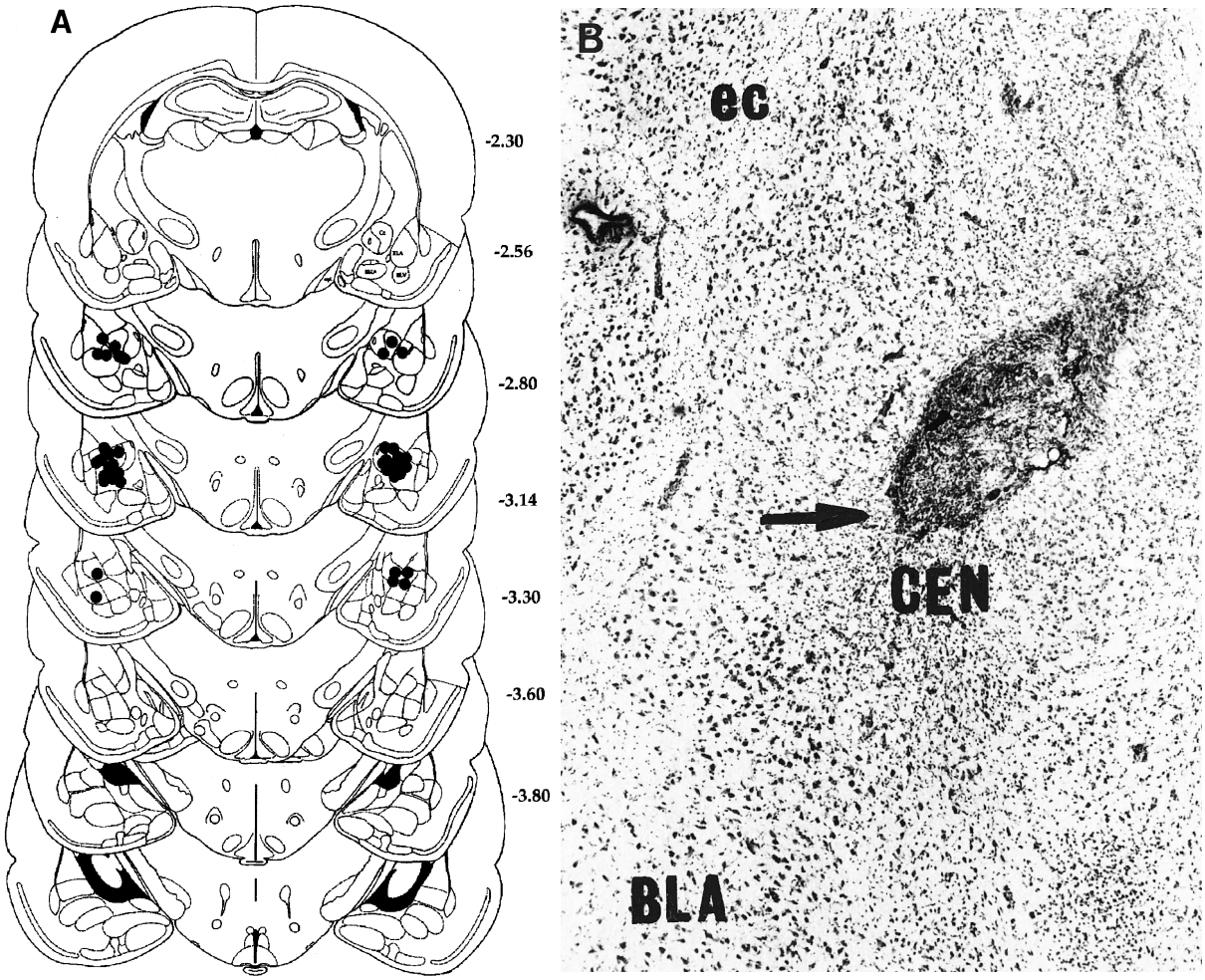

Fig. 1 A, B Infusion sites within the amygdala. **A** Coronal sections through the rat brain, based upon the atlas of Paxinos and Watson (1986). Numbers adjacent to each section represent distances from Bregma (mm) in the anterior-posterior plane. Infusion sites shown as *filled circles*. **B** Photomicrograph of infusion site (*arrowhead*) of one animal. *CEN* central nucleus; *BLA* basolateral area; *ec* external capsule. The region of necrosis appeared to be restricted to that of the infusion site itself

Results

Histological examination verified that all infusion sites intended for the amygdala were located within basolateral or central nuclei and were within ± 0.5 mm of the intended coordinates in the rostral-caudal dimension (see Fig. 1, left panel). Inspection under high power magnification revealed only very limited gliosis around the immediate infusion site (Fig. 1, right panel).

Acquisition of discriminative approach

As shown in Fig. 2 (left panels), post-session intra-amygdala infusions of the D1 dopamine receptor agonist, SKF-38393, had no effect on the number of alcove

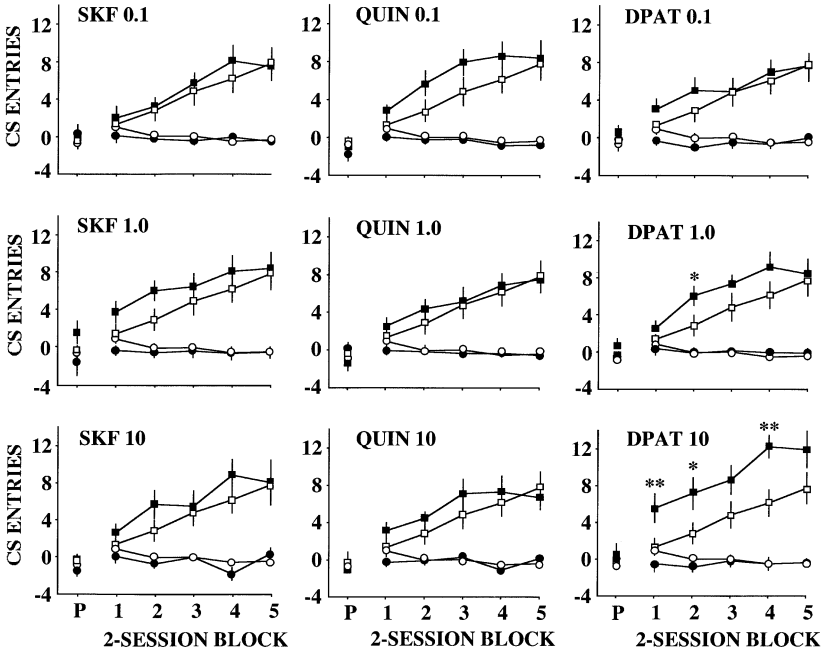

Fig. 2 Effect of bilateral post-session intra-amygdala infusions of SKF-38393, quinpirole and 7-OH-DPAT on the acquisition of discriminative approach. *Left panels*: SKF-38393 0.1, 1.0 and 10-nmol. *Centre panels*: quinpirole 0.1, 1.0 and 10 nmol. *Right panels*: 7-OH-DPAT 0.1, 1.0 and 10 nmol. Points on horizontal axes labelled "P" refer to responding on the first training session only, i.e. before the first drug infusion. Total number of CS+ or CS− entries per session are presented. Note the lack of difference between vehicle- and drug-treated groups either on CS+ or on CS− performance. Subsequent points refer to the appropriate two-session block. *Filled squares*: drug group CS+ performance; *open squares*: Vehicle group CS+ performance; *filled circles*: drug group CS− performance; *open circles*: vehicle group CS− performance. Values represent the mean ± 1 SEM of the number of alcove entries (CS entries minus pre-CS entries) per two-session block. *Stars* indicate statistically significant comparisons with the appropriate vehicle performance. $*P < 0.05$, $**P < 0.01$

entries made either during the CS^+ [Group $F(3,40) = 0.52$, NS; Group × Session $F(15,200) = 0.54$, NS] or CS^- periods [Group $F(3,40) = 0.43$, NS; Group × Session $F(15,200) = 1.16$, NS]. In addition, there was no effect on alcove entries that occurred during the US period [Table 1; Group $F(3,38) = 1.01$, NS; Group × Session $F(15,190) = 0.93$, NS], indicating an absence of effect on the response to primary reward. Similarly, post-session intra-amygdala infusions of the D_2/D_3 dopamine receptor agonist, quinpirole (Fig. 2, centre panels), had no effect on the number of alcove entries made either during the CS^+ [Group $F(3,40) = 0.59$, NS; Group × Session $F(15,200) = 0.89$, NS] or CS^- periods [Group $F(3,40) = 0.67$, NS; Group × Session $F(15,200) = 1.57$, NS]. Closer inspection of the data revealed some suggestion of an enhancement of CS^+alcove entries at the lowest dose of quinpirole administered (0.1 nmol). Data for this dose alone were therefore analysed separately; however, this revealed that the main effect of quinpirole (0.1 nmol) failed to reach statistical significance [$F(1,20) = 1.41$, NS; Group × Session interaction $F(5,100) = 1.35$, NS]. There was no effect of post-session quinpirole on alcove approach during the US period [Table 1; Group $F(3,40) = 0.19$, NS; Group × Session $F(15,200) = 1.17$, NS], again indicating an absence of effect on the response to primary reward. In contrast, post-session intra-amygdala infusions of the relatively selective D_3 dopamine receptor agonist, 7-OH-DPAT, increased the number of alcove entries made during the CS^+ period [Group $F(3,38) = 2.99$, $P < 0.05$; Group × Session $F(15,190) = 1.15$, NS]. Separate analysis for each dose indicated that this was due to a significant effect of the 10 nmol dose only [Group $F(1,18) = 6.33$, $P < 0.05$], although there was a marginal effect of the 1 nmol dose [Group $F(1,20) = 2.38$, $P = 0.14$]. Thus, post-hoc analyses revealed that both doses significantly increased discriminative approach on at least one two-trial block during training (see Fig. 2, right panels). However, intra-amygdala 7-OH-DPAT failed to affect the number of alcove entries made during the CS^- period [Group $F(3,38) = 0.61$, NS; Group × Session $F(15,190) = 0.75$, NS], and failed to alter alcove approach during the US period [Table 1; Group $F(3,38) = 0.03$, NS; Group × Session $F(15,190) = 0.93$, NS]. Thus, intra-amygdala infusions of 7-OH-DPAT selectively enhanced approach toward the CS^+, without affecting approach toward either the control stimulus (CS^-) or the primary reward (US) itself.

Extraneous activity

Post-session intra-amygdala infusions of SKF-38393, quinpirole or 7-OH-DPAT had no effect on horizontal

Table 1 Effect of bilateral post-session intra-amygdala infusions of SKF-38393 (*SKF*), quinpirole (*QUIN*) and 7-OH-DPAT (*DPAT*) on the total number of alcove approach responses per session made during the US period. Numbers adjacent to drug labels refer to doses infused (nmol). Session *P* refers to responding on the first, pre-drug session. Subsequent cells refer to the appropriate two-session block. Values represent the mean ± 1 SEM

Group	US period alcove entries					
	P	1	2	3	4	5
Vehicle	4.8 ± 0.6	8.6 ± 2.1	8.4 ± 1.1	9.3 ± 1.4	9.6 ± 1.2	9.5 ± 0.9
SKF 0.1	3.9 ± 0.5	8.9 ± 1.1	10.7 ± 1.2	10.8 ± 1.0	10.3 ± 1.2	9.8 ± 1.0
SKF 1	3.9 ± 0.6	8.0 ± 0.8	9.1 ± 0.6	9.1 ± 0.8	9.7 ± 0.9	9.8 ± 0.6
SKF 10	3.5 ± 0.6	8.0 ± 0.6	8.3 ± 1.2	7.8 ± 1.6	7.8 ± 1.4	7.1 ± 1.1
QUIN 0.1	4.2 ± 0.7	10.2 ± 1.4	10.1 ± 1.3	8.9 ± 1.0	8.9 ± 0.8	8.8 ± 0.9
QUIN 1	3.5 ± 0.3	8.8 ± 0.4	9.3 ± 0.9	8.1 ± 0.6	9.7 ± 1.0	8.7 ± 0.4
QUIN 10	4.3 ± 0.6	8.8 ± 0.6	7.7 ± 0.2	7.9 ± 0.6	8.5 ± 0.7	8.7 ± 0.9
DPAT 0.1	3.9 ± 0.5	8.5 ± 0.6	8.8 ± 0.6	10.1 ± 0.8	9.7 ± 1.0	9.5 ± 0.8
DPAT 1	4.5 ± 0.6	8.9 ± 0.7	9.3 ± 0.9	9.7 ± 1.0	8.9 ± 0.9	7.5 ± 1.0
DPAT 10	4.6 ± 0.6	8.1 ± 0.4	8.7 ± 0.3	9.2 ± 0.4	9.6 ± 0.7	9.8 ± 0.6

Table 2 Conditioned instrumental performance. Note: subjects were tested drug-free 48 h after the last session of discriminative training in the absence of primary (sucrose) reward. Subjects had received previously post-training intra-amygdala infusions of SKF-38393 (*SKF*), quinpirole (*QUIN*) and 7-OH-DPAT (*DPAT*). Numbers adjacent to drug labels refer to doses previously infused (nmol). CS+, total responses upon the lever resulting in a 0.5 s presentation of the CS+ (probability 0.5); CS-, total responses upon the lever resulting in a 0.5-s presentation of the CS− (probability 0.5); *ALCOVE*, total alcove entries; *HORI*, horizontal activity (total number of beam breaks); *VERT*, vertical activity (total number of beam breaks). Values represent the mean ± 1 SEM

Group	Conditioned instrumental performance				
	CS^+	CS^-	ALCOVE	HORI	VERT
Vehicle	46.1 ± 6.8	22.9 ± 5.9	43.8 ± 3.4	220 ± 23	21 ± 4
SKF 0.1	45.1 ± 6.2	33.9 ± 5.5	44.9 ± 7.7	206 ± 17	16 ± 2
SKF 1	42.0 ± 11.4	16.9 ± 2.4	56.2 ± 10.9	181 ± 20	14 ± 3
SKF 10	36.6 ± 6.5	24.3 ± 6.9	55.6 ± 10.5	238 ± 25	15 ± 2
QUIN 0.1	48.6 ± 5.6	37.1 ± 11.5	64.0 ± 10.0	267 ± 31	23 ± 3
QUIN 1	30.2 ± 5.7	14.6 ± 4.3	42.9 ± 4.4	190 ± 22	19 ± 4
QUIN 10	31.6 ± 5.7	15.8 ± 2.1	38.1 ± 5.8	182 ± 21	14 ± 2
DPAT 0.1	45.6 ± 4.8	30.6 ± 6.9	48.1 ± 7.5	215 ± 14	17 ± 3
DPAT 1	35.4 ± 5.2	24.5 ± 6.0	41.7 ± 7.3	255 ± 26	18 ± 2
DPAT 10	44.2 ± 10.2	29.0 ± 6.2	39.1 ± 6.3	228 ± 21	17 ± 3

or vertical activity. Thus, there was no main effect of SKF-38393 on either measure [horizontal: $F(3, 40) = 0.43$, NS; vertical: $F(3, 40) = 0.74$, NS] and no significant Group × Session interactions [horizontal: $F(15, 200) = 1.15$, NS; vertical: $F(15, 200) = 1.31$, NS]. Similarly, there was no main effect of quinpirole on either measure [horizontal: $F(3, 40) = 2.21$, NS; vertical: $F(3, 40) = 2.32$, NS] and no significant Group × Session interactions [horizontal: $F(15, 200) = 1.28$, NS; vertical: $F(15, 200) = 0.72$, NS]. Intra-amygdala 7-OH-DPAT had no effect on horizontal or vertical activity. Thus, there was no main effect of 7-OH-DPAT on either measure [horizontal: $F(3,38) = 0.30$, NS; vertical: $F(3, 38) = 0.97$, NS] and no significant Group × Session interactions [horizontal: $F(15,190) = 1.08$, NS; vertical: $F(15,190) = 0.42$, NS].

Conditioned reward

The conditioned rewarding efficacy of the sucrose-associated stimulus was assessed 48 h after the last session of discriminative approach training, in the absence of sucrose reward and in drug-free animals. Rates of response upon the CS^+ lever exceeded rates of response upon the CS^- lever in the PBS-vehicle group [Table 2; Lever $F(1, 10) = 16.3$, $P < 0.005$], indicating that the sucrose-associated stimulus (CS^+) had acquired significant rewarding efficacy. Post-session, intra-amygdala administration of SKF-38393, quinpirole or 7-OH-DPAT during discriminative training failed to affect subsequently the conditioned rewarding properties of the CS^+as indicated by non-significant Group × Lever interactions [SKF-38393 $F(3, 30) = 0.77$, NS; quinpirole $F(3, 33) = 0.4$, NS; 7-OH-DPAT $F(3, 38) = 0.4$, NS].

Alcove entries, measured concurrently with responding for conditioned reward, were unaffected by post-session, intra-amygdala administration of SKF-38393 [$F(3, 30) = 0.6$, NS] or 7-OH-DPAT [$F(3, 38) = 0.4$, NS] during discriminative training. Post-session, intra-amygdala administration of quinpirole during discriminative training altered significantly alcove approach [$F(3, 33) = 3.3$, $P < 0.05$]. However, while this effect appeared to be due to an increase in alcove entries by the 0.1 nmol group (Table 2), post-hoc pairwise comparisons (Dunnett's-*t*) of each quinpirole

group versus the PBS-vehicle group failed to reach significance in each case.

There were no significant main effects of Group on horizontal or vertical activity during conditioned reward testing for SKF-38393 [horizontal: $F(3,30) = 1.2$, NS; vertical: $F(3, 30) = 0.84$, NS], quinpirole [horizontal: $F(3, 33) = 2.3$, NS; vertical: $F(3, 33) = 1.1$, NS] or 7-OH-DPAT [horizontal: $F(3, 38) = 0.7$, NS; vertical: $F(3, 38) = 0.3$, NS].

Discussion

The results of the present study indicate that post-session intra-amygdala administration of the relatively selective D_3 dopamine receptor agonist 7-OH-DPAT dose-dependently enhanced the acquisition of an approach response to a conditioned stimulus (CS^+) predictive of 10% sucrose reward. Furthermore, 7-OH-DPAT was without effect upon the approach response to a conditioned stimulus (CS^-) unpaired with sucrose reward, or on responding during presentation of the sucrose reward (US) itself. In addition, there was no effect of 7-OH-DPAT on measures of activity extraneous to the approach response. By contrast, SKF-38393 and quinpirole failed to affect the rate of acquisition of CS^+ approach, or indeed of any other measure recorded during discriminative approach training. Therefore, these findings indicate a highly selective effect of intra-amygdala 7-OH-DPAT, and presumably therefore the D_3 receptor subtype, in the modulation of stimulus-reward learning .

In addition, the data facilitate interpretation of a recent study in which we reported that post-session intra-amygdala infusions of *d*-amphetamine enhanced acquisition of a discriminated approach response (Hitchcott et al. 1997). The pharmacological mechanism of *d*-amphetamine involves a potentiation of catecholamine transmission by a presynaptic action to increase both dopamine and noradrenaline release (Scheel-Krüger 1971). However, the present data suggest that activation of dopamine D_3 receptors is sufficient for the enhancement of stimulus-reward learning. The neural mechanism by which 7-OH-DPAT, or indeed *d*-amphetamine, acts to enhance stimulus-reward learning is, however, unclear. Previous studies indicate that lesions to either the basolateral area (Cador et al. 1989; Everitt et al. 1991; Burns et al. 1993), or central nucleus (Gallagher et al. 1990) of the amygdala impair acquisition of appetitive Pavlovian conditioning in tasks very similar to that used here. The cannulae placements and volume of drug infusion used in the present study do not discriminate conclusively between an action of 7-OH-DPAT within a specific nucleus of the amygdaloid complex. In a recent study, Hatfield et al. (1996) reported that excitotoxic lesions of the basolateral area, but not the central nucleus, of the amygdala reduced the ability of a food-paired light CS to act as a reinforcer for second-order conditioning of a tone. In contrast, lesions of the central nucleus have been reported to retard the acquisition of conditioned orienting ("CS-generated CRs") responses while having little effect on conditioned approach ("US-generated CRs") responses (Gallagher et al. 1990). Thus, there is reason to believe that the basolateral area, rather than the central nucleus, of the amygdala might mediate the effects of 7-OH-DPAT observed in the present study.

However, other evidence suggests this may not be the case. It has been demonstrated that both the D_1 and D_2 dopamine receptor families are present within the amygdaloid complex but show non-overlapping distributions within the various nuclei. Thus, D_2-like binding shows a preferential location in the central nucleus compared to the basolateral area, while the reverse is true in the case of the D_1 receptor family (Scibilia et al. 1992). In the case of the D_1 receptor family, this pattern corresponds closely with the distribution of D_1 receptor mRNA (Fremeau et al. 1991). In contrast, the patterns of D_2-like binding and D_2 receptor mRNA show considerably less overlap since D_2 mRNA is present in the central nucleus only in very low levels (Mansour et al. 1990), suggesting that the D_2-like binding reported in the central nucleus may reflect the presence of other subtypes of the D_2 family, presumably of either the D_3 or D_4 subtypes, both of which have been identified as being present in the mammalian amygdala (Sokoloff et al. 1990; Van Tol et al. 1991). Thus, while highly speculative, it is possible that the central nucleus of the amygdala contains a relatively high density of D_3 dopamine receptors, at least with respect to the basolateral area, and may therefore be the locus of action of 7-OH-DPAT on stimulus-reward learning.

It should be noted that on the basis of receptor binding studies, 7-OH-DPAT can be considered only relatively selective for the D_3 dopamine receptor over the D_2 subtype (Levesque et al. 1992; Damsma et al. 1993). Furthermore, functional indices determined in cultured cells indicate lower selectivity than the radioligand binding data (Chio et al. 1994). However, intra-amygdala administration of quinpirole had no effect on the discriminative approach response despite its reported poor selectivity (~2 fold) for the D_2 dopamine receptor over the D_3 subtype in vitro (Chio et al. 1994). Therefore, at least in terms of the behavioural data, 7-OH-DPAT produced a highly selective enhancement of the acquisition of discriminative approach. However, while the present results suggest a selective role of D_3 dopamine receptors in the amygdala in the modulation of stimulus-reward learning, additional data indicate that this may not be a function unique to the D_3 dopamine receptor. Thus, infusions of an antagonist of the NMDA glutamate receptor subtype AP5 into the basolateral region of the amygdala have been found to impair acquisition of stimulus-reward learning as

measured in a discriminative approach procedure similar to that presently employed (Burns et al. 1994). In addition, it is yet to be determined whether intra-amygdala administration of drugs which have been shown to affect aversively motivated conditioning, such as opiate or GABA-A receptor antagonists (Brioni et al. 1989; Introini-Collison et al. 1989), also modulate appetitive stimulus-reward learning.

There are differing opinions concerning the involvement of the amygdala as a permanent site of memory storage. Certainly, the demonstration, in vivo, of long-term potentiation within the basolateral area of the amygdala (Clugnet and LeDoux 1990) suggests that plastic changes consistent with the storage of information within this structure can occur. In addition, other findings indicate that antagonists of the NMDA subtype of glutamate receptor impair fear conditioning and discriminative approach (Miserendino et al. 1990; Burns et al. 1994). As it is well established that NMDA receptors are required for the induction of long-term potentiation (Collingridge 1987), these findings suggest that plastic changes within the amygdala may mediate the effects of fear conditioning and/or discriminative approach. Evidence for an alternative view, namely that the amygdala modulates storage of information elsewhere in the brain, is also available. Thus, intra-amygdala infusions of *d*-amphetamine facilitated retention of both cued and spatial versions of a water maze task, an effect that was unaltered in rats given intra-amygdala infusions of lidocaine to cause reversible inactivation of the amygdala, immediately before a retention test (Packard et al. 1994). Thus, the enhanced performance following *d*-amphetamine administration during training was not dependent upon neural activity within the amygdala. It is not clear, however, whether this finding, based on water maze learning, indicates that plastic changes outside the amygdala were responsible for the effect of 7-OH-DPAT.

Subsequent conditioned reward efficacy was also unaffected by prior intra-amygdala D_1, D_2 or D_3 dopamine receptor agonist administration during training. This observation is consistent with previous data indicating that prior intra-amygdala administration of *d*-amphetamine, which enhanced acquisition of a discriminated approach response (Hitchcott et al. 1997), failed to affect subsequently the control over behaviour exerted by a conditioned reward. This is perhaps to be expected, since all groups displayed similar asymptotic levels of performance during the latter stages of discriminative training. Hence, it is possible that 7-OH-DPAT treated rats would have shown enhanced acquisition of operant responding for conditioned reward if tested after fewer sessions of discriminative training. Previous data demonstrated that the amygdala may determine the degree of discriminative control over behaviour exerted by natural- (Burns et al. 1993) or drug- (Hitchcott and Phillips 1997) associated conditioned rewards. In contrast, amygdala lesions have been shown to be without effect upon the ability of intra-accumbens *d*-amphetamine to enhance the instrumental efficacy of the conditioned reward (Burns et al. 1993). Hence, there is evidence that while the amygdala is a necessary substrate for Pavlovian stimulus-reward learning, it may not be involved to the same degree in the instrumental aspect of conditioned rewards.

In conclusion, the present findings confirm previous evidence (Hitchcott et al. 1997) suggesting a role of the mesoamygdaloid dopamine projection in stimulus-reward learning. Since 7-OH-DPAT, but not quinpirole or SKF-38393, enhanced such learning, the data implicate the selective involvement of the D3 dopamine receptor subtype in this effect. Confirmation of this selective involvement awaits the availability of compounds possessing a higher degree of selectivity for, most importantly, the D_2 and D_3 dopamine receptor subtypes.

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