The Journal of Neuroscience, ? 1997, 17(21):8580–8587

Role of Dopamine D_1 and D_2 Receptors in the Nucleus Accumbens in Mediating Reward

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The objectives of this study were to examine the involvement of D_1 and D_2 receptors within the nucleus accumbens (ACB) in mediating reinforcement. The intracranial self-administration (ICSA) of D_1 and D_2 agonists was used to determine whether activating D_1 and/or D_2 receptors within the ACB of Wistar rats is reinforcing. At concentrations of 0.25, 0.50, and 1.0 mM (25, 50, and 100 pmol/100 nl of infusion), neither the D_1 agonist *R*(+)-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol [SKF 38393 (SKF)] hydrochloride nor the D_2 agonist (−)-quinpirole (Quin) hydrochloride was self-administered into the shell region of the ACB. On the other hand, equimolar mixtures of SKF and Quin (SKF+Quin), at concentrations of 0.25, 0.50, and 1.0 mM each, were significantly self-infused into the ACB shell. The core region of the ACB did not support the ICSA of SKF+Quin at any of these concentrations. Rats increased lever pressing when the response requirement was increased from a fixed ratio 1 (FR1) to FR3, and they responded significantly more on the infusion lever than they did on the control lever. Coadministration of either 0.50 mM *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SCH 23390) hydrochloride, a D_1 antagonist, or 0.50 mM *S*(−)-sulpiride, a D_2 antagonist, completely abolished the ICSA of the mixture of SKF+Quin (each at 0.50 mM) into the ACB shell. The present results suggest that concurrent activation of D_1- and D_2-type receptors in the shell of the ACB had a cooperative effect on DA-mediated reward processes.

Key words: dopamine D_1 receptor; dopamine D_2 receptor; SKF 38393; quinpirole; nucleus accumbens; intracranial self-administration; reward; reinforcement

Dopamine (DA) systems of the brain have been implicated in mediating reward-related behavior (see Fibiger and Phillips, 1986; Koob and Bloom, 1988; Wise and Rompre, 1989; Le Moal and Simon, 1991). In particular, the DA pathway projecting from the ventral tegmental area (VTA) to the nucleus accumbens (ACB) is thought to play a major role in mediating the rewarding effects of many stimuli, such as electrical brain stimulation and drugs of abuse (see Wise and Bozarth, 1987; Di Chiara, 1995). ACB DA depletion, produced by 6-OHDA, abolishes or attenuates intravenous self-administration of the indirect DA agonists amphetamine and cocaine (Roberts et al., 1977; Lyness et al., 1979; Pettit et al., 1984). Microinjection of DA antagonists into the ACB disrupts operant responding maintained by electrical brain stimulation (Mora et al., 1975; Mogenson et al., 1979; Stellar et al., 1983; Stellar and Corbett, 1989) and food (Ikemoto and Panksepp, 1996). Amphetamine microinfused into the ACB facilitates brain electrical self-stimulation (Broekkamp et al., 1975; Colle and Wise, 1988) and produces place-preference conditioning (Carr and White, 1983, 1986). Moreover, rats self-administer amphetamine (Hoebel et al., 1983; Phillips et al., 1994) and the DA uptake inhibitor nomifensine (Carlezon et al., 1995) directly into the ACB.

Received June 24, 1997; revised Aug. 11, 1997; accepted Aug. 20, 1997.

This work was supported in part by United States Public Health Service Grants AA10721 and AA09619.

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The interaction of DA D_1 and D_2 receptors has been reported for a number of electrophysiological and behavioral measures (see Clark and White, 1987; Waddington et al., 1994). In the ACB, synergistic effects of D_1 and D_2 agonists have been reported for locomotor activity (Dreher and Jackson, 1989; Essman et al., 1993; Koshikawa et al., 1996a), jaw movements (Cools et al., 1995; Koshikawa et al., 1996b), and neuronal firing (White, 1987). However, there is no clear information whether the interaction of D_1 and D_2 receptors within the ACB can produce a cooperative or synergistic effect on reinforcement processes. In addition, it is not clear whether activating DA receptors within the shell and/or core of the ACB is reinforcing. There is evidence that the shell portion of the ACB is involved in mediating reward because both amphetamine (Hoebel et al., 1983) and nomifensine (Carlezon et al., 1995) were self-infused in this subregion. However, there is also evidence that amphetamine could be self-infused into the core portion of the ACB (Phillips et al., 1994).

Therefore, one objective of the present study was to determine, using the intracranial self-administration (ICSA) technique, whether activation of both D_1 and D_2 receptors was required for the processing of reward-relevant information mediated by DA within the ACB. A second objective was to determine whether processing of this information occurred in the shell and/or core of the ACB.

MATERIALS AND METHODS

Subjects

Experimentally naive, female Wistar rats (weighing 250–300 gm at the time of surgery) were obtained from Harlan Industries (Indianapolis, IN). Female rats were used in the present study because their growth rate maintained their size within a range that aided the stereotaxic placements (Ikemoto et al., 1997). Although not systematically examined, the

estrous cycle did not seem to have any obvious effect on ICSA behavior in this or a previous study (Ikemoto et al., 1997). Animals were singly housed and maintained on a 12 hr light/dark cycle (lights on at 9:00 A.M.) with constant temperature and relative humidity. Food and water were available *ad libitum* except in the test chamber. The treatments of the subjects were approved by the institutional review board and are in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.

Test agents

R(+)-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol (SKF 38393) HCl, (−)-quinpirole HCl (LY 171555), *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SCH 23390) HCl, and *S*(−)-sulpiride were purchased from Research Biochemicals (Natick, MA). Test agents were dissolved in an artificial CSF (aCSF) consisting of (in mM): 120.0 NaCl, 4.8 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25.0 $NaHCO_3$, 2.5 $CaCl_2$, and 10.0 D-glucose. When necessary, the pH was adjusted to 7.3 ± 0.2 with 0.1 M HCl.

Apparatus

The apparatus used in the present study has been described previously (Ikemoto et al., 1997). The operant chamber (30 cm in width × 30 cm in height × 26 cm in depth) was equipped with two identical levers (3.5 × 1.8 cm) and was situated in a sound-attenuating cubicle (64 × 60 × 50 cm; Coulbourn Instruments, Inc., Allentown, PA) with a ventilating fan. A dim house light illuminated the operant chamber during testing. Unintended lever presses by the rat brushing against the levers or stepping on them were avoided by mounting the levers 15 cm above the grid floor and by separating them by 12 cm. This arrangement required rats to rear to press the levers. The delivery of infusate in relation to lever responses was controlled by a personal computer equipped with an operant control system (L2T2 system; Coulbourn Instruments, Inc.).

An electrolytic microinfusion transducer (EMIT) system (see Criswell, 1977; Bozarth and Wise, 1980; Goeders and Smith, 1987) was used for infusing the test agents. Briefly, two platinum electrodes were placed in an infusate-filled cylinder (28 mm in length × 6 mm in diameter) equipped with a 28 gauge injection cannula (C323ICT; Plastic One, Roanoke, VA). The electrodes were connected via spring-protected cable (Plastic One) and a swivel (Model 205; Mercotac, Inc., Carlsbad, CA) to a constant current generator (MNC, Inc.; Shreveport, LA), which delivered 6 μA of quiescent current and 200 μA of infusion current between the electrodes. Depression of the infusion lever activated the constant current generator and delivered the infusion current for 5 sec, which led to the rapid generation of H_2 gas in the gas tight cylinder and, in turn, forced 100 nl of the infusate through the injection cannula.

Animal preparation

Under Halothane anesthesia, a unilateral 22 gauge guide cannula (C313G; Plastic One) was stereotaxically implanted in the right hemisphere of each subject and aimed 1.0 mm above the shell or core of the ACB. The coordinates for the shell were 1.8 mm anterior to bregma, 3.4 mm lateral to the midline with a 16° lateral angle to the vertical, and 7.6 mm ventral from the skull surface. The coordinates for the core were 1.8 mm anterior to bregma, 4.0 mm lateral to the midline with a 16° lateral angle to the vertical, and 7.0 mm ventral from the skull surface. The incisor bar was set at −3.3 mm below horizontal zero (Paxinos and Watson, 1986). When the system was not in use, a 28 gauge stylet (C313DC; Plastic One) was inserted and extended 0.5 mm beyond the tip of the guide cannula.

At least 7 d were allowed for recovery from the surgery, during which animals were brought daily to the testing room and handled for 5 min. On the day before the experimental sessions were started, subjects were placed in the test chamber for 30 min to acclimate them to the novel environment.

General test condition

To obtain stable voltage readings with the different test solutions, it was necessary to condition the electrodes before starting the test sessions. To accomplish this, electrodes were placed in the test solution, and the quiescent current was applied overnight. Immediately before a test session was started, the infusate was replaced with a fresh solution. All test sessions were conducted during the light phase of the cycle.

Subjects were brought to the testing room and were placed individually in the operant chambers. To avoid trapping air at the tip of the injection cannula and to minimize clogging of the injector tip, we delivered the infusion current for 5 sec as the injection cannula was inserted into the guide cannula. The injection cannula extended 1.0 mm beyond the tip of the guide. Depression of the infusion lever resulted in the delivery of 100 nl of infusate over a 5 sec period followed by a time-out period (60 sec for experiments 1, 2, and 4, and 5 sec for experiment 3), during which depression of the infusion lever produced no programmed consequence. Depression of the control lever had no programmed consequence at any time. The assignment of infusion and control levers with respect to the left and right locations was counterbalanced among subjects. For each subject, however, the assignment of the levers remained the same throughout the experiment. No shaping technique was used to facilitate the acquisition of lever responses. The number of infusions and responses on the infusion and control levers (including responses during the time-out period) were recorded.

General test procedure for experiments 1, 2, and 4

An infusion was delivered contingent to the depression of the infusion lever, which was followed by a 60 sec time-out period. The session lasted for 3 hr but was terminated early if rats self-administered 40 infusions. Sessions were separated by 48–72 hr.

Experiment 1, intra-ACB self-administration of D_1 and D_2 agonists: dose–response analyses

Rats with a cannula placement in the shell were assigned one of three infusate treatments: the D_1 agonist SKF 38393 alone (SKF), the D_2 agonist quinpirole alone (Quin), or a mixture of SKF 38393 and quinpirole (SKF+Quin). Rats with cannula placements in the core received only the mixture of SKF+Quin. During four sessions, the animals were given the opportunity to self-infuse four concentrations (0.0, 0.25, 0.50, and 1.0 mM) of the test solution. These concentrations produced infusions of 0, 25, 50, and 100 pmol/100 nl of the individual SKF and Quin solutions or 25, 50, and 100 pmol each of SKF and Quin in 100 nl of the mixture (SKF+Quin). The order of testing different concentrations and vehicle was counterbalanced among subjects. A one-way within-subject design ANOVA followed by a Newman–Keuls test was conducted on the data obtained with the four different concentrations.

Experiment 2, intrashell self-administration of D_1 and D_2 agonists: an interactive effect

Experimentally naive rats with a cannula placement in the shell were assigned to one of three groups for intrashell self-administration of the DA agonists. The SKF group received infusions of 0.5 mM SKF alone during the first three sessions and infusions of 0.5 mM SKF plus 0.5 mM Quin for sessions 4 and 5. The Quin group was given 0.5 mM Quin for sessions 1–3 and 0.5 mM SKF plus 0.5 mM Quin in sessions 4 and 5. The SKF+Quin group received infusions of 0.5 mM SKF plus 0.5 mM Quin for sessions 1–3 and infusions of vehicle in session 4; 0.5 mM SKF plus 0.5 mM Quin was reinstated in session 5. To evaluate differential effects of these three infusate treatments among the three groups, we conducted a three × three mixed ANOVA on infusions with the three groups (SKF vs Quin vs SKF+Quin groups) over the first three sessions. Differences in infusion levels after the introduction of 0.5 mM SKF plus 0.5 mM Quin in the SKF and Quin groups were analyzed using a 3 × 2 mixed ANOVA followed by a simple effects test comparing the data obtained in sessions 3 and 5. Paired *t* tests were conducted between sessions 3 and 4 and between sessions 4 and 5 on infusions of the SKF+Quin group to evaluate the effects of the removal and reinstatement of 0.5 mM SKF plus 0.5 mM Quin in the SKF+Quin group. In addition, preference for the infusion or control lever in the SKF+Quin group was examined using a 2 × 3 ANOVA for the two levers during the first three sessions. The removal and reinstatement effects of SKF+Quin on responding were evaluated using 2 × 2 ANOVAs for the two levers between sessions 3 and 4 and between sessions 4 and 5.

Experiment 3, effects of increased lever–response requirements on the self-infusion of SKF+Quin into the ACB shell

Test condition. To make it easier for rats to learn the response–stimulus contingency, auditory and visual cues were provided, and the time-out period was shortened. The initiation of an infusion was accompanied by activation of a high frequency tone (Sonalert; Coulbourn Instruments, Inc.) and by the extinguishing of the dim house light. The high frequency tone and the extinguished house light persisted during the 5 sec infusion period and the subsequent 5 sec time-out period. Depression of the

infusion lever did not produce additional infusions during the infusion and time-out periods. The termination of the auditory cue and reinstatement of the house light signaled the availability of another infusion. Depression of the control lever had no programmed consequence at any time. With the fixed-ratio 1 (FR1) schedule, a single depression of the infusion lever resulted in the delivery of the infusate. With the FR3 schedule, three depressions of the infusion lever were required to deliver one infusion, except for the first four infusions and the second four infusions, which were delivered after a single response and after two responses, respectively. Sessions were 90 min in duration; they were terminated early if rats self-administered 24 infusions.

Test procedure. Experimentally naive rats ($n = 10$) were prepared as described above. Animals were given the opportunity to self-infuse 0.5 mM SKF plus 0.5 mM Quin into the shell portion of the ACB during three sessions. The first session was used to acclimate the rats to the test condition. In the first session, subjects were given the opportunity to self-infuse SKF+Quin with the FR1 schedule. During the second and third sessions, the effects of increasing the response requirements were evaluated; each rat was allowed to self-administer the SKF+Quin mixture with the FR1 and the FR3 schedules. The order of testing the FR1 and FR3 schedules was counterbalanced among subjects.

To evaluate the effects of the two different response requirements, we conducted a 2×2 ANOVA for responses on the two levers during the two response requirement schedules. Paired t tests were conducted to evaluate the effects of the two response requirements on infusions and the time to complete the session.

Experiment 4, effects of D_1 and D_2 antagonists on the self-administration of the SKF+Quin mixture into the ACB shell

Experimentally naive rats were prepared as described above. In session 1, the rats were given the opportunity to self-infuse 0.5 mM SKF plus 0.5 mM Quin into the shell region of the ACB; this session was used to acclimate the subjects to the general test condition described above. During sessions 2–4, subjects were given the opportunity to self-administer 0.5 mM SKF plus 0.5 mM Quin, 0.5 mM SKF plus 0.5 mM Quin containing 0.5 mM SCH 23390, a D_1 antagonist (SKF+Quin+SCH), and 0.5 mM SKF plus 0.5 mM Quin containing 0.5 mM sulpiride, a D_2 antagonist (SKF+Quin+Sul). The order of testing these three solutions was counterbalanced among subjects. To evaluate the effects of the D_1 and D_2 antagonists on the number of self-infusions of the 0.5 mM SKF plus 0.5 mM Quin mixture, a one-way within-subject design ANOVA was conducted for the three infusion solutions, followed by a Newman–Keuls *post hoc* test.

Histology

At the termination of each experiment, the animals were killed by CO_2 inhalation. Black India ink (0.5 μl) was injected into the infusion site; the brain was removed and frozen. The frozen brain was sliced into 40 μm sections, using a cryostat microtome. Sections were stained with cresyl violet.

RESULTS

Experiment 1, intra-ACB self-administration of D_1 and D_2 agonists: dose–response analyses

Figure 1 depicts injection sites with shell (*left*) and core (*right*) placements. Figure 2 provides photomicrographic evidence demonstrating placements within the shell and core of the ACB.

Figure 3 shows the number of infusions of SKF, Quin, and SKF+Quin, at concentrations of 0.0, 0.25, 0.50 and 1.0 mM, in the shell and core of the ACB. Rats reliably self-administered SKF+Quin into the shell region of the ACB, whereas the solutions of SKF or Quin alone were not reliably self-infused into the shell. In addition, the SKF+Quin mixture was not significantly self-administered into the core of the ACB.

Experiment 2, intrashell self-administration of D_1 and D_2 agonists: an interactive effect

Figure 4 shows a comparison of the number of infusions obtained during the first three sessions of groups of rats given SKF alone, Quin alone, or the mixture of SKF+Quin. The effects of giving the SKF and Quin groups the mixture of SKF+Quin in sessions 4 and 5 and of substituting vehicle for the mixture in the SKF+Quin group in session 4 are also shown in Figure 4. The mixture of SKF+Quin was much more effective in supporting ICSA behavior than was either compound alone. A between-subject design comparison revealed that the SKF+Quin group obtained more infusions than the SKF or Quin groups during the first three sessions [$F_{(2,20)} = 10.53$; $p < 0.001$]. A mixed ANOVA with the three groups between sessions 3 and 5 revealed a group $\times$ session interaction [$F_{(2,20)} = 4.32$; $p = 0.04$]. The SKF and Quin groups obtained more infusions in session 5 when the mixture of SKF+Quin was made available ($p = 0.01$), whereas the SKF+Quin group that obtained SKF+Quin in both sessions 3 and 5 maintained similar levels of infusions between the sessions.

The effect of substituting vehicle for the SKF+Quin mixture was evaluated in the SKF+Quin group by comparing the number of infusions in session 3 with the number obtained in session 4. The rats obtained much lower infusion levels when the SKF+Quin solution was replaced with vehicle [$t(5) = 2.92$; $p = 0.02$]. When the SKF+Quin mixture was reinstated in session 5, the rats again obtained higher levels of infusions compared with session 4 [$t(5) = 2.15$; $p = 0.04$].

Figure 5 shows the number of responses on the infusion and control levers by the SKF+Quin group during the first three

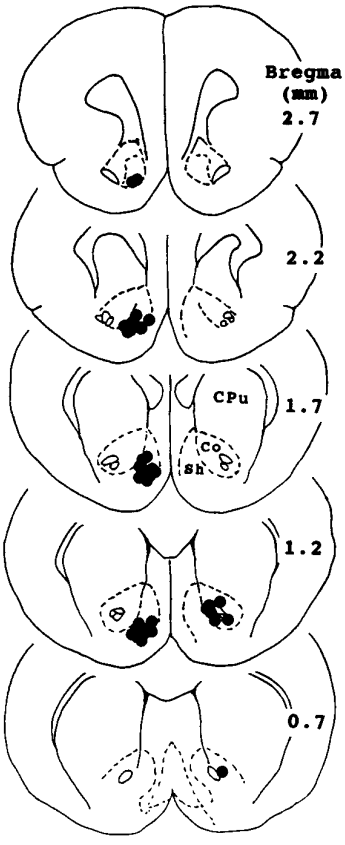

Figure 1. Injection placements in experiment 1. Cannula placements that were included for shell infusions were depicted on the *left*, whereas cannula placements included for core infusions were depicted on the *right*. The *numbers* on the *right* indicate distances (in millimeters) from bregma. The drawings are based on the rat brain atlas of Paxinos and Watson (1986), and the divisions between the shell and core are based on the study by Jongen-Relo et al. (1994). *Co*, Core; *CPu*, caudate putamen; *Sh*, shell.

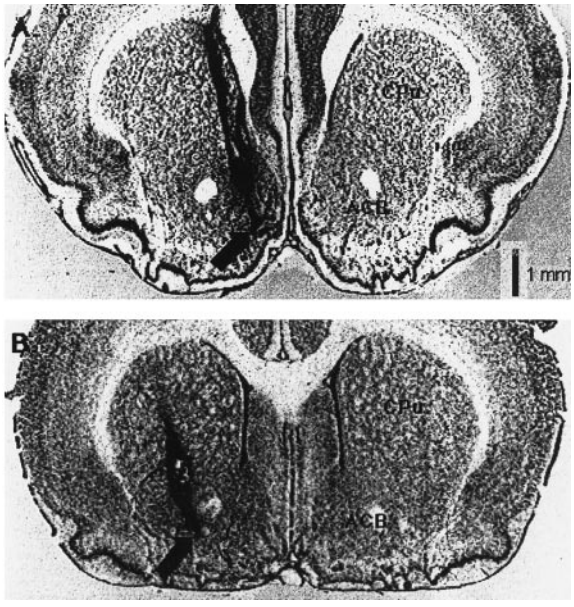

Figure 2. Photomicrographs showing a shell and core placement in the ACB. *A*, A typical shell placement that supported a high level of self-infusion of SKF+Quin. *B*, A core placement that supported a low level of self-infusion of SKF+Quin.

sessions when the SKF+Quin mixture was available, during session 4 when vehicle was substituted for the mixture, and after reinstatement of the mixture in session 5. When a within-subject design was used, rats exhibited a statistically reliable preference ($p = 0.01$) for the infusion lever over the control lever during the first three sessions. This difference was not evident when vehicle was substituted for the mixture in session 4.

Experiment 3, effects of increased lever–response requirements on the self-infusion of SKF+Quin into the ACB shell

Figure 6 illustrates the effects of increasing the response requirements from FR1 to FR3 on the number of responses on the infusion and control levers, on the number of infusions, and on the time needed to complete the test sessions. The two schedules had a differential effect on response levels with the two levers collapsed together ($p = 0.02$). In addition, rats responded more on the infusion lever than on the control lever [$F_{(1,9)} = 6.24$; $p = 0.03$]. The lever × schedule interaction was not reliable. The number of infusions delivered with the two different schedules was not different [$t(9) = 1.47$; $p = 0.2$]; the time needed to complete the sessions with either schedule was not significantly different [$t(9) = 1.90$; $p = 0.09$].

Experiment 4, effects of D_1 and D_2 antagonists on the self-administration of the SKF+Quin mixture into the ACB shell

Figure 7 shows the effects of including D_1 and D_2 antagonists in the infusate on the ICSA of the SKF+Quin solution into the ACB shell. Coadministration of either the D_1 antagonist SCH 23390 or the D_2 antagonist sulpiride abolished the intrashell self-administration of SKF+Quin [$F_{(2,17)} = 12.84$; $p = 0.002$].

DISCUSSION

The major findings of the present study suggest that concurrent activation of D_1- and D_2-type receptors in the shell, but not core, region of the ACB produces a cooperative effect on operant reinforcement behavior. This conclusion is supported by the findings that none of the individual concentrations of SKF and Quin were capable of supporting ICSA behavior in the ACB, whereas the combination of SKF plus Quin produced reliable self-infusions only in the shell portion of the ACB (Fig. 3). Furthermore, the low infusions obtained with either the D_1 or D_2 agonist alone were not caused by misplacement of the injection cannula because rats in the SKF and Quin groups exhibited heightened levels of infusions when given the SKF+Quin mixture (Fig. 4).

Interaction of D_1- and D_2-type receptors

The interaction of DA D_1- and D_2-type receptors has been reported on a variety of measures (see Clark and White, 1987; Waddington et al., 1994). The cooperative effect of the SKF+Quin mixture on intra-accumbens self-infusions is in general agreement with findings on motor activation produced by manipulations of ACB DA or DA receptors. In general, microinjection of either a D_1 or D_2 agonist alone into the ACB had little or no effect on motor activity, whereas concurrent injection of a D_1 and D_2 agonist into the ACB increased motor activity (Plaznik et al., 1989; Essman et al., 1993; Koshikawa et al., 1996a). The heightened locomotor activity produced by concurrent D_1 and D_2 agonists was attenuated by coadministration of either a D_1 or D_2 antagonist (Plaznik et al., 1989; Koshikawa et al., 1996a).

Coadministration of either the D_1 antagonist SCH 23390 or the D_2 antagonist sulpiride abolished the intrashell self-infusion of the mixture of D_1 and D_2 agonists (Fig. 7). These data support the idea that concurrent activation of D_1- and D_2-type receptors is involved in DA-mediated reinforcement processes within the ACB. Findings from the ICSA studies of Phillips et al. (1994) and the brain electrical self-stimulation experiments of Kurumiya and Nakajima (1988) and Nakajima (1989) are in agreement with this interpretation. Phillips et al. (1994) reported that coadministration of the D_1 antagonist SCH 23390 or the D_2 antagonist sulpiride decreased the rewarding effects of self-infusion of amphetamine into the ACB. Microinjection of either the D_2 antagonist raclopride or the D_1 antagonist SCH 23390 alone into the ACB diminished brain electrical self-stimulation behavior (Kurumiya and Nakajima, 1988; Nakajima, 1989).

Mechanisms underlying the cooperative effects of activating DA D_1 and D_2 receptors have not been clearly identified. One possible mechanism to explain the present results is that concurrent activation of both D_1 and D_2 receptors on certain populations of neurons may be needed to mediate reinforcement. There are subpopulations of ACB neurons that seem to contain both D_1- and D_2-type receptors (White and Wang, 1986; Le Moine and Bloch, 1996; Shetreat et al., 1996). In addition, there are some neurons in the ACB that are inhibited to a greater extent with simultaneous application of D_1 and D_2 agonists than with either agonist alone (White and Wang, 1986; White, 1987).

In the present study, both agonists need to be given to maintain reliable self-infusions because the endogenous extracellular levels of DA may be too low at key synapses to activate a sufficient number of D_1 receptors to enable D_2 receptors when only the D_2 agonist is available. A similar argument could be used for the low self-infusions of the D_1 agonist alone. Furthermore, intra-accumbens infusion of Quin is likely to reduce endogenous DA release by stimulating presynaptic D_2 autoreceptors (Imperato and Di Chiara, 1988).

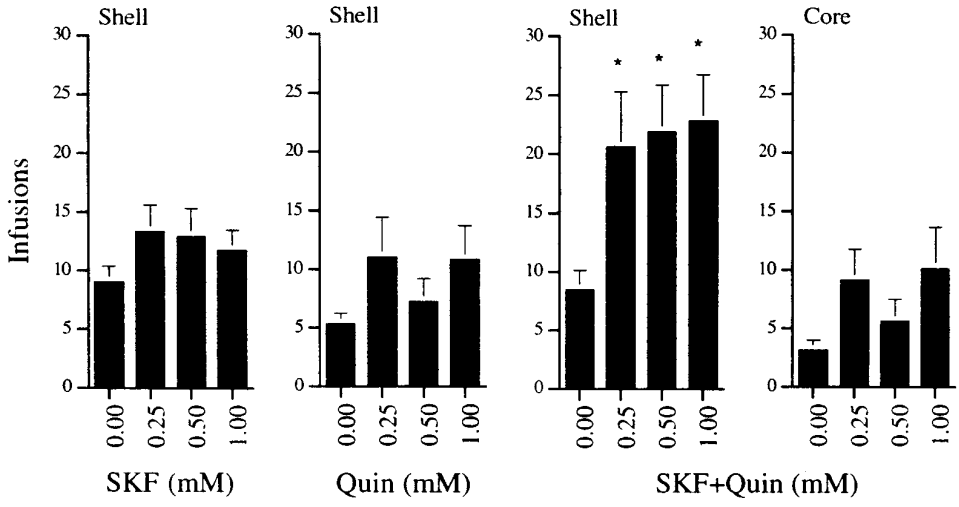

Figure 3. Intra-ACB self-administration of D_1 and D_2 agonists: dose–response analyses. Rats were assigned to one of three infusate groups: SKF, Quin, or SKF+Quin. During four sessions (3 hr/session), animals were given the opportunity to respond to vehicle and three concentrations (25, 50, and 100 mM) of one of the infusate types. One-way ANOVAs over four concentrations of infusion solutions revealed that the treatment of SKF alone [$n = 9$; $F_{(3,24)} = 2.50$] or Quin alone [$n = 10$; $F_{(3,27)} = 2.01$] into the shell or SKF+Quin into the core [$n = 7$; $F_{(3,18)} = 2.57$] did not produce a statistically reliable effect on infusions, whereas the treatment of SKF+Quin into the shell ($n = 9$) produced heightened levels of infusions [$F_{(3,24)} = 7.28$; $p = 0.001$]. *$p < 0.01$ compared with vehicle. Data are the mean ± SEM.

Contrary to the present findings, White et al. (1991) reported that injection of either a D_1 or D_2 agonist alone into the ACB produced place-preference conditioning. Some factors that could contribute to the differences between the two studies were the high doses used in the place-preference study and the types of behaviors being measured. White et al. (1991) injected nanomole quantities (2–10 nmol/0.5 μl) of SKF 38393 and quinpirole into the anterior and middle regions of the ACB, primarily within the shell, to produce conditioned place preference. This concentration is several-fold higher than the picomole amounts that were used in the present study. At these higher doses, SKF and Quin could be having nonselective effects. In the present experiments, the concentrations of the individual agonists seem to be high enough to produce a pharmacological effect. For example, the combination of 0.25 mM SKF plus 0.25 mM Quin produced significantly more self-infusions above vehicle than did either 1.0 mM SKF or 1.0 mM Quin alone (Fig. 3). If 0.25 mM agonist in the mixture is sufficient to produce self-administration behavior, then 1.0 mM agonist, when given alone, should also be effective, unless activation of both D_1 and D_2 receptors is required to maintain ICSA behavior.

A second explanation for the apparent contradictory findings of the study of White et al. (1991) and the present study is that different neural mechanisms may be regulating reinforcement measured with operant responding and reinforcement measured with place conditioning. In the case of the place-conditioning task, activation of only one type of DA receptor may be needed.

Nanomole amounts of amphetamine (Hoebel et al., 1983; Phillips et al., 1994) and nomifensine (Carlezon et al., 1995) were needed to support ICSA behavior, whereas, in the present study, the amounts of SKF and Quin required in the mixture to support ICSA were approximately 5–20-fold lower. The likely reason for this is that lower amounts of the direct-acting agonists are needed to produce effects similar to those caused by the indirect-acting DA agonists.

There are reports that rats and monkeys can maintain intravenous self-administration of either the D_1- or D_2-type agonist alone (e.g., Woolverton et al., 1984; Wise et al., 1990; Self and Stein, 1992; Grech et al., 1996). These findings suggest that activation of only one subtype of receptor may be needed to maintain DA-mediated reinforcement behavior. However, comparing results from systemic self-administration experiments with data obtained using the ICSA procedure is difficult, because any behavioral effect observed after systemic administration may be caused by a net effect produced by a compound acting at multiple CNS sites.

Functional dissociation of the shell and core

The present study found the shell region of the ACB supported the self-administration of the mixture of DA agonists, whereas

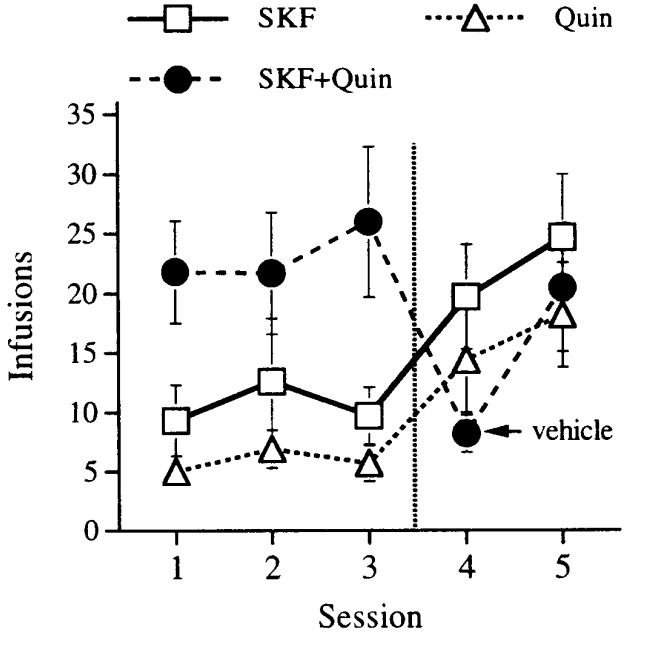

Figure 4. Intrashell self-administration of D_1 and D_2 agonists: an interactive effect. Rats were assigned to one of three groups. The SKF group ($n = 7$) received 0.5 mM SKF during the first three sessions and the mixture of 0.5 mM SKF plus 0.5 mM Quin (SKF+Quin) in sessions 4 and 5. Similarly, the Quin group ($n = 10$) received infusions of 0.5 mM Quin in sessions 1–3, followed by infusions of 0.5 mM SKF plus 0.5 mM Quin in sessions 4 and 5. The SKF+Quin group ($n = 6$) received infusions of 0.5 mM SKF plus 0.5 mM Quin in sessions 1–3 and 5; in session 4, only vehicle was available. During sessions 1–3, rats receiving SKF+Quin obtained more infusions than did rats receiving SKF or Quin alone ($p < 0.001$). The SKF and Quin groups exhibited higher levels of self-infusion in session 5, when SKF+Quin was given in place of SKF or Quin alone, than in session 3 ($p = 0.01$). The replacement of SKF+Quin with vehicle in the SKF+Quin group in session 4 diminished self-infusions ($p = 0.02$), whereas the group exhibited higher self-infusions in session 5 when SKF+Quin was reinstated ($p = 0.04$). Data are the mean ± SEM.

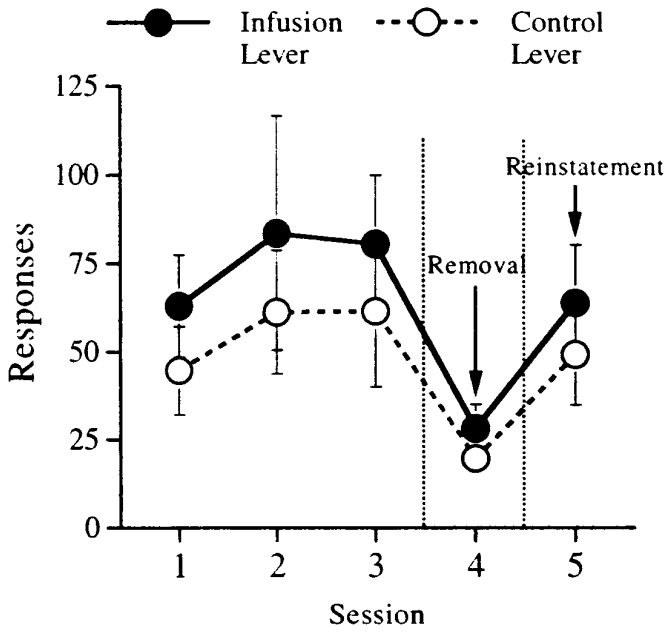

Figure 5. Lever responses by the SKF+Quin group during acquisition, extinction, and reinstatement sessions. Data are the mean ± SEM. A within-subject experimental design revealed that rats given 0.50 mM SKF plus 0.50 mM Quin showed reliable preference for the infusion lever over the control lever during the first three sessions [$F_{(1,3)} = 13.55$; $p = 0.01$]. It should be noted that the relatively large SEM values were mainly because of the variability among subjects. Within subjects, preference for the infusion lever over the control lever was consistent. The rats exhibited a reduction in lever responses when SKF+Quin was replaced with vehicle in session 4 [the main effect of schedules, $F_{(1,5)} = 7.31$; $p = 0.04$]. Although not statistically reliable, rats tended to increase lever responses when SKF+Quin was reinstated in session 5 [the main effect of schedules, $F_{(1,5)} = 4.61$; $p = 0.085$].

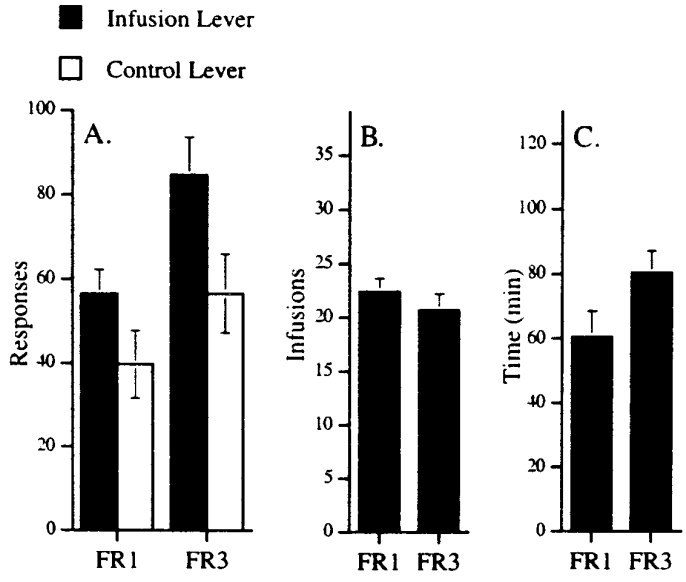

Figure 6. Effects of increased lever–response requirements on the self-infusion of the SKF+Quin mixture into the ACB shell. Rats ($n = 10$) were given the opportunity to self-administer the mixture of 0.5 mM SKF plus 0.5 mM Quin over two sessions. As shown in *A*, rats exhibited a higher level of lever responses under the FR3 schedule than under the FR1 schedule with the levers collapsed together [$F_{(1,9)} = 8.19$; $p = 0.02$]; rats also exhibited a reliable lever preference for the infusion lever over the control lever with the schedules collapsed together [$F_{(1,9)} = 6.24$; $p = 0.03$]; and the lever × FR schedule interaction was not reliable [$F_{(1,9)} = 2.56$]. There was no reliable difference between the schedules in the number of infusions [*B*; $t(9) = 1.47$] or in the time needed to complete the session [*C*; $t(9) = 1.90$]. Data are the mean ± SEM.

the core did not (Fig. 3). These results are in agreement with reports that rats self-administer nomifensine (Carlezon et al., 1995) and amphetamine (Hoebel et al., 1983) into the ACB shell region.

Contrary to the above findings, the study of Phillips et al. (1994) suggested that the core portion of the ACB may mediate amphetamine self-administration. However, their injection sites appeared to be near the boundary of the core and shell, and diffusion of the amphetamine into the shell region could account for their results.

The shell portion of the ACB receives its major DA input from the VTA and is considered to be involved in mediating motivated behaviors; the core receives significant DA inputs from the substantia nigra and is considered to be important in regulating motor activity (for review, see Kalivas et al., 1993). Thus, the present ICSA studies are consistent with an interpretation that activation of DA receptors within the shell portion of the ACB enhances goal-directed behavior.

Goal-directed effect of ACB infusions

One major concern of the ICSA paradigm is whether self-administration behavior is an artifact produced by an enhancement of general motor activity. Indeed, microinjection of DA agonists into the ACB has been shown to produce heightened locomotor activity (see references cited above). Disoriented motor arousal, however, does not explain the heightened infusions and responses observed in the present study. First, the levers were

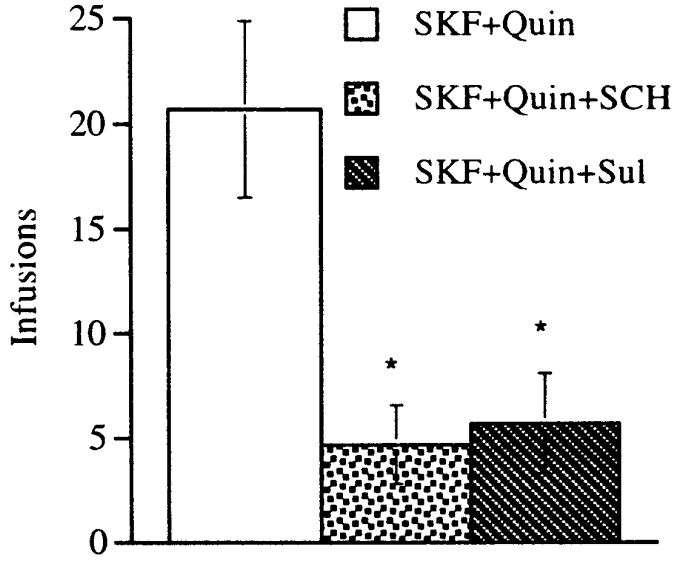

Figure 7. Effects of D_1 and D_2 antagonists on the intrashell self-administration of SKF+Quin. During three sessions, rats ($n = 6$) were given the opportunity to self-administer the mixture of 0.5 mM SKF plus 0.5 mM Quin (SKF+Quin) or the SKF+Quin mixture containing either 0.5 mM SCH 23390 (SKF+Quin+SCH) or 0.5 mM sulpiride (SKF+Quin+Sul). The presence of the D_1 antagonist SCH or the D_2 antagonist Sul significantly reduced intrashell self-infusion of SKF+Quin (*$p < 0.01$). Data are the mean ± SEM.

placed on a relatively high level from the floor; rats needed to rear to depress the lever. Thus, it seems unlikely that simply moving about the operant chamber could result in reliable high lever responding by many subjects across multiple sessions. Second, in experiment 2, the rats self-administrating the mixture of the D_1 and D_2 agonists exhibited a reliable preference for the lever producing infusions over the lever without consequence (Fig. 5). In addition, this preference cannot be explained by asymmetrical movements that unilateral infusions may have produced, because the position of the infusion lever between left and right was counterbalanced among subjects. Third, in experiment 3, rats exhibited an adaptive response when a higher response requirement was instituted. Enhanced lever responding was observed when the fixed-ratio requirement was increased from FR1 to FR3; no changes in infusion levels and the time to complete sessions were observed between the FR1 and FR3 schedules (Fig. 6). A reliable preference for the infusion lever over the control lever was observed under both schedules in this experiment. In summary, the results suggest a goal-directed effect of the combination of D_1 and D_2 agonists infused into the ACB.

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