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Neuropeptide Y: Intraaccumbens Injections Produce a Place Preference That Is Blocked by *cis*-Flupenthixol

SHEENA A. JOSSELYN AND RICHARD J. BENINGER[1]

Department of Psychology, Queen's University, Kingston, Ontario K7L 3N6, Canada

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JOSSELYN, S. A. AND R. J. BENINGER. *Neuropeptide Y: Intraaccumbens injections produce a place preference that is blocked by* cis-*flupenthixol.* PHARMACOL BIOCHEM BEHAV **46**(3) 543–552, 1993.—Neuropeptide Y (NPY) has been localized in the nucleus accumbens (NAcc), where it may influence dopamine (DA) neurotransmission. Extensive data implicate NAcc DA in reward-related learning, raising the possibility that NPY microinjected into the NAcc may induce rewarding effects mediated by DA. This hypothesis was tested using the conditioned place preference (CPP) paradigm. Each experiment consisted of three distinct phases: preconditioning (three 15-min exposures to an apparatus with two compartments connected by a tunnel); conditioning (four 30-min pairings of one compartment with drug and four similar pairings of the other compartment with vehicle); and test (three 15-min exposures to the apparatus). A significant increase in the time spent in the drug-paired compartment from preconditioning to test was taken as evidence of a CPP. Two experiments showed that systemic (2.0 mg/kg, IP) or intraaccumbens amphetamine (10.0 μg in 0.5 μl on each side) produced a CPP. The third experiment showed that intraaccumbens NPY (0.1 μg in 0.5 μl on each side) produced a CPP. This CPP was blocked by pretreatment with a dose of the DA receptor blocker *cis*-flupenthixol (20.0 μg in 0.5 μl on each side in the NAcc) that, alone, produced no CPP effect. These results strongly suggest that NPY applied to the NAcc is rewarding. In addition, these rewarding properties of NPY may be mediated by DA neurotransmission.

Neuropeptide Y Reward Dopamine Place conditioning Flupenthixol

NEUROPEPTIDE Y (NPY), originally isolated by Tatemoto and colleagues, is the most abundant of the neuropeptides (71, 73,74). As yet, no physiological function has been conclusively established for NPY, although its widespread distribution within both the central and peripheral nervous systems (1,2, 19,26,45) suggests that it may perform a regulatory role (3).

NPY may interact with the catecholamines. In the peripheral nervous system, NPY is colocalized with norepinephrine (NE) (45,46,54) and potentiates the postsynaptic response to NE while presynaptically inhibiting its release (45). In the brain, NPY is partially localized with NE and epinephrine (EPI) (2,26,48) and, further, the physiological effects of central NPY are comparable to some of the established consequences of central EPI (15,28,33) and NE (4,21,22,44,56,68). Thus, the peripheral and central effects of NPY appear to parallel those of the catecholamine with which it coexists.

Although immunocytochemical analyses have localized NPY-containing neurons in the nucleus accumbens (NAcc) and dorsal striatum (1,2,19,24–26), there is currently no confirmation of the coexistence of dopamine (DA) with NPY in these areas. Several lines of indirect evidence, however, lend support to the notion of an interaction between these two compounds. For example, synaptic contact between tyrosine hydroxylase (a marker for DA neurons)-positive and NPY-immunoreactive neurons has been visualized in the NAcc (7) and dorsal striatum (7,26,41). As well, biochemical studies have shown that NPY increases DA levels (10,34,75). These data provide some indication for a functional interaction between NPY and DA.

Recently, studies from our laboratory (53) examined the functional relationship between NPY and DA in the dorsal striatum using the circling paradigm. Lateralized imbalances in striatal DA activity produce circling behavior, rats typically rotating away from the striatum with higher activity (59). As expected, unilateral intrastriatal administration of amphetamine induced a contraversive circling bias. Similarly applied NPY also produced contraversive circling. Moreover, circling biases produced by amphetamine or NPY were antagonized by intrastriatal pretreatment with the DA receptor blocker, *cis*-flupenthixol. These data indicated that the functional activity of dorsal striatal NPY was dependent upon DA neurotransmission.

Results from the self-administration paradigm suggest that the mesolimbic DA system plays a critical role in the reward-

[1] To whom requests for reprints should be addressed.

ing properties of psychomotor stimulants (47,58,61). In addition, results accumulated from the conditioned place preference (CPP) procedure clearly indicate the critical role of NAcc DA in the neurochemical substrate of psychomotor stimulant reward (67). In one version of the CPP, two neutral environmentally distinct compartments are paired, one with drug and the second with vehicle treatment. When drug free, animals are subsequently presented with an opportunity to spend time in the presence of environmental cues previously associated with drug or vehicle injection. The drug treatment is inferred to be rewarding if the animal spends more time in the environment associated with drug treatment (37,55,66). Animals are tested in a drug-free state; hence, conclusions can be made regarding a drug's effects on reward-related learning avoiding the confounding effects of interfering drug actions on motor behavior. A second advantage is that preferences or aversions can be expressed, as animals will approach appetitive stimuli and avoid aversive stimuli (38).

As NPY has been localized in the NAcc and may have an action complimentary to DA, the predominant catecholamine of this region, the following experiments were designed to assess the potentially rewarding properties of NPY using the CPP paradigm. The first and second experiments showed that amphetamine induced a place preference when administered either systemically or into the NAcc; the third experiment showed that NPY would induce a similar place preference and evaluated the effects of the DA receptor blocker *cis*-flupenthixol on this preference.

METHOD

This research has been done with due regard for the Animals for Research Act, the Guidelines of the Canadian Council on Animal Care, and relevant University policy. The Queen's University Animal Care Committee has reviewed and approved this protocol.

Animals

Eighty male albino Wistar rats (Charles River, Canada) were individually housed in hanging wire cages and maintained in a controlled environment on a 12 L : 12 D cycle (light on at 0600 h) at a temperature of 21°C. Food and water were continuously available in the home cages. Animals weighed between 300 and 450 g when the experiment commenced.

Surgeries

Those animals requiring surgery were prepared with chronic indwelling cannulae. Rats were anesthetized with sodium pentobarbital (65 mg/ml; 1 ml/kg body weight, IP) and stereotaxically implanted with bilateral stainless steel cannulae (0.64 mm in diameter) aimed at the center of the NAcc. Coordinates of the guide cannulae tips were 1.7 mm anterior (A) to bregma, 1.5 mm lateral (L) to the midline, and 5.6 mm ventral (V) to the surface of the skull, with the incisor bar set at 3.2 mm below the horizontal plane passing through the interaural line (57). The cannulae were anchored to the skull with stainless steel screws and dental cement. When not in use, the guide cannulae were occluded with wire pins.

Central Injections

A Hamilton microsyringe (10.0 μl) (Hamilton Co., Reno, NV) mounted in an infusion pump (Sage Instrument Pump Model 355) was used to infuse the drug at a constant rate of 1.0 μl/min. The volume of all injections, including vehicle, was 0.5 μl. Injection cannulae, made of stainless steel tubing (0.31 mm in diameter), were cut to extend 1.0 mm beyond the tips of the guide cannulae and were attached to the microsyringe by polyethylene tubing. To ensure diffusion of the drug, the injection cannulae were maintained in position for an additional 1 min following injection.

Drugs

Porcine NPY (Sigma Chemical Co., St. Louis, MO) was dissolved in double-distilled water, while (+)-amphetamine (Smith, Kline & French, Canada) and *cis*-flupenthixol (H. Lundbeck, Copenhagen, Denmark) were dissolved in normal saline. All drugs were prepared daily.

Apparatus

Four similar shuttleboxes consisted of two conditioning compartments (38 × 27 × 36 cm) that were connected by a tunnel (8 × 8 × 8 cm), which could be closed by means of guillotine-type doors. In each shuttlebox, one conditioning compartment had urethane-sealed wooden walls while the second had walls covered with black-and-white vertical stripes (1 cm width). The floors of these compartments were either wire grid (1.0 × 1.0 cm) or wire rod (1.0 cm apart). The wall and floor patterns were arranged in such a way that each shuttlebox had a unique configuration. Six infrared beams, two located in each conditioning compartment (height 5 cm) and one at each end of the tunnel (height 3 cm), were connected to a single-board computer that recorded the number of beam breaks and amount of time spent in each area of the shuttlebox.

General Experimental Procedure

Behavioral evaluation was carried out in three distinct phases, taking place over 14 consecutive days.

Preconditioning. Rats were familiarized with the apparatus for 15 min on 3 consecutive days. Rats were placed in a particular conditioning compartment (designated the start compartment) and allowed free access to the entire shuttlebox (guillotine doors removed). The designation of the start compartment was counterbalanced across rats, that is, one half of the rats was started in one of the compartments and the other half in the other compartment. The amount of time spent in each compartment was recorded as a baseline measure of unconditioned initial preference.

Conditioning. During eight daily 30-min sessions, vehicle injections were paired with animals' start compartment (on days 2, 4, 6, and 8) and drug injections were paired with animals' nonstart compartment (days 1, 3, 5, and 7). Animals received injections of vehicle or drug and were confined to the designated compartment by guillotine doors, blocking the entrance to the tunnel.

Test. During three daily 15-min sessions, animals' preference for each conditioning compartment again was measured. No injections preceded these sessions. Animals were introduced into the shuttlebox in the start compartment, with the guillotine barriers removed. The time spent in each conditioning compartment was recorded.

Specific Experimental Procedures

Three experiments were conducted. In a replication of previous studies (16,20,30,38,39,52,70), amphetamine (2.0 mg/

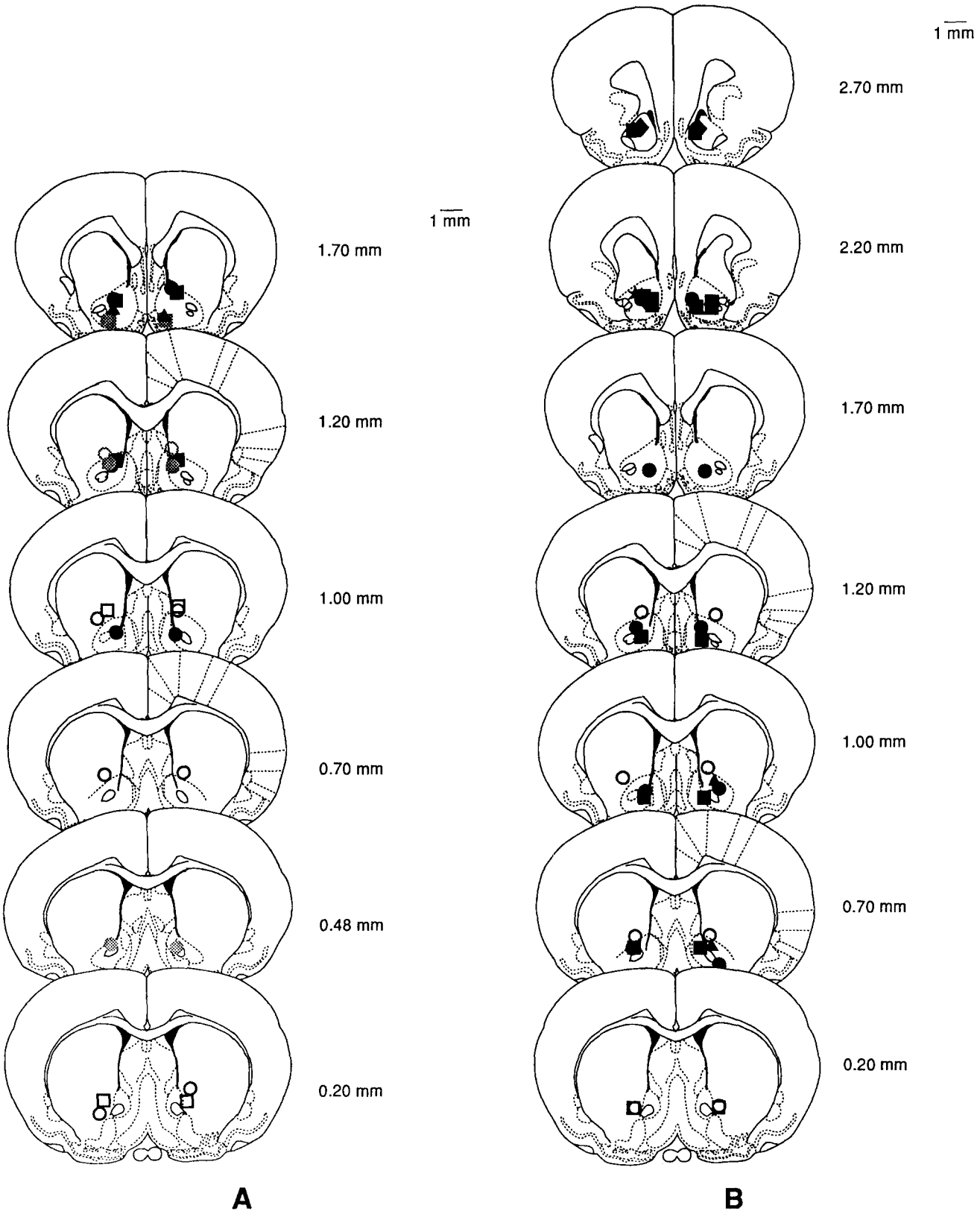
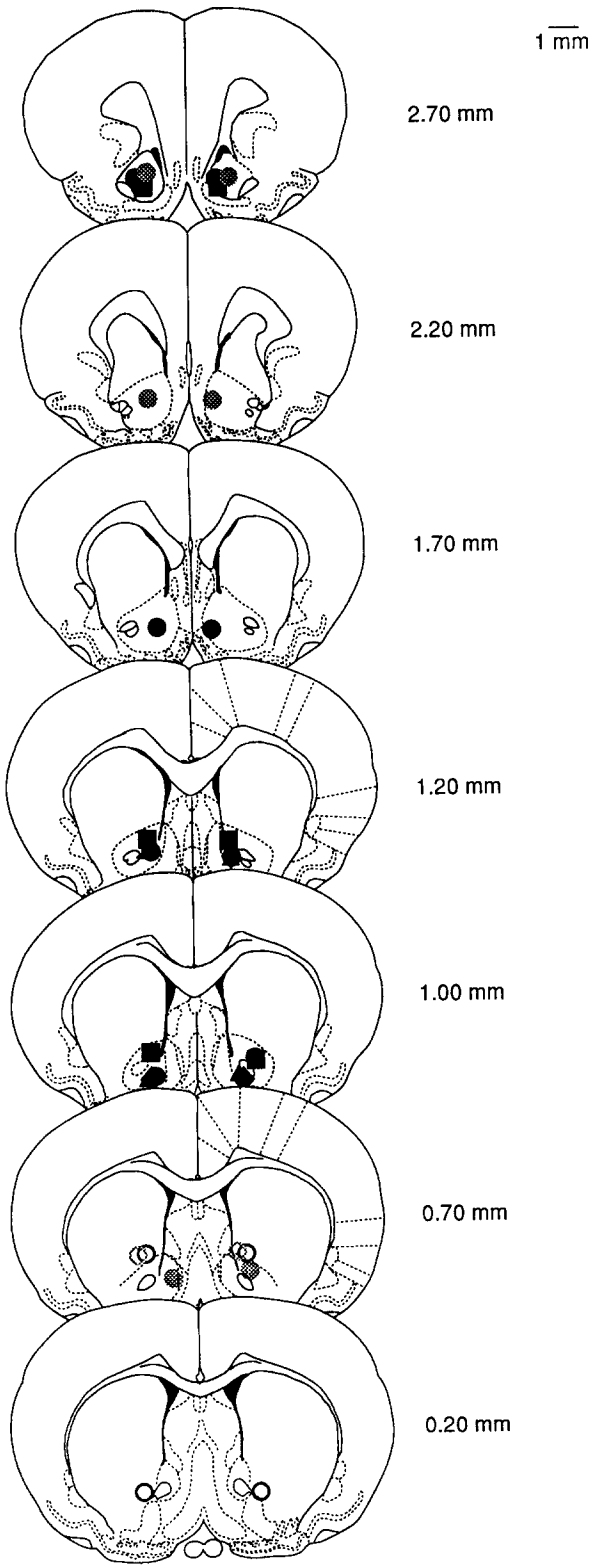


FIG. 1. Location of intracerebral injection sites. The position of the cannulae aimed at the N. Acc. for experiments 2 (intra-accumbens saline or amphetamine) and groups 2 (saline + NPY), 3 (*cis*-flupenthixol + saline) and 4 (*cis*-flupenthixol and NPY) from experiment 3 are shown in A, B, C, and D, respectively. Figure 1C also shows the 4 animals from experiments 3 that received saline + saline injections. Coronal sections represented are adapted from the atlas of Ref 57. The anterior-posterior co-ordinates (relative to Bregma) are located to the right of the sections. Open symbols represent misses, closed symbols represent hits, solid symbols represent drug animals and stippled symbols represent vehicle animals. Each animal was implanted with two cannulae and the shape of the symbols (triangle, circle, square, etc.) indicates the pairs of placements for a particular animal. A representative brain section showing cannulae tracts and nucleus accumbens placements is shown in E.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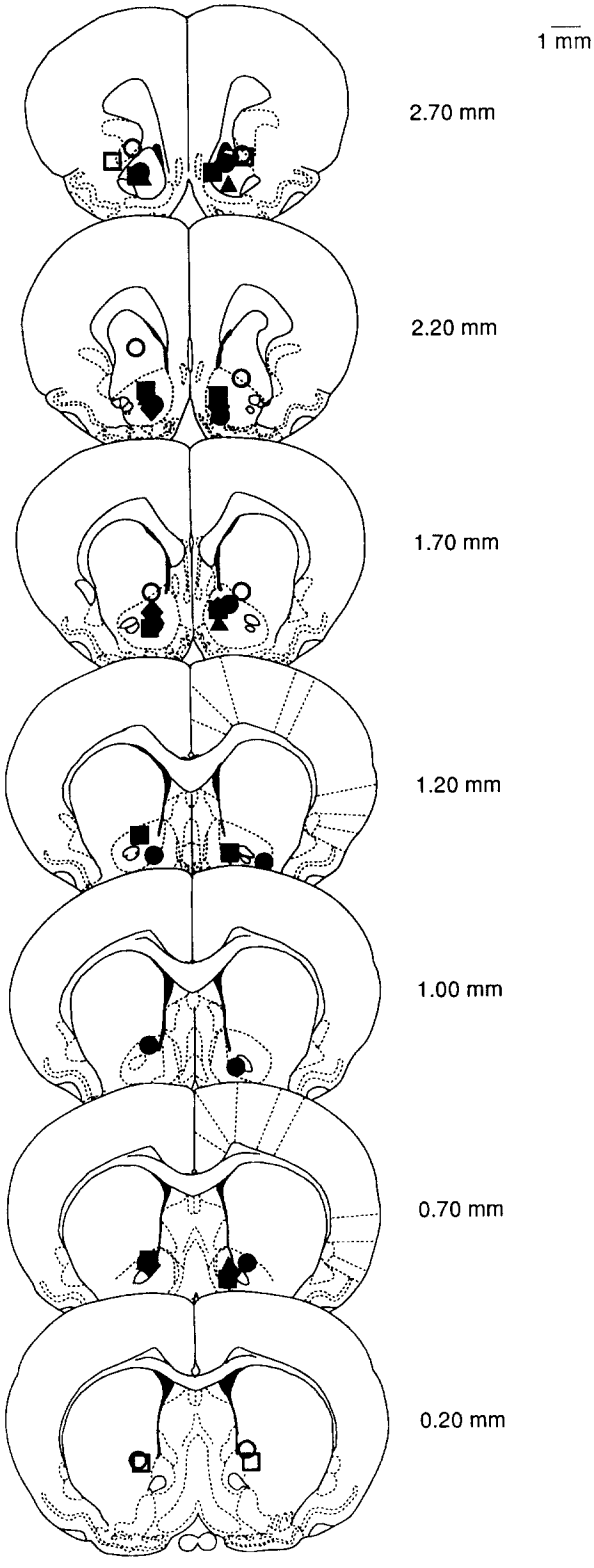
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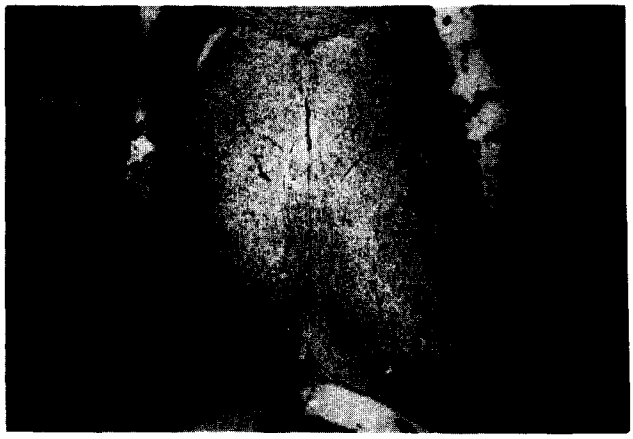

FIG. 1. Cont.

kg, IP) was administered systemically to unoperated animals (n = 8) while control animals (n = 4) received similarly administered saline in Experiment 1. This experiment was included to confirm that systemic amphetamine produces a CPP.

Experiment 2 examined whether bilateral microinjections of amphetamine (10.0 μg/side) or saline (0.5 μl/side) into the NAcc of rats (ns = 12 and 4, respectively) would yield a CPP. On nondrug days (days 2, 4, 6, and 8), animals in both groups received intraaccumbens saline (0.5 μl).

Experiment 3 evaluated whether NPY (0.1 μg per side), injected into the NAcc, would induce a CPP and whether this effect could be blocked by the DA antagonist, *cis*-flupenthixol (20.0 μg/side). The choice of doses of NPY and *cis*-flupenthixol was guided by the results of Moore et al. (53), who reported that 0.1 μg NPY in the dorsal striatum produced the strongest contraversive circling bias and a dose of 20.0 μg *cis*-flupenthixol blocked the NPY effect while manifesting no significant effect when applied on its own. Experiment 3 included four groups. On drug days, all rats received two intraaccumbens injections separated by a 15-min interval: group 1 (n = 4), saline + saline; group 2 (n = 24), saline + NPY; group 3 (n = 12), *cis*-flupenthixol + saline; and group 4 (n = 24), *cis*-flupenthixol + NPY. On nondrug days, all groups received two intraaccumbens injections of saline (0.5 μl) separated by 15 min.

Histology

Upon completion of the experiment, confirmation of the cannulae placements was accomplished through histological analysis. Animals were given a lethal injection of sodium pentobarbital (60 mg/ml; 1.0 ml, IP) and infused through the heart with saline followed by a solution of 4% formalin. Brains were removed, frozen, and sliced in 50-μm sections that were mounted and stained. Rats were included in the statistical analyses if both cannulae tips were within the boundaries of the NAcc (coordinates were: A 1.7–2.7 mm, V 6.0–7.5 mm, and L 1.0–2.0 mm).

Statistical Analyses

Statistical analyses were carried out according to Keppel (40), using the BMDP 4V software package. Time spent in the drug-paired compartment over the 3 preconditioning days was compared to the time spent in this compartment over the 3 test days. An increase in the amount of time spent in the drug-paired compartment of the shuttlebox from the preconditioning to the test phase was taken as evidence for a CPP. Thus, analyses of variance (ANOVAs) with two repeated measures—phase (pre conditioning vs. test) and day (days 1 vs. 2 vs. 3)—were performed. Establishment of a CPP would be statistically reflected in a significant main effect of, or interaction involving, phase. Posthoc Dunnett's tests, if warranted, compared each test day to the mean of the preconditioning days to determine which test day exhibited the greatest effect.

RESULTS

Histology

Figure 1 displays the cannulae placements for Experiments 2 and 3. Results obtained from improperly cannulated animals (see below) were discarded. One control animal from Experi-

TABLE 1

SUMMARY OF MEAN TIME IN DRUG-PAIRED COMPARTMENT DURING PRECONDITIONING AND TEST PHASES FOR SALINE ANIMALS IN ALL EXPERIMENTS

		Time in Drug-Paired Compartment (seconds)					
		Phase					
		Preconditioning			Test		
Exp.	Name	P1	P2	P3	T1	T2	T3
1	IP AMPH (n = 3)	384.0 (20.6)*	322.3 (23.3)	344.3 (75.9)	347.7 (65.2)	328.7 (97.3)	241.0 (38.5)
2	NAcc AMPH (n = 3)	485.0 (58.0)	363.0 (68.9)	435.0 (7.5)	320.3 (92.1)	261.7 (54.3)	291.3 (66.1)
3	NAcc NPY (n = 3)	442.3 (9.3)	446.7 (41.9)	505.0 (117.8)	441.3 (92.7)	393.0 (131.6)	334.7 (106.7)

AMPH, amphetamine.
*Numbers in parentheses are SEMs.

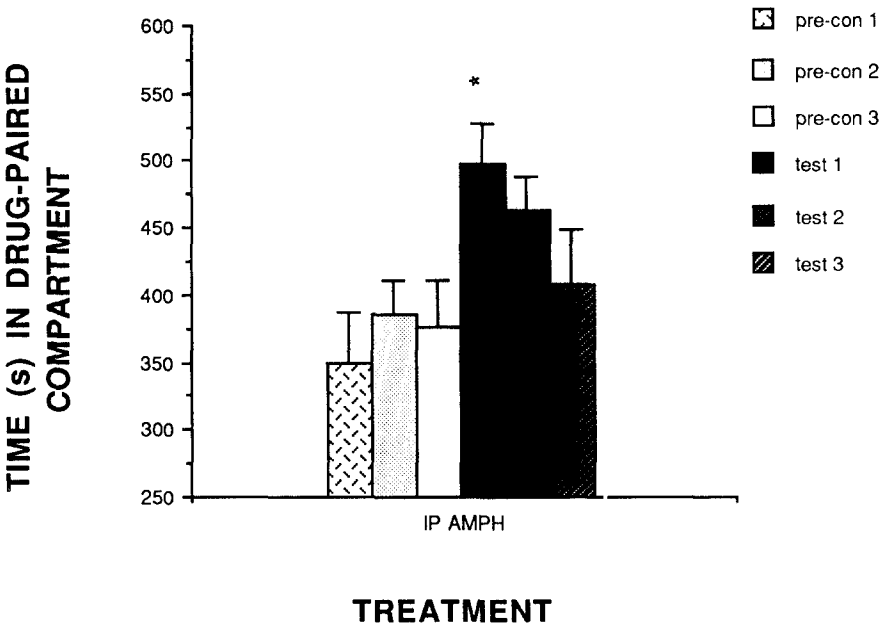

FIG. 2. Mean (+SEM) time (s) spent in the drug-paired compartment during 3 preconditioning (pre-con) and 3 test days for rats treated with amphetamine (AMPH: 2.0 mg/kg, ip). Rats spent significantly more time in the drug-paired compartment on the first test day. $*p < .05$.

ment 1 did not complete the experiment due to illness. Of the 16 animals implanted with cannulae in Experiment 2, 3 saline control and 7 amphetamine animals had placements in the target region (see Fig. 1A). Of the 4, 24, 12, and 24 rats implanted with cannulae in the four groups tested in Experiment 3, the number of animals with cannulae placements in the target region was 3, 19, 10, and 18, respectively (see Figs. 1B–1D). A photograph of a brain section showing the cannu-

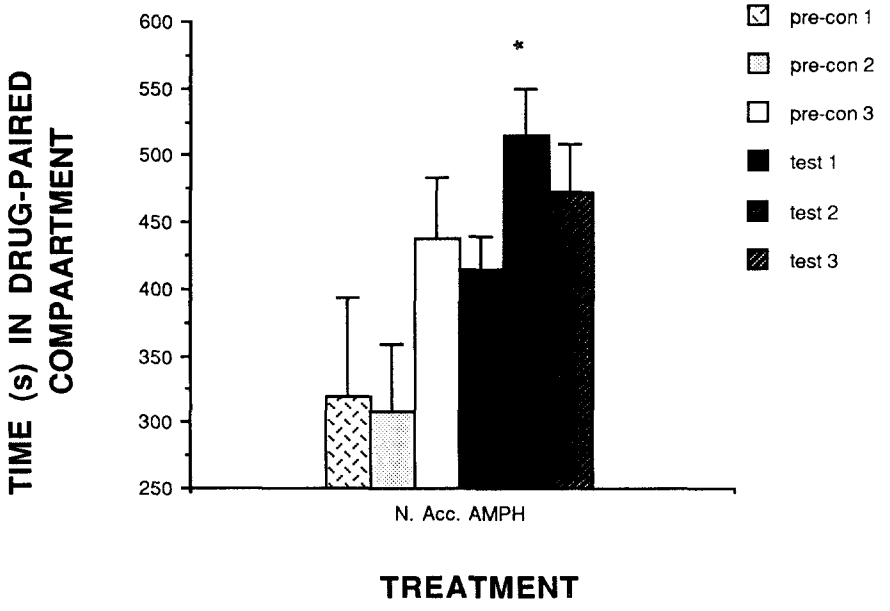

FIG. 3. Mean (+SEM) time (s) spent in the drug-paired compartment during 3 preconditioning (pre-con) and 3 test days for rats treated with amphetamine (AMPH: 1.0 μg in 0.5 μl bilaterally in the nucleus accumbens [N. Acc.]). Rats spent significantly more time in the drug-paired compartment on the second test day. $*p < .05$.

lae tracts and nucleus accumbens placements is presented in Fig. 1E.

Saline Groups

Saline administration in each experiment differed slightly. In Experiment 1 (n = 3) the saline was administered IP, in Experiment 2 (n = 3) saline was centrally administered (0.5 μl), while in the third experiment (n = 3) saline (0.5 μl) was centrally administered twice with a 15-min interval between injections. The saline data were analyzed to determine if saline administration had an effect on place preference. The mean (+ SEM) time (seconds) spent in the nominal drug-paired compartment for each experiment can be seen in Table 1. (In fact, animals received saline injections on both sides.) There appeared to be a decrease in the amount of time spent in the so-called drug-paired compartment from the preconditioning to the test phase. However, the results of an ANOVA with two within factors, phase and day, revealed no significant main effects or interaction. Thus, saline did not condition a place preference.

Experiment 1: Systemic Administration of Amphetamine

Figure 2 shows the mean (+ SEM) time (seconds) spent in the drug-paired compartment over the 3 preconditioning and test days for animals receiving systemic amphetamine. Amphetamine produced a CPP, as animals spent more time in the drug-paired compartment during the test phase.

The statistical evaluation of the results yielded a significant phase × day interaction, $F(2, 14) = 4.89$, $p < 0.05$, and a significant main effect of phase, $F(1, 7) = 8.23$, $p < 0.05$. To isolate the source of the two-way interaction, one-way ANOVAs were conducted on each phase; the variable day was significant in the test phase only, $F(2, 6) = 3.57$, $p < 0.05$. A Dunnett's posthoc test comparing the time spent in the drug-paired compartment to the mean of the preconditioning days revealed that rats spent significantly more time in the drug-paired compartment on the first test day.

Experiment 2: Intraaccumbens Amphetamine

Figure 3 illustrates the mean (+ SEM) amount of time (seconds) spent in the drug-paired compartment over the 3 preconditioning and test days for animals receiving NAcc amphetamine. Although there was considerable variability from day to day in both phases, intraaccumbens amphetamine appeared to increase time on the drug-paired side from phase to phase, indicating a CPP. A two-factor ANOVA revealed a significant main effect of phase, $F(2, 12) = 3.84$, $p < 0.05$, supporting this description of the data. The interaction of phase with day approached significance, $F(2, 12) = 3.84$, $p < 0.052$. Posthoc Dunnett's analysis comparing the test days to the mean of the preconditioning days revealed that on the second test day animals spent significantly more time in the drug-paired side. Thus, amphetamine, applied to the NAcc, produced a CPP.

Experiment 3: Intraaccumbens NPY

Figure 4 illustrates the mean (+ SEM) amount of time (seconds) spent in the drug-paired compartment over the preconditioning and test days. Saline followed by NPY produced a CPP; this CPP was blocked by pretreatment with a dose of *cis*-flupenthixol that on its own produced no CPP effect.

Two-factor ANOVAs conducted separately on each group revealed no significant interactions or main effects in the *cis*-

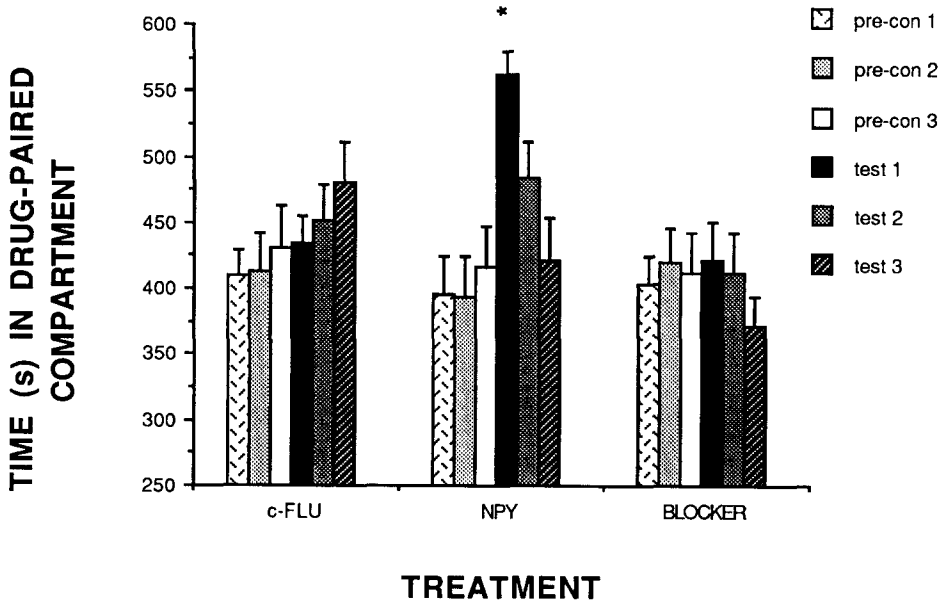

FIG. 4. Mean (+SEM) time (s) spent in the drug-paired compartment during 3 pre-conditioning (pre-con) and 3 test days for rats treated with (a) *cis*-flupenthixol (c-FLU; 20.0 μg in 0.5 μl, bilaterally in the nucleus accumbens) + saline (0.5 μl bilaterally in the nucleus accumbens), (b) saline + neuropeptide Y (NPY; 0.1 μg in 0.5 μl, bilaterally in the nucleus accumbens) (c) *cis*-flupenthixol + NPY (BLOCKER). Rats spent significantly more time in the drug-paired compartment during the first day of test in the saline + NPY condition. *$p < .05$.

flupenthixol + saline or *cis*-flupenthixol + NPY groups. However, a significant interaction of phase with day, $F(2, 26) = 4.38$, $p < 0.05$, as well as a main effect of phase, $F(1, 13) = 7.40$, $p < 0.05$, was noted in the saline + NPY group. This interaction was further analyzed and the simple main effect of day was found to be significant in the test phase only, $F(2, 12) = 7.98$, $p < 0.05$. Posthoc Dunnett's analysis comparing the test days to the mean of the preconditioning days revealed that animals spent significantly more time in the drug-paired side on the first test day.

DISCUSSION

The present experiments demonstrated that both systemic and intraaccumbens amphetamine and intraaccumbens NPY generated a CPP. In general, the greatest preference was exhibited on the first test day, followed by a steady decline. This pattern has been described in previous studies using this paradigm (9,20,38,52,62) and may reflect an extinction-like process. None of the three small subgroups receiving systemic ($n = 3$) or nucleus accumbens ($n = 6$) saline showed an increase in time spent on the nominal drug-paired side and the change in time from the preconditioning to the test phase was not statistically significant. Thus, although the saline control subgroups were small their combined results suggest the conclusion that the CPP observed with amphetamine or NPY resulted from some action of the drugs.

The demonstration of place conditioning produced by systemic amphetamine is in agreement with several previous reports (16,30,38,39,43,49,67,70). The finding that intraaccumbens amphetamine produced a CPP in rats replicates the results of Carr and White (17,18) and Hiroi and White (35). These researchers examined a number of anatomic sites in the brain and found only the NAcc to be active. This suggests that our results can be attributed to the action of amphetamine in the NAcc rather than more dorsal structures that may have been influenced by the spread of amphetamine along the cannulae tracts. Thus, dopaminergic agents, whether administered peripherally or centrally, produce a CPP. Hoebel et al. (36) and Lenard et al. (42) reported that rats would self-administer dopaminergic compounds directly into the NAcc. Place preferences conditioned by peripheral amphetamine were blocked by 6-hydroxydopamine (6-OHDA) lesions of the NAcc (66). Microinjections of the D_2 agonist, quinpirole, directly into the NAcc produced a CPP (78). Together, these findings emphasize the critical role of the DA projections to the nucleus accumbens in mediating the rewarding effects of psychomotor stimulants.

The finding that *cis*-flupenthixol alone had no significant effect on place preference replicates earlier findings using systemic *cis*-flupenthixol (49) and other DA antagonists such as haloperidol (51,67), pimozide (14,67), and SCH23390 (39) [but, see (63,64)]. Thus, while an increase in mesolimbic DA seems to produce a place preference, decreases in DA are not necessarily reflected in conditioned place aversions.

The finding that intraaccumbens NPY produced a CPP has not been reported previously. The present study included only one dose of NPY; it will be the task of future studies to examine the effects of a range of doses of NPY. The blockade of the CPP effect by pretreatment with *cis*-flupenthixol is consistent with the notion that DA neurotransmission mediated the rewarding effect of NPY. Further, the results are congruous with those reported by Aulisi and Hoebel (8) and Hiroi and White (35), who found the CPP generated by intraaccumbens amphetamine was attenuated by adding *cis*-flupenthixol to the injection fluid.

The observation that the behavioral consequence of NPY could be blocked by a DA antagonist is in accordance with previous studies from this laboratory. Moore et al. (53) found that NPY produced contraversive circling when unilaterally applied to the dorsal striatum, an outcome also induced by amphetamine. Further, these effects were both blocked by intrastriatal pretreatment with *cis*-flupenthixol. Collectively, these studies suggest that NPY, in the dorsal or ventral striatum, requires intact DA neurotransmission for manifestation of some of its behavioral consequences.

It is not possible to identify the mechanism by which NPY produces DA release from the present or previous (10,34,75) studies. Whether NPY produces DA release via an action at the NPY receptor or via some other mechanism awaits further study with NPY receptor antagonists. Such studies may eliminate the possibility that NPY is producing its effects on DA release via nonpharmacological actions (e.g., osmolarity, pH) not examined in the present study.

It is interesting that other endogenous nonopiate neuropeptides also have been characterized as rewarding based upon the results of the CPP paradigm. Neurotensin, microinjected into the ventral tegmental area (VTA), produces a CPP (31, 32). Further, neurotensin produces excitatory effects on the neurophysiological activity of DA neurons (6) and has been localized in the VTA (60,74). Thus, both NPY in the ventral striatum and neurotensin in the VTA produce reward, presumably through a dopaminergic action. It would be interesting to examine whether NPY or neurotensin are capable of supporting self-administration. It has been postulated that the CPP and self-administration paradigms measure analogous drug properties (37,69). It therefore would be predicted that animals could be trained to self-inject these neuropeptides. The peptidergic involvement in reward processes clearly illustrates the important role of neuropeptides and further broadens the possible modulatory actions of neuropeptides.

NPY is localized, along with somatostatin, in some medium aspiny neurons in the striatum (12,29,65,76). Recent evidence has shown that the somatostatin–NPY neurons are unique in that they are selectively spared in Huntington's disease (1,5,11,23,27), unlike a wide spectrum of other neurotransmitters (13). Specifically, Dawburn et al. (23) reported the density of NPY cells to be increased threefold in the caudate of patients with Huntington's disease. Clinical observations suggest an exacerbation of choreic symptoms with DA (50) and the present results provide a neuropeptide mechanism for this exacerbation. It is possible that the selective sparing of the NPY- containing neurons of the striatum could partially account for the choreic symptoms of this disease, as the present results suggest that NPY enhances DA function. In conclusion, the evidence obtained from the present experiments suggests that NPY produces reward-related learning. Further, the results suggest that the functions of the DA and NPY systems in the NAcc may be intimately entwined.

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