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Research report

Effects of selective dopamine D1 or D2 receptor blockade within nucleus accumbens subregions on ingestive behavior and associated motor activity

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Abstract

Two anatomically and neurochemically distinguishable regions of the nucleus accumbens (Acb), the core and the shell, have been shown to differentially regulate feeding behavior. Nevertheless, despite the well-known role of Acb dopamine in the modulation of motivated behaviors, there have been no studies directly comparing the effects of acute dopamine receptor blockade in the Acb core versus the Acb shell on feeding. In this study, D1- or D2-selective dopamine receptor antagonists were infused bilaterally into the Acb core or shell of hungry rats, whereupon feeding, drinking, and spontaneous motor activity were monitored. Both the D1 antagonist SCH 23390 (0, 1, and 2 μg/0.5 μl) and the D2 antagonist raclopride (0, 1, and 2 μg/0.5 μl) markedly suppressed ambulation and rearing when infused into either the Acb core or shell. Total food intake and latency to begin feeding were unaffected by either drug in either site. SCH 23390 in the Acb shell, and raclopride in the Acb core or shell, significantly decreased the total number of feeding bouts. In the Acb core, raclopride produced a small but statistically significant increase in overall feeding duration. Dopamine receptor blockade in either site tended to increase mean feeding bout duration. Measures of drinking behavior were generally unaffected. Hence, dopamine receptor blockade in either the Acb core or shell of hungry rats suppressed spontaneous motor activity and shifted the structure of feeding towards longer bout durations, but did not alter the total amount of food consumed. In the Acb shell, the effects of D1 receptor blockade tended to be of greater magnitude than the effects of D2 receptor blockade, although major differences between core and shell effects were not observed. These results are discussed with regard to current theories of dopaminergic control of feeding behavior, and with reference to the functional heterogeneity of Acb subregions.

Keywords: Nucleus accumbens shell; Nucleus accumbens core; SCH 23390; Raclopride; Dopamine D1 receptor; Dopamine D2 receptor; Feeding; Motor activity

1. Introduction

It has long been known that large 6-hydroxydopamine (6-OHDA)-induced lesions of the ascending dopaminergic pathways or administration of high doses of dopamine receptor antagonists produce, among other deficits, profound aphagia (e.g. [11,20,27,33]); these observations have recently been confirmed using gene knockout technology [42]. Several early studies identified nucleus accumbens (Acb) as an important anatomical substrate for the dopaminergic modulation of feeding behavior [12,18,22], although the precise nature of this modulation has remained a source of debate for many years.

Detailed analyses of the pattern of behavioral changes produced by restricted 6-OHDA-induced lesions or dopamine receptor antagonism in the Acb revealed that these treatments either did not affect or increased food intake, while decreasing adjunctive motor behavior or motorically demanding food-reinforced instrumental behaviors [1,2,18,28]. These observations supported the notion that dopamine in Acb may be involved in the motivational activation and motor output associated with the proximity of or imminent access to food, rather

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than with the rewarding aspects of the consummatory act (see also [6,7]). Some support for this idea came from neurochemical studies showing increased extracellular Acb dopamine levels preceding food-reinforced lever presses [17], after presentation of a conditioned stimulus for food delivery [25], or during food presentation schedules accompanied by high levels of motor activation [21]. Nevertheless, other experiments indicated that the largest increases in extracellular levels of Acb dopamine occurred during feeding, rather than during the presentation of stimuli predicting food delivery [14,26,36], even when these stimuli elicited "sniffing, locomotion, and rearing" (see [36]).

One factor that might contribute to these seemingly discrepant findings is that the Acb might contain functionally heterogeneous zones that are involved preferentially in either the anticipatory or consummatory aspects of feeding. In the past several years, it has been proposed that the Acb can be divided into anatomically and neurochemically distinguishable territories (for reviews, see [8,39]). Two of these regions, the core and the shell, have been shown to be differentially involved in feeding behavior. For example, infusion of glutamate receptor antagonists or gamma-amino butyric acid (GABA) agonists into the Acb shell but not the Acb core produced intense hyperphagia [19,30]. Moreover, and particularly germane to the discrepancies among the neurochemical studies cited above, a recent microdialysis study showed that extracellular dopamine levels were dramatically elevated in the Acb shell, and to a much lesser extent in the Acb core, during first-time consumption of a highly palatable food [3]. In contrast, presentation of conditioned stimuli associated with the palatable food (conditioned stimuli that elicited "orienting and approach ... sniffing and licking") increased dopamine efflux in the Acb core but not the shell [3]. Hence, the Acb core and shell may be recruited to different degrees in the various stages of feeding behavior.

Given these recent findings, one might predict that the effects of dopamine receptor blockade on feeding and associated motor activity might be different in the Acb core than in the Acb shell. Somewhat surprisingly, there have been no studies directly comparing the feeding- and activity-related effects of dopamine antagonist infusions into these two structures. To address this question, subtype-selective dopamine receptor antagonists were infused into the Acb core or shell of hungry rats, and spontaneous motor activity and ingestive behavior were monitored. We had reason to suspect that subtype-selective dopamine antagonists might have different effects in the two Acb subregions based on our earlier finding that a D1-selective agonist produced a stronger enhancement of motor activity when infused into the Acb shell versus the Acb core [32]. Of particular interest in the present study was the question of whether the previously observed profile of dopamine antagonist effects in the Acb (i.e. strong suppressive effects on motor activity, little or no effect on food intake, and a shift of the structure of feeding towards fewer but longer feeding bouts—see [2]) would be reproduced in both Acb subregions.

2. Methods

2.1. Subjects

Subjects in all experiments were male Sprague–Dawley rats, obtained from Harlan (Indianapolis), weighing 260–280 g upon arrival at the laboratory. The rats were housed in clear polycarbonate cages (9.5 in. width × 17 in. length × 8 in. height), with cob bedding, in a light- and temperature-controlled vivarium. Animals were maintained under a 12 h light/dark cycle (lights on at 07:00 AM). Food and water were available ad libitum, except during the drug-testing phase of the experiment. Animals were handled daily to reduce stress.

All facilities and procedures were in accordance with the guidelines regarding animal use and care put forth by the National Institutes of Health, were supervised and approved by the Institutional Animal Care and Use Committee of the University of Wisconsin.

2.2. Surgery

Rats (weighing 330–370 g at the time of surgery) were anesthetized with a xylazine/ketamine mixture (13 mg/kg xylazine, 87 mg/kg ketamine; Research Biochemicals International, Natick, MA) injected intraperitoneally. Animals were then secured in a Kopf stereotaxic frame, and 10 mm bilateral stainless steel cannulae cut from 23 gauge stainless steel tubing (Small Parts, Inc., Miami Lakes, FL) were implanted according to standard stereotaxic surgical procedures. Cannulae were aimed at either the core or shell subregion of the Acb. For the core placements, the coordinates were A-P: 1.4 mm anterior to bregma, M-L: ±1.8 mm, D-V: −5.5 mm from skull surface; the toothbar was set at −4 mm below interaural zero. For the shell placements, the coordinates were A-P: 3.1 mm anterior to bregma, M-L: ±1.0 mm, D-V: −5.3 mm from skull surface; the toothbar was set at 5 mm above interaural zero. Cannulae were fixed in place using self-curing dental acrylic (New Truliner, Bosworth Co., Skokie, IL) and three anchoring stainless steel screws (Plastics One, Inc., Roanoke, VA). Wire stylets (10 mm long, 30 gauge) were placed in the cannulae to prevent blockage. After surgery, each animal was given an intramuscular injection of penicillin (0.3 ml of a 300,000 unit/ml suspension; Phoenix Pharmaceuticals, St. Joseph, MO), and placed

in a warm recovery cage. Upon awakening, rats were returned to their home cages and given a recovery period of no less than 10 days (with daily health checks) before behavioral testing commenced.

2.3. Microinfusion and behavioral testing procedures

For intracerebral microinfusions, rats were held gently, and the wire stylets were removed from the guide cannulae. Stainless steel injectors, fashioned from 30 gauge stainless steel tubing, were lowered to the site of infusion; injectors protruded 2.5 mm below the tips of the guide cannulae. Thus, the final D-V coordinate for Acb core microinfusions was −8.2 mm from the skull surface, and the final D-V coordinate for Acb shell microinfusions was −7.8 mm from the skull surface. Injectors were attached to 10 μl capacity glass Hamilton syringes (Hamilton Co., Reno, NV) with polyethylene tubing (PE-10, Becton Dickinson and Co., Sparks, MD). The Hamilton syringes were attached to a Harvard microdrive pump. The rate of infusion was 0.32 μl/min; the total infusate volume for all experiments was 0.5 μl/side. After the infusion, the injectors were left in place for 1 min to allow the infusate to diffuse away from the injector tip and penetrate into the tissue. All infusions were made bilaterally.

Behavioral testing was carried out in clear polycarbonate cages (9.5 in. width × 17 in. length × 8 in. height) with wire grid floors. A pre-weighed quantity of food (standard rat chow pellets) was placed on the cage floors, and water was available from an overhead bottle. A sheet of paper was placed underneath each testing cage to collect food spillage.

On testing days, animals were moved from the vivarium into the testing room, given an infusion of a D1 or D2 receptor antagonist (see below), and returned to their home cages. Ten minutes after the infusion, animals were placed into the testing cages for 30 min. During that time, the rats' behavior was monitored and recorded by an experimenter blind to treatment. The behaviors monitored were: *crossovers*, defined as ambulations across the center of the cage; *rears*, *feeding*, and *drinking*. These parameters were recorded using a switch box connected to a microprocessor (Paul Fray Ltd., Cambridge, UK). Using this system, the experimenter monitored the frequency and duration for all behaviors except for crossovers, for which only frequency was recorded. From the frequency and duration data, the mean duration of each rearing, feeding, or drinking bout was calculated. Data for the 30 min testing session were recorded in three 10 min time bins. At the conclusion of the session, uneaten food and food spillage was weighed and recorded.

2.4. Drugs

The D1 receptor antagonist SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride) and the D2 receptor antagonist raclopride ((*S*)-3,5-dichloro-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2-hydroxy-6-methoxy-benzamide-L-tartrate) were obtained from Research Biochemicals International, Natick, MA. The drugs were dissolved in sterile 0.9% saline and stored in 150 μl aliquots at −20 °C.

2.5. Experimental design

Four experiments were carried out, each in a separate group of rats. In the first two experiments, the behavioral effects of SCH 23390 ($n = 7$) or raclopride ($n = 7$) infusion into the Acb core were tested; in the second two experiments, the effects of SCH 23390 ($n = 8$) or raclopride ($n = 8$) infusion into the Acb shell were explored.

In all experiments, rats were habituated to the injection and testing procedures before drug testing began. First, rats received two "mock" infusions on two sequential days, in which injectors that did not protrude beyond the ends of the guide cannulae were lowered, and animals were placed into the testing cages for 30 min. The day after the second mock infusion, animals received an intracerebral saline infusion, and were then placed into the testing cages for 30 min.

The day after the saline infusion, drug testing commenced. Rats were subjected to intracerebral injections of either SCH 23390 or raclopride (0, 1, and 2 μg/side) administered in counterbalanced orders according to Latin square designs. Each drug dose was tested on a separate day; drug-testing days were separated from each other by at least one treatment-free day. Each rat in an experiment received all drug doses.

During the drug-testing phase of the experiment, rats were maintained at 80% of their free-feeding weight with a food restriction regimen (15 g/rat per day of standard laboratory chow). Water was available ad libitum during all stages of the experiments.

2.6. Histology

Rats were deeply anesthetized with sodium pentobarbital and perfused transcardially with a 0.9% saline solution followed by a solution of 10% formalin in phosphate buffer (Sigma Diagnostics, St. Louis, MO). Brains were removed and stored in 10% formalin. The brains were then cut on a cryostat microtome; 60 μm sections were taken through the injection site. Brain slices were mounted on slides, stained with cresyl violet, and subsequently reviewed to verify correct placement of the intracerebral injections. Images of representative

sections from each experiment were captured using Scion Image software on a computer interfaced with a microscope-mounted Hitachi HV-C20 CCD camera.

2.7. Statistical analyses

Scores for crossovers, rears, feeding bouts, feeding duration, and mean feeding bout duration were analyzed in several ways (each of these behavioral measures was analyzed separately). First, data were analyzed with two-factor repeated-measures ANOVAs (dose × time). There were three levels for the dose factor (corresponding to the three drug doses) and three levels for the time factor (corresponding to the three 10 min time bins of the 30 min testing sessions). Each of the four experiments (i.e. SCH 23390-core, raclopride-core, SCH 23390-shell, raclopride-shell) was analyzed separately. Following significant dose × time interactions, data were further analyzed for simple effects with two-factor ANOVAs (dose × time). Pre-planned contrasts (0 μg versus 1 μg, 0 μg versus 2 μg) were used for specific comparisons among means on data collapsed across the time factor. For the drinking measures, scores were summed across the three 10 min time bins of the 30 min testing sessions, and subjected to one-factor (dose) repeated-measures ANOVAs. Pre-planned contrasts (see above) were used for specific comparisons among means. Latency scores were also analyzed with one-factor (dose) repeated-measures ANOVAs and pre-planned contrasts.

3. Results

3.1. Spontaneous motor behavior (crossovers and rears)

3.1.1. Nucleus Acb core

Both SCH 23390 and raclopride significantly decreased crossovers when infused into the core subregion of the nucleus Acb (main effects for the dose factor; SCH 23390: $F(2,12) = 8.36$, $P < 0.006$; raclopride: $F(2,12) = 16.39$, $P < 0.0005$; see Fig. 1). Pre-planned contrasts revealed that both doses of both drugs differed significantly from their respective vehicle controls (for alpha values, see legend for Fig. 1). There was a dose × time interaction for the raclopride experiment ($F(4,24) = 8.69$, $P < 0.0003$); two-factor ANOVAs for simple effects revealed dose × time interactions for comparisons between the effects of vehicle and the 1 μg raclopride dose ($F(2,24) = 11.81$, $P < 0.003$) and between the effects of vehicle and the 2 μg raclopride dose ($F(2,24) = 13.83$, $P < 0.002$). As can be seen in Fig. 1, both doses of raclopride depressed locomotor activity throughout the 30 min testing session relative to vehicle, although the effects were most pronounced at the 10 and 30 min time points.

The effects of intra-Acb core infusions of SCH 23390 or raclopride on rearing behavior were similar to the effects of those two drugs on ambulatory activity (see Fig. 2). Both drugs reduced the total number of rears in the 30 min testing session (main effect of dose; SCH 23390: $F(2,12) = 12.25$, $P < 0.002$, raclopride: $F(2,12) = 7.02$, $P < 0.01$). Pre-planned contrasts indicated that the effects of both doses of SCH 23390, and the effects of both doses of raclopride, differed significantly from their respective vehicle controls (for alpha values, see legend for Fig. 1). In the raclopride experiment, there was a significant dose × time interaction ($F(4,24) = 3.45$, $P < 0.03$); two-factor ANOVAs for simple effects revealed a dose × time interaction for the comparison between the effect of vehicle and the effect of both raclopride doses (vehicle versus 1 μg: $F(2,24) = 6.09$, $P < 0.03$; vehicle versus 2 μg: $F(2,24) = 3.66$, $P < 0.05$). As shown in Fig. 2, raclopride-induced depression of rearing behavior was most evident in the first 10 min of the testing session, where the vehicle-associated mean was considerably higher than means associated with both raclopride doses. At the 20 and 30 min time points, the effects of vehicle and 1 μg were similar, whereas the means associated with the 2 μg/side dose remained lower than the vehicle-associated means.

3.1.2. Nucleus Acb shell

As in the Acb core, infusion of SCH 23390 or raclopride into the Acb shell significantly decreased crossovers. For both drugs, there was a main effect of dose (SCH 23390: $F(2,14) = 26.06$, $P < 0.0002$; raclopride: $F(2,14) = 3.89$, $P < 0.05$). Pre-planned contrasts on data collapsed across the time factor indicated that the effects of both doses of both drugs differed significantly from their respective vehicle controls (for alpha values, see legend for Fig. 2). There were dose × time interactions for both drugs (SCH 23390: $F(4,28) = 9.53$, $P < 0.0002$; raclopride: $F(4,28) = 7.01$, $P < 0.0006$). Two-factor ANOVAs for simple effects yielded dose × time interactions on comparisons between both doses and vehicle, for both SCH 23390 (vehicle versus 1 μg: $F(2,28) = 13.96$, $P < 0.001$; vehicle versus 2 μg: $F(2,28) = 14.58$, $P < 0.001$) and raclopride (vehicle versus 1 μg: $F(2,28) = 12.36$, $P < 0.002$; vehicle versus 2 μg: $F(2,28) = 7.81$, $P < 0.02$). Fig. 1 shows that for both SCH 23390 and raclopride, vehicle-associated means for crossovers were higher in the beginning of the testing session and declined over time, whereas drug-associated values remained low throughout the testing session.

For rearing behavior, there was a main effect of dose in the SCH 23390 experiment ($F(2,14) = 7.24$, $P < 0.007$); however, for the raclopride experiment, the main effect of dose did not quite reach statistical significance ($F(2,14) = 3.51$, $P < 0.06$). Pre-planned contrasts on data collapsed across the time factor revealed that both doses of SCH 23390 significantly depressed

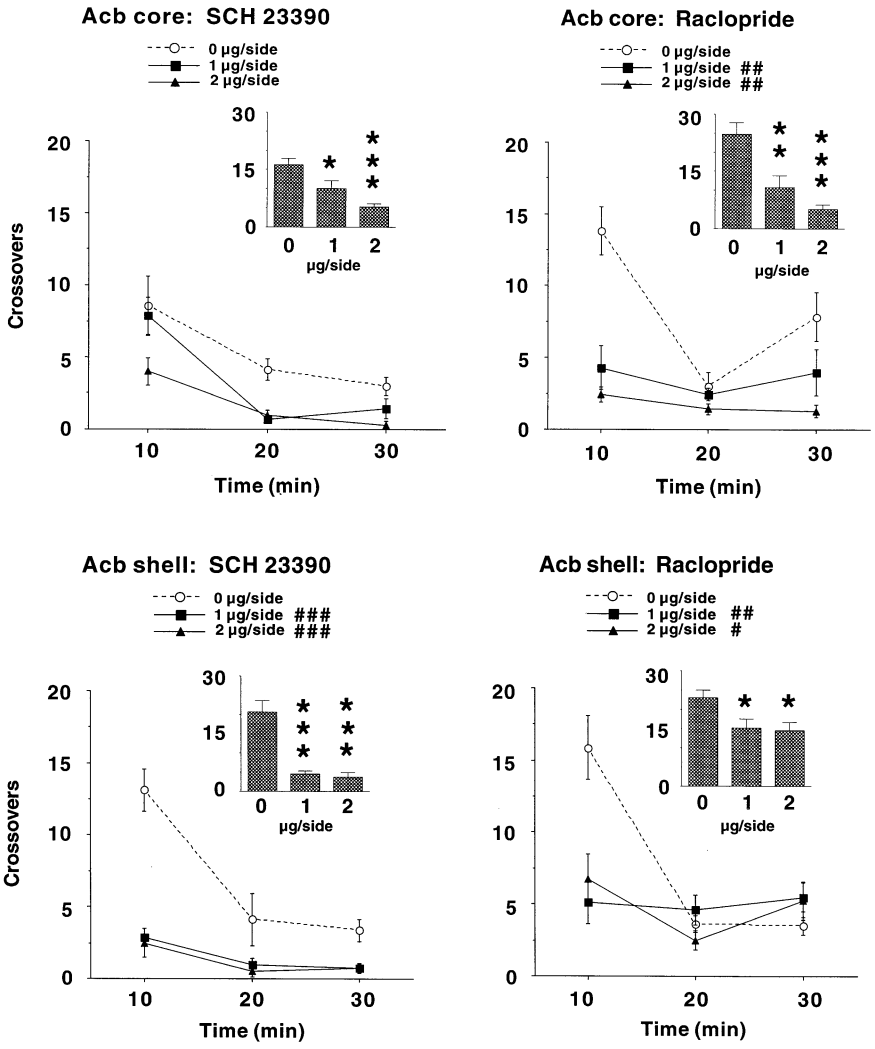

Fig. 1. Effects of infusions of SCH 23390 or raclopride into the nucleus Acb core or shell on ambulatory activity (crossovers). Main graphs represent the time course of activity over the 30 min testing session presented in 10 min time bins. Insets represent activity summed over the 30 min testing session. Error bars represent one S.E.M. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, different from 0 µg/kg on data collapsed across the 30 min testing session. $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$, dose × time interaction when compared to 0 µg/side (see Section 2).

rearing behavior (for alpha values, see legend for Fig. 2). There was a dose × time interaction only in the raclopride experiment ($F(4,28) = 5.95$, $P < 0.002$). Analyses for simple effects revealed significant dose × time interactions (vehicle versus 1 µg: $F(2,28) = 9.64$, $P < 0.005$; vehicle versus 2 µg: $F(2,28) = 7.82$, $P < 0.006$). As can be seen in Fig. 2, vehicle-associated means were higher than drug-associated means in the first 10 min of the testing session, and then decline towards the drug-associated values over the time course of testing.

3.2. Feeding behavior

In contrast to the clear exploratory activity-suppressing effects of SCH 23390 and raclopride described above, neither drug affected total food intake when infused into either the core or shell subregion of the Acb (F: 0.15–1.25, n.s.; see Fig. 3).

3.2.1. Nucleus Acb core

When infused into the Acb core, neither SCH 23390 ($F(2,12) = 0.20$, n.s.) nor raclopride ($F(2,12) = 0.63$, n.s.) influenced latency to begin feeding (see Table 1). Nevertheless, the two drugs had distinguishable effects on the number of feeding bouts and the total duration of feeding in the 30 min testing session. As summarized in Table 1, whereas there was no statistically significant effect of dose or dose × time interaction of SCH 23390 on those two measures (F: 0.54–2.86, n.s.), raclopride significantly decreased the number of feeding bouts and increased total feeding duration (main effect of dose, feeding bouts: $F(2,12) = 28.49$, $P < 0.0002$; feeding duration: $F(2,12) = 8.76$, $P < 0.005$). Pre-planned contrasts revealed that for both these behavioral measures, the effects of both doses of raclopride differed significantly from vehicle control (for alpha values, see legend for Table 1).

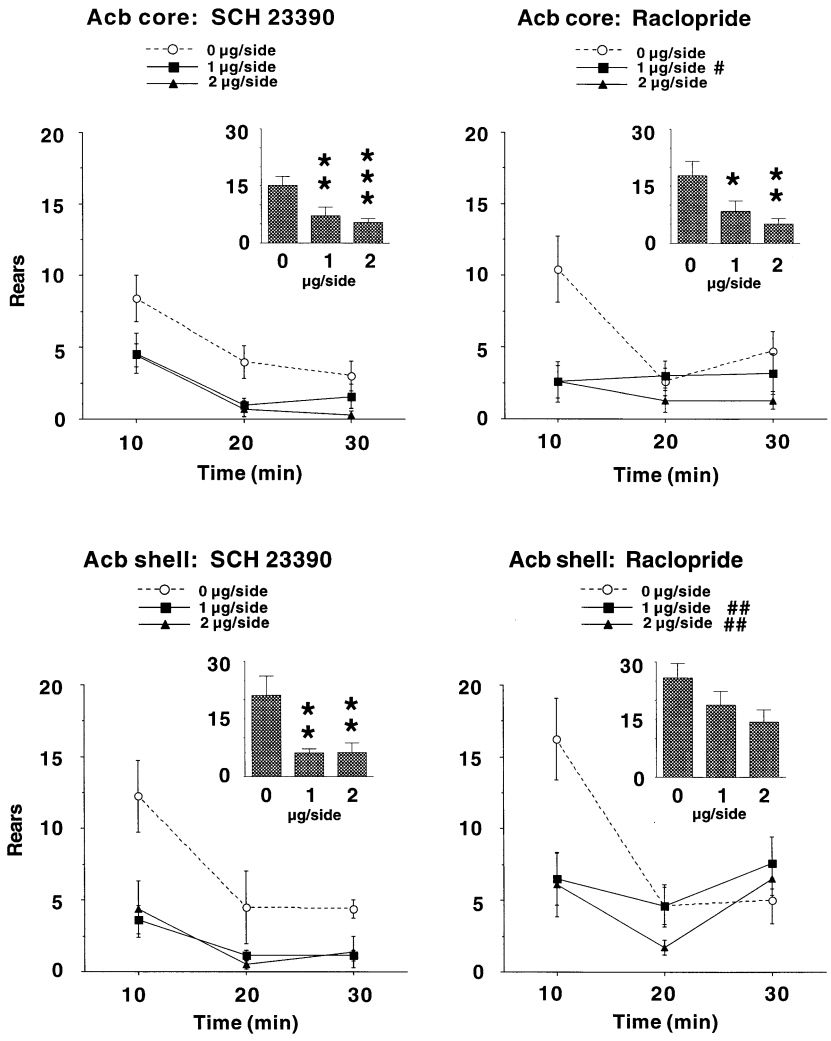

Fig. 2. Effects of infusions of SCH 23390 or raclopride into the nucleus Acb core or shell on rearing behavior. Main graphs represent the time course of rearing behavior over the 30 min testing session presented in 10 min time bins. Insets represent activity summed over the 30 min testing session. Error bars represent one S.E.M. $*P<0.05$, $**P<0.01$, $***P<0.001$, different from 0 µg/kg on data collapsed across the 30 min testing session. $\#P<0.05$, $\#\#P<0.01$, dose × time interaction when compared with 0 µg/side (see Section 2).

Additionally, in the raclopride experiment, there was a dose × time interaction for feeding bouts ($F(4,24)=6.00$, $P<0.002$); analyses for simple effects yielded significant dose × time interactions for the comparison between the effects vehicle and the 1 µg dose ($F(2,24)=10.27$, $P<0.005$), and the comparison between the effects of vehicle and the 2 µg dose ($F(2,24)=7.26$, $P<0.01$). In the first 10 min of the testing session, vehicle-associated means were higher than drug-associated means, and then decline towards the drug-associated values over the remainder of the testing session (time course data not shown).

As shown in Fig. 4, raclopride significantly increased mean feeding bout duration (main effect of dose: $F(2,12)=5.01$, $P<0.03$); however, the effect of SCH 23390 did not quite achieve statistical significance ($F(2,12)=3.68$, $P<0.06$). Pre-planned contrasts indicated that the effect of the 2 µg/side raclopride dose differed significantly from the vehicle control. As can be seen in Fig. 4, bout sizes tended to be largest in the second 10 min time bin of the 30 min testing sessions, after the rats switched from active exploration of the testing environment to feeding behavior, but before they approached satiety.

3.2.2. Nucleus Acb shell

Neither SCH 23390 nor raclopride affected feeding latency when infused into the Acb shell (F=1.05–3.09, n.s.; see Table 1); however, both drugs significantly decreased the number of feeding bouts when injected into this structure (main effect of dose, SCH 23390: $F(2,14)=62.27$, $P<0.0002$; raclopride: $F(2,14)=3.99$, $P<0.05$; see Table 1). Pre-planned contrasts on data collapsed across the time factor indicated that the effects of both doses of both drugs on feeding bouts differed significantly from their respective vehicle controls (for

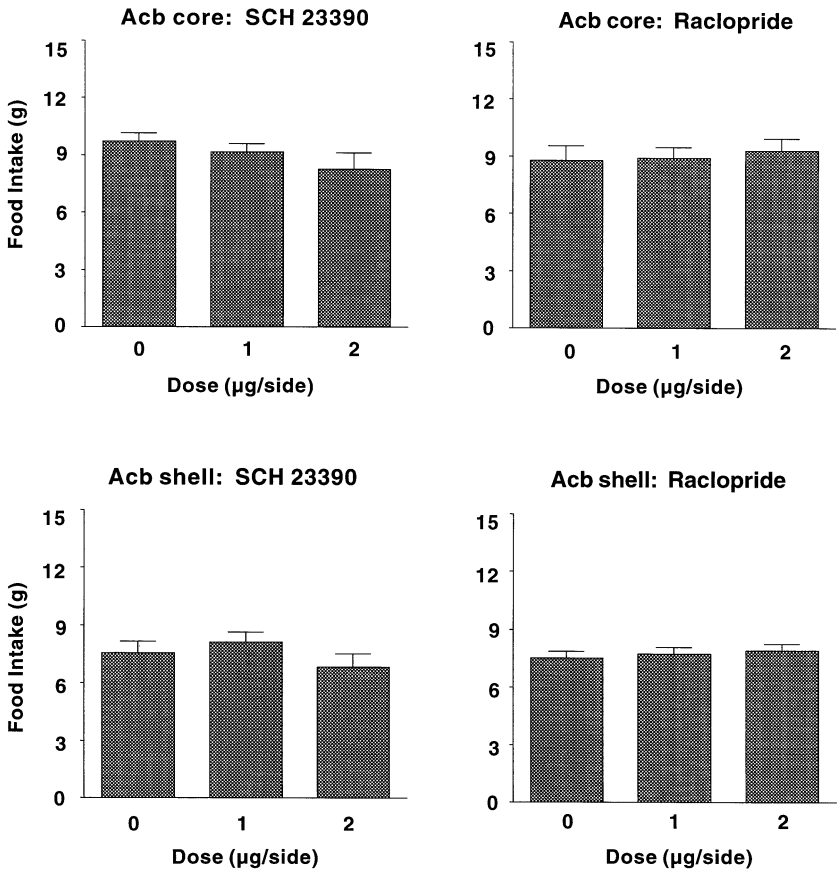

Fig. 3. Effects of infusions of SCH 23390 or raclopride into the nucleus Acb core or shell on total food intake in the 30 min testing sessions. Error bars represent one S.E.M.

alpha values, see legend for Table 1). For both drugs, there were dose × time interactions for feeding bouts (SCH 23390: $F(4,28) = 12.12$, $P < 0.0002$; raclopride: $F(4,28) = 8.45$, $P < 0.0002$). Analyses for simple effects revealed significant dose × time interactions for both SCH 23390 (vehicle versus 1 µg: $F(2,28) = 15.90$, $P < 0.001$; vehicle versus 2 µg: $F(2,28) = 20.20$, $P < 0.0003$) and raclopride (vehicle versus 1 µg: $F(2,28) = 12.32$, $P < 0.002$; vehicle versus 2 µg: $F(2,28) = 11.91$, $P < 0.002$). For both drugs, the effects of the drug doses relative to vehicle were most evident in the first 10 min of the testing session (time course data not shown).

Interestingly, in contrast to the results from the Acb core experiments (see above), there were no main effects of drug dose on feeding duration for either SCH 23390 or raclopride ($F = 1.17$–2.06, n.s.; see Table 1). There was, however, a significant dose × time interaction on this measure in the SCH 23390 experiment ($F(4,28) = 2.76$, $P < 0.05$); analyses for simple effects revealed a dose × time interaction for the comparison between vehicle and the 2 µg dose ($F(2,28) = 5.07$, $P < 0.03$). The 2 µg/side dose of SCH 23390 produced an increase in feeding duration relative to vehicle in the first 10 min of the testing session; however, for the last 10 min of the session, the mean associated with the 2 µg/side dose was lower than the vehicle-associated mean (time course data not shown).

In contrast to the results obtained in the Acb core (see above), SCH 23390, but not raclopride, significantly increased mean feeding bout duration when infused into the Acb shell (main effect of dose, SCH 23390: $F(2,14) = 6.73$, $P < 0.009$; raclopride: $F(2,14) = 1.94$, n.s.; see Fig. 4). Pre-planned contrasts revealed that the effects of both doses of SCH 23390 differed significantly from the vehicle control. In both the SCH 23390 and raclopride experiments, there were no treatment × time interactions ($F = 0.70$–1.22, n.s.).

3.3. Drinking behavior

As summarized in Table 2, SCH 23390 infusion into the Acb core decreased the total number of drinking bouts in the 30 min testing session (main effect of dose: $F(2,12) = 7.15$, $P < 0.01$). Pre-planned contrasts revealed that the effect of the 2 µg/side dose differed significantly from vehicle control (for alpha value, see legend for Table 2).

There were no statistically significant effects on any other drinking measure for either drug infused into either brain region ($F = 0.40$–3.05, n.s.), although as can

Table 1
Effects of SCH 23390 or raclopride infusion into the nucleus Acb core or shell, on latency to feed, total number of feeding bouts, and total feeding duration

	Latency to feed (s)	Feeding bouts	Total feeding duration (s)
Acb core			
SCH 23390			
0 μg	14.6±3.25	26.7±4.03	1265.1±61.01
1 μg	12.9±2.44	19.1±1.90	1395.4±32.98
2 μg	15.7±3.36	16.9±2.12	1320.9±119.14
Raclopride			
0 μg	21.1±3.51	36.0±3.27	1126.6±48.23
1 μg	16.4±1.96	20.1±2.53**	1312.9±40.82**
2 μg	18.3±4.70	18.9±3.44***	1335.1±47.95**
Acb shell			
SCH 23390			
0 μg	17.4±3.22	28.0±1.58	1220.8±69.32
1 μg	17.3±2.39	12.4±1.60***	1363.4±72.67
2 μg	22.0±3.91	12.1±2.25***	1193.3±90.32
Raclopride			
0 μg	36.9±6.19	24.9±3.01	1206.0±55.41
1 μg	22.5±4.56	15.4±2.63*	1280.6±50.53
2 μg	20.9±4.78	14.0±2.18*	1356.6±49.47

Data is presented as mean±S.E.M.
* $P < 0.05$; different from 0 μg (see Section 2 for details of the statistical analyses).
** $P < 0.01$; different from 0 μg (see Section 2 for details of the statistical analyses).
*** $P < 0.001$; different from 0 μg (see Section 2 for details of the statistical analyses).

be seen in Table 2, latency to drink tended to be increased by both drugs in both sites.

3.4. Histological analyses

As can be seen in Fig. 5, the injector tips for the Acb shell placements were located in the ventro-medial sector of that structure. Injector tips did not penetrate into the lateral ventricles. For the core placements, injector tips were located just ventral and medial to the anterior commissure. No excessive or unexpected tissue damage was noted in any of the experiments.

4. Discussion

Infusion of dopamine receptor antagonists into the Acb core or shell produced dissociable effects on spontaneous motor activity versus feeding behavior. Infusion of either SCH 23390 or raclopride into the Acb core or shell significantly decreased ambulatory activity and rearing, although the effects of SCH 23390 in the shell were considerably more pronounced than the effects of raclopride in that structure. Neither SCH 23390 nor raclopride significantly affected total food intake or latency to eat when infused into either the Acb core or shell; however, alterations in the microstructure of feeding were noted. Thus, infusion of raclopride, but not SCH 23390, into the Acb core significantly reduced the number of feeding bouts per 30 min session and produced a small but statistically significant increase in the overall feeding duration, although it should be noted that for the raclopride group, vehicle baseline values were slightly higher for feeding bouts and slightly lower for total feeding duration relative to other control groups. In the Acb shell, infusion of either SCH 23390 or raclopride decreased the number of feeding bouts, but neither drug influenced total feeding duration. Mean feeding bout duration was increased by both drugs in both sites, although these effects achieved statistical significance only for raclopride in the Acb core, and SCH 23390 in the Acb shell. Drinking was unaffected except for a significant decrease in total drinking bouts produced by the highest dose of SCH 23390 in the Acb core, and a non-significant tendency for latencies to be increased by both drugs in both sites. Hence, dopamine antagonist-treated rats ate the same amount of food as vehicle-treated rats in fewer but longer feeding bouts,

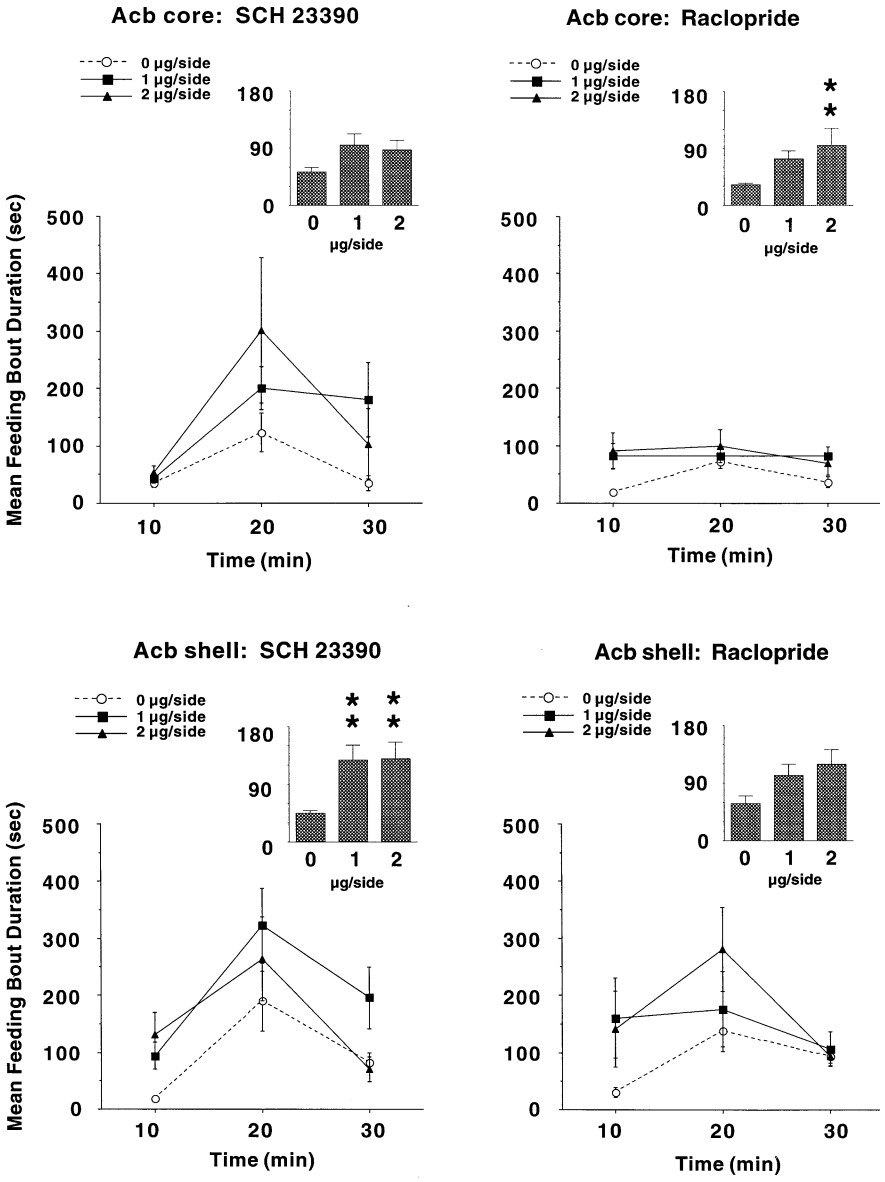

Fig. 4. Effects of infusions of SCH 23390 or raclopride into the nucleus Acb core or shell on mean feeding bout duration. Main graphs represent the change over time of mean bout duration over the 30 min testing sessions presented in 10 min time bins. Insets represent overall mean bout duration for the entire 30 min testing session. Error bars represent one S.E.M. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, different from 0 µg/kg on data collapsed across the 30 min testing session.

while exhibiting little change in drinking behavior and a marked decrease in locomotor activity and rearing.

In agreement with the present results, Koob et al. [18] observed that 6-OHDA lesions of the Acb and olfactory tubercle failed to decrease food intake in hungry rats; indeed, these investigators observed increased intake of wet mash during restricted testing sessions in lesioned rats. Nevertheless, the lesioned rats displayed marked hypoactivity. Similarly, Bakshi and Kelley [2] found that infusions of the mixed dopamine D1/D2 receptor antagonist haloperidol into the Acb (the injector tips were located mainly in the ventromedial Acb) produced a statistically non-significant trend toward increasing food intake in food-restricted rats, a significant increase in mean feeding bout duration, and a significant decrease in locomotor activity. Taken together, these findings indicate that manipulations that interrupt dopaminergic neurotransmission in the Acb spare feeding behavior in rats that are motivated to eat, while strongly diminishing motor behaviors such as ambulation and rearing. The present results extend these earlier findings by demonstrating that the same overall pattern is observed regardless of whether dopamine transmission is blocked specifically in the Acb core or shell.

It is interesting to evaluate the present results according to current theories on the role of dopamine in

Table 2
Effects of SCH 23390 or raclopride infusion into the nucleus Acb core or shell, on latency to drink, total number of drinking bouts, total drinking duration, and mean drinking bout duration

	Latency to drink (s)	Drinking bouts	Total drinking duration (s)	Mean bout duration (s)
Acb core				
SCH 23390				
0 μg	441.9±30.44	8.4±1.11	174.3±30.03	21.3±3.34
1 μg	395.2±52.64	6.3±1.27	170.0±20.88	31.9±5.66
2 μg	562.7±211.60	3.9±1.10*	56.6±21.41	22.0±5.10
Raclopride				
0 μg	358.9±25.31	6.3±1.04	163.4±12.40	30.6±5.79
1 μg	630.8±87.68	6.1±1.06	141.0±22.24	30.1±4.13
2 μg	720.2±220.19	4.4±1.07	136.7±20.80	47.1±16.41
Acb shell				
SCH 23390				
0 μg	511.3±111.87	6.4±0.68	180.1±21.36	29.8±3.90
1 μg	478.2±64.36	3.5±0.54	125.5±29.13	55.0±19.71
2 μg	890.3±228.30	3.9±1.13	102.3±33.80	22.5±7.65
Raclopride				
0 μg	389.3±79.78	7.6±1.36	166.9±23.32	25.1±4.80
1 μg	613.5±86.43	6.0±0.91	141.1±7.90	27.9±4.70
2 μg	729.7±164.62	5.4±1.19	143.8±17.06	35.3±6.76

Data is presented as mean±S.E.M.
* $P < 0.01$; different from 0 μg (see Section 2 for details of the statistical analyses).

motivated behavior. One older but important theory, the anhedonia hypothesis, posits that dopaminergic neurotransmission (particularly involving the meso-Acb projection) mediates the rewarding properties of goal objects (food, drugs of abuse, rewarding electrical brain stimulation, etc.), thereby enabling them to serve as positive reinforcers ([37,38]; see also [9]). Another hypothesis, the incentive salience theory of dopamine function, holds that dopaminergic substrates are involved in the process of assigning positive motivational significance to stimuli (“wanting”), while not mediating the hedonic effects (“liking”) of rewarding stimuli [5]. The present observation that dopamine-blocking manipulations in the Acb of hungry rats did not diminish the amount of food consumed, and shifted the microstructure of feeding towards longer eating bouts, seems inconsistent with the anhedonia hypothesis, at least as it relates to food reward. The present data are perhaps

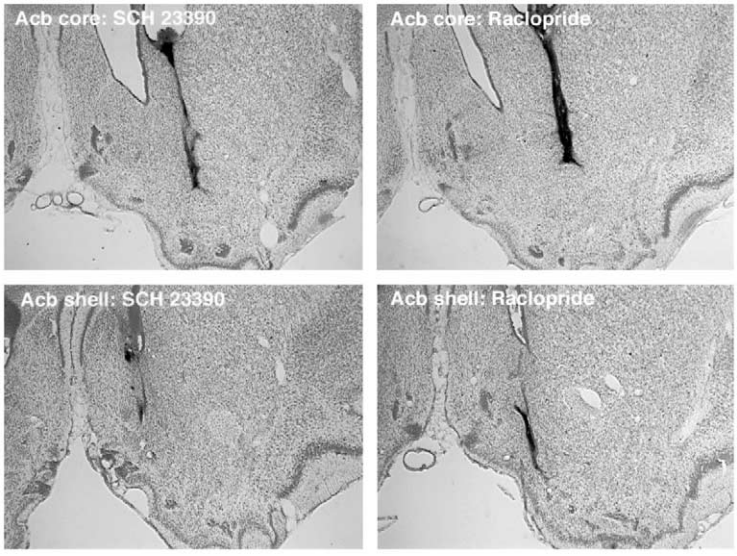

Fig. 5. Photomicrographs depicting representative intra-Acb injector placements for each of the four experiments. Injections were made bilaterally in all experiments; injectors are shown here on one side for clarity.

more compatible with the incentive salience theory, insofar as the lack of dopamine antagonist effects on food intake can be interpreted as evidence that dopamine transmission does not mediate the hedonic properties of food once the food has been contacted. An important caveat to this interpretation is that the doses of dopamine antagonists used in the present studies may not have been high enough to completely block the effects of dopamine in reward mediation. Indeed, it is likely that higher antagonist doses would have decreased feeding. Nevertheless, the present doses were sufficiently high to markedly reduce ambulation and rearing, thereby revealing a dissociation between the effects of intra-Acb dopamine antagonist treatment on deprivation-induced feeding versus ambulatory motor behavior.

The present results also seem consistent with a preferential role of Acb dopamine in the motor output associated with the proximity of a salient incentive. Previous studies showed that 6-OHDA-induced depletion of dopamine in the Acb of rats impaired performance on food-reinforced instrumental tasks with a high motoric "cost", such as climbing over obstacles to obtain food or lever-pressing on a high fixed ratio schedule, while sparing the eating of readily available food or lever-pressing on a continuous reinforcement schedule [1,28]. These effects were interpreted as reflecting dopaminergic lesion-induced anergia [29]. Additionally, extracellular dopamine levels in the Acb were elevated during eating in a schedule of periodic food presentation that resulted in high levels of locomotor activation, but not during a schedule of food presentation that did not elicit locomotor hyperactivity, even though all the food was eaten under both schedules [21]. Taken together, these results were interpreted as evidence that Acb dopamine transmission is involved in facilitating motor processes and instrumental behaviors associated with the presence of a desired goal object, but does not mediate the rewarding aspects of the consummatory act (see [21,28]). This theory is consistent with the present observation that an intra-Acb dopamine antagonist treatment strongly reduced motor behaviors (ambulation and rearing) but did not affect food intake in hungry animals.

Alternatively, the present results could be interpreted as demonstrating a role for dopamine in switching between competing behavioral repertoires [2,10,18,35]. Thus, the largest proportion of a hungry rat's behavioral output consists of responses directed at obtaining food or consuming readily available food. According to the switching hypothesis, blockade of dopamine receptors would prolong this feeding behavior by inhibiting dopamine-mediated facilitation of switching to other behaviors such as rearing, locomotion, or grooming. This formulation can account for the present finding that dopamine receptor antagonism in either the Acb core or shell increased mean feeding bout duration.

It is important to note that the switching hypothesis and the anergia hypothesis are neither mutually exclusive, nor incompatible with the incentive salience hypothesis; indeed, these are interrelated concepts. Hence, a certain level of exploratory motor output may be advantageous even in situations when a hungry animal has already encountered food in a safe environment; for example, to ensure that all the food in the immediate vicinity has been located. In this study, it was common for hungry vehicle-treated animals to drop a half-eaten food pellet, engage in sniffing and rearing, and then ambulate to the opposite side of the cage to commence eating a second food pellet. However, in a hungry animal in which dopamine function has been compromised, nucleus Acb-mediated switching away from feeding behavior in a safe, well-known environment may be less adaptive because the animal's overall capacity to engage in dopamine-facilitated motor acts, whether they be feeding responses or local foraging activities, has been reduced. As such, it would be less advantageous to trade off feeding behavior for local foraging activities, because feeding is the physiologically more strongly driven behavior. Hence, diminution of switching in a "free-feeding" test such as employed in the present study, or a reallocation of available motor effort to less demanding instrumental tasks (see above), could be interpreted as adaptive early consequences of compromised dopamine function, which are manifested before more severe symptoms of sensorimotor incapacity appear (such as the inability to eat).

Although there were subtle differences in the present study between the effects of dopamine antagonist infusions into the Acb core versus shell, the overall pattern of reduced motor activity, no change in food intake, and a shift towards longer feeding bouts was largely similar in both Acb subregions. This was somewhat unexpected, considering recent evidence on the functional heterogeneity of the Acb with regard to feeding behavior. For example, infusions of glutamate receptor antagonists or GABA agonists into the Acb shell of rats, but not the core, elicited intense hyperphagia in ad lib-fed rats [4,15,19,30]. This effect was hypothesized to be subserved, at least in part, by the Acb shell projection to the lateral hypothalamus [13,19,31,34,40,41]. In contrast, blockade of glutamate receptors in the Acb core prevented learning of food-reinforced lever pressing but did not inhibit lever pressing in rats that had already learned the response [16]. Moreover, selective excitotoxic lesions of the Acb core but not the Acb shell disrupted the formation of a Pavlovian association between a light cue and the delivery of a sucrose solution [23].

A recent microdialysis study provided further evidence for core–shell distinctions in feeding behavior. Thus, Bassareo and Di Chiara [3] showed that extracellular dopamine levels in the Acb shell of rats rose

during first-time consumption of a highly palatable snack food; the effect in the Acb core was of considerably smaller magnitude. However, extracellular dopamine levels rose in the Acb core but not the shell when the rats were presented with stimuli they had previously learned to be associated with the snack food. Taken together, these studies indicate that the Acb shell might preferentially mediate unconditioned responses to novel foods and the generation of eating-related behavioral patterns, whereas the core might mediate associations that are relevant to food-motivated instrumental behaviors. Hence, one might have expected distinguishable effects of dopamine receptor antagonists, particularly on the food intake measure, in the Acb core versus shell. In contrast, the present results support a similar role for dopamine in both Acb subregions in motor activity and feeding behavior in hungry rats presented with familiar lab chow, and further suggest that dopamine might have a general modulatory role rather than mediating the specialized functions of the Acb core or shell. In this study, however, animals were well habituated to the food; therefore, factors that contributed to core–shell differences in the Bassareo and Di Chiara [3] study were minimized. It would be interesting in future studies to compare the effects of dopamine antagonists infused into the Acb core versus shell on the intake of novel, highly palatable food in non-deprived rats.

It was interesting to note that in the Acb shell, the effects of the D1 receptor antagonist SCH 23390 were considerably more pronounced than the effects of the D2 receptor antagonist raclopride. Taken together with previous data showing that the D1 receptor-selective agonist SCH 82598 elicited stronger locomotor activation and rearing when infused into the Acb shell versus the core [32], the present findings suggest that the regulation of D1 receptors might differ between the two Acb subregions. Although the neurophysiological bases for these observations are unknown, it has been shown that the inhibitory effect of dopamine on elicited postsynaptic potentials in the Acb shell was blocked by the D1 receptor antagonist SCH 23390, but not by the D2 receptor antagonist sulpiride [24]. Thus, D1 receptors may have a particularly important role in mediating dopamine-mediated electrophysiological activity and behavior in the Acb shell.

In summary, infusion of a D1 or D2 receptor antagonist into either the Acb core or shell of hungry rats did not alter the amount of food eaten, but lengthened the duration of individual feeding bouts and concomitantly depressed ambulatory activity and rearing. Hence, blocking dopamine receptors in the Acb core or shell to a degree that depresses motor behavior does not abolish the primary motivation to eat. Instead, interfering with dopamine transmission in either Acb subregion might diminish switching among competing behaviors, prolonging the organisms focus on those behaviors most strongly driven by physiological need.

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