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ORIGINAL INVESTIGATION

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Increases in food intake or food-seeking behavior induced by GABAergic, opioid, or dopaminergic stimulation of the nucleus accumbens: is it hunger?

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Abstract *Rationale:* Previous work has shown that stimulation of GABAergic, opioid, or dopaminergic systems within the nucleus accumbens modulates food intake and food-seeking behavior. However, it is not known whether such stimulation mimics a motivational state of food deprivation that commonly enables animals to learn a new operant response to obtain food. *Objectives:* In order to address this question, acquisition of lever pressing for food in hungry animals was compared with acquisition in non-food-deprived rats subjected to various nucleus accumbens drug treatments. *Methods:* All animals were given the opportunity to learn an instrumental response (a lever press) to obtain a food pellet. Prior to training, ad lib-fed rats were infused with the γ-aminobutyric acid $(GABA)_A$ agonist muscimol (100 ng/0.5 µl per side) or the mu-opioid receptor agonist D-Ala^2, *N*-me-Phe^4, Gly-ol^5-enkephalin (DAMGO, 0.25 µg/0.5 µl per side), or saline into the nucleus accumbens shell (AcbSh). The indirect dopamine agonist amphetamine (10 µg/0.5 µl per side) was infused into the AcbSh or nucleus accumbens core (AcbC) of ad lib-fed rats. An additional group was food deprived and infused with saline in the AcbSh. Chow and sugar pellet intake responses after drug treatments were also evaluated in free-feeding tests. *Results:* Muscimol, DAMGO, or amphetamine did not facilitate acquisition of lever pressing for food, despite clearly increasing food intake in free-feeding tests. In contrast, food-deprived animals rapidly learned the task. *Conclusions:* These findings suggest that pharmacological stimulation of any of these neurochemical systems in isolation is insufficient to enable acquisition of a food-reinforced operant task. Thus, these selective processes, while likely involved in control of food intake and food-seeking behavior, appear unable to recapitulate the conditions necessary to mimic the state of negative energy balance.

Keywords Muscimol · DAMGO · Amphetamine · Incentive motivation · Learning · Ingestive behavior

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Introduction

Over the past several years, evidence has accrued indicating that the nucleus accumbens is comprised of three distinct subregions: the core, the shell, and the rostral pole. These subregions can be distinguished based on differences in efferent and afferent connections, cytoarchitectonics, histochemical features, and varied functional responses to environmental stimuli (Deutch and Cameron 1992; Zahm and Brog 1992; Voorn et al. 1994; Kelley 1999). The core (AcbC) and the shell (AcbSh) are the most extensively studied regions of the nucleus accumbens; these subregions have been shown to subserve distinct behavioral processes. For example, stimulation of gamma-aminobutyric acid (GABA) receptors within the AcbSh produces intense hyperphagia, while a similar manipulation of the AcbC does not (Stratford and Kelley 1997; Basso and Kelley 1999). The behavioral responses observed after GABA agonist infusion into the AcbSh are similar to those seen when the lateral hypothalamus (LH) is stimulated (Stanley et al. 1993) suggesting an association between these regions. Accordingly, pharmacological inactivation of the lateral hypothalamus blocks the feeding response induced by muscimol injections into the AcbSh (Maldonado-Irizarry et al. 1995; Stratford and Kelley 1999).

In addition to the GABA system, mu opioid and dopamine systems within the nucleus accumbens also modulate feeding behavior. Infusion of mu-opioid receptor agonists into the Acb preferentially increases intake of high-sugar and high-fat foods, while an opioid receptor antagonist infused directly into the Acb decreases fat

intake in hungry rats (Zhang et al. 1998). In addition, stimulation of mu opioid receptors in the Acb increases the break point for food reinforcement in a progressive ratio task (Zhang et al. 2003). Like mu-opioid stimulation, intra-Acb infusion of the dopamine releaser, amphetamine, also increases break point for food reinforcement; however, intra-Acb amphetamine does not increase chow intake in a free-feeding test. Moreover, blockade of dopamine receptors in either the AcbC or AcbSh diminishes ambulation and rearing at doses that completely spare feeding behavior in food-deprived rats (Baldo et al. 2002). Thus, although GABAergic, opioid, and dopaminergic systems within the accumbens are all involved in the modulation of food-seeking behavior in a general sense, the nature of the responses associated with each of these neuromodulator systems are distinguishable.

One issue that has not been resolved, however, relates to the similarities or differences among these three neurochemically coded systems in the Acb in mediating the acquisition of novel food-seeking behaviors. It has long been known that, in addition to augmenting the output of both unconditioned consummatory behaviors and previously learned food-reinforced instrumental responses, food deprivation establishes a condition under which novel food-reinforced behaviors are acquired more readily (Skinner 1938; Clark 1958; for review, see Toates 1986). The latter phenomenon therefore represents an important criterion with which to evaluate candidate neural substrates for 'hunger.' Wise (1974) raised a similar point in a seminal paper reviewing the similarities and differences between the motivational state produced by food deprivation and that produced by electrical stimulation of the lateral hypothalamus. In this review, the ability of stimulation to establish food as a reinforcer was used as an important index of homology between food deprivation and hypothalamic stimulation. Likewise, the present study was designed to obtain corroborative evidence for the involvement of Acb-localized GABA, opioid, or dopamine systems in the motivational state associated with food deprivation, by evaluating the ability of these systems to enable food-reinforced instrumental learning.

To explore this question, ad lib-fed rats were trained in a food-reinforced lever-pressing task after receiving infusions of the $GABA_A$ agonist (muscimol) or the mu-opioid agonist (DAMGO) in the AcbSh; or the indirect dopamine agonist, amphetamine, in the AcbSh or AcbC. The rate at which drug-treated rats acquired the task was compared with that observed in food-deprived, vehicle-treated rats. As proof of the principle that these manipulations elicit feeding, and to demonstrate that behaviorally active doses of pharmacological compounds were used, muscimol, DAMGO, or amphetamine were infused into the AcbSh or AcbC of sated rats, and spontaneous feeding was measured.

Methods

Animals

A total of 53 male Sprague-Dawley rats (Harlan, Madison, WI), housed in groups of two or three in acrylic cages, were used in this experiment. Rats were given food (Harlan Teklad Rat Diet) and water ad libitum, with the exception of one group, which was food restricted to maintain approximately 85% of free-feeding weight. Also, the non-deprived groups were mildly food deprived for 2 days prior to training to promote consumption of the sugar pellets. Prior to surgery, all rats weighed between 300 g and 360 g. Lights were maintained on a 12-h/12-h light/dark cycle. All animal procedures and facilities were reviewed and approved by the IACUC of the University of Wisconsin-Madison, and were inspected and accredited by AALAC.

Surgery

Animals were anesthetized with a ketamine/xylazine mixture (100 mg/kg ketamine and 10 mg/kg xylazine-i.p.). Bilateral 10-mm stainless-steel guide cannulae (23 gauge) were implanted using standard stereotaxic techniques and were secured to the skull with stainless-steel screws and dental cement. Guide cannulae were aimed at the nucleus accumbens shell [anteroposterior (AP), +3.1 mm from bregma; mediolateral (ML), −1.0 mm; dorsoventral (DV), −5.4 mm from skull surface] with nosebar 5 mm above interaural zero; or at the nucleus accumbens core (AP, +1.3 mm from bregma; ML, −1.7 mm; DV, −5.1 mm from skull surface) with flat skull. After surgery, stainless-steel wire stylets were placed in the guide cannulae to prevent occlusion. Each rat received an intramuscular injection (0.3 ml) of sterile penicillin immediately following surgery. Rats were allowed a week of recovery before behavioral testing.

Drugs and microinjections

Muscimol, the $GABA_A$ agonist, was obtained from Sigma (St. Louis, MO), the mu-opioid receptor agonist, D-Ala^2, *N*-me-Phe^4, Gly-ol^5-enkephalin (DAMGO), from Research Biochemicals (Natick, MA), and the indirect dopamine agonist, amphetamine, from the National Institute on Drug Abuse. All drugs were dissolved in sterile 0.9% saline.

After several days of handling and habituation to the experimental paradigm (see *Behavioral testing and experimental design*), rats were taken into the testing room, their stylets removed, and bilateral intracerebral injections of drug or vehicle (total volume of 0.5 µl per side infused over 1 min 33 s) were administered through a 12.5-mm injector cannulae (30-gauge) with a microinfusion pump (Harvard Apparatus, South Natick, MA). Injector cannulae were left in place for an additional minute post-infusion to allow for diffusion of injectate into the tissue. Injectors were then removed, and stylets replaced.

The total unilateral muscimol dose used in these studies was 100 ng (876 pmol), the total DAMGO dose, 0.25 µg (1.25 pmol), and the total amphetamine dose, 10 µg (50 pmol). Previous experiments demonstrated that these doses elicit feeding behavior or alter food-reinforced operant responding in rats (Stratford and Kelley 1997; Zhang et al. 1998, 2003). Muscimol-, amphetamine-, and vehicle-treated animals were tested within five minutes of injection. DAMGO-treated animals were placed in home cages without food for 30 min prior to testing and then placed into the testing cages. This delay was introduced based on past studies indicating the maximum effect of DAMGO on food intake occurs 30–60 min post-infusion (Zhang and Kelley 1997).

Behavioral testing and experimental design

Behavioral testing occurred in standard operant chambers (W: 9.5 in., L: 17 in., H: 8 in.). These chambers (Coulbourn Instruments, Allentown, PA) contained two levers, a food trough with photosensors, a pellet delivery system, a house light, and a red signal light. Prior to introduction to operant chambers, the animals were habituated to sugar pellets in their home cages in order to familiarize them with this novel food. Rats were then habituated to the infusion process and the operant testing chambers. On the first two days of habituation, the rats received a mock injection in which 10-mm injectors were lowered into the guide cannulas. These shortened injectors did not protrude beyond the infusion cannualae. The rats were then placed in the operant chambers for 10 min with food pellets in the trough, but with the levers retracted. On the third day of habituation, the rats received a saline infusion into the injection site with 12.5-mm injectors, and were placed in the operant chambers as described above. After the third habituation day, testing commenced. On the first two testing days, arbitrarily assigned correct levers had sugar pellets placed on them to help shape the lever pressing response. Responding on the correct lever resulted in the following sequence of events: the house light switched off, the red signal light switched on for 3 s, and the food pellet was delivered to the food trough. Pressing the incorrect lever had no scheduled consequences. Testing sessions were 15 min in length. Correct lever presses, incorrect lever presses and nosepokes into the food trough were recorded.

Rats (total n=53) received bilateral infusions aimed at the AcbSh or AcbC and were divided into seven groups. The first group (n=8) received intra-AcbSh infusions of saline and was food restricted. The next six groups were not food-deprived. These groups received intra-AcbSh infusions of 0.9% sterile saline (n=6), muscimol (100 ng, n=6), DAMGO (0.25 μg, n=8), amphetamine (10 μg, n=8); or were given intra-AcbC infusions of amphetamine (10 μg, n=9) or saline (n=8). In all cases, infusions were given before the start of each testing session, according to the pretreatment times noted above (see *Drugs and Microinjections*). All rats received drug or vehicle infusions on the first 5 days of the experiment, and were tested for an additional 5–7 days without drug treatment to ensure that asymptotic responding in the operant task had been achieved.

One day after the end of testing in the operant chambers, the six non-food-deprived groups were tested in a 30-min laboratory chow intake paradigm. In this paradigm, rats were habituated for 30 min to the clear polycarbonate observation cages, similar to the home cage, the day before testing. On the test day, the rats were infused with the respective drug treatments they had received in the previous phase of the experiment, and were placed into the cages with a pre-weighed portion of rat chow, water bottles, and paper under the cages to collect food spillage. After 30 min, the amount of laboratory chow intake was calculated. A separate food-deprived group (n=8) was also tested and shown in Fig. 3 for comparison.

A separate group of rats (n=8) were tested in a 15-min sugar pellet intake paradigm in the operant chambers. Rats received bilateral intra-AcbSh infusions of saline, muscimol (100 ng), DAMGO (0.25 μg), and amphetamine (10 μg) administered in a counterbalanced order according to a within-subjects Latin-square design. Injections were performed as described above (*Drugs and microinjections*). Each rat received all treatments, and injections were separated by at least one treatment-free day. After habituation to sugar pellets and microinjection process (see above), testing commenced. On the first test day, rats had been maintained at 85% of free-feeding weight and received intra-AcbSh infusions of 0.9% sterile saline. Subsequently, rats resumed free-feeding for 3 days and then received treatment injections followed by testing. Each test day a pre-measured amount of sugar pellets were placed in the trough of operant chambers in which both levers were retracted and the house light was continually on. At the end of the 15-min session, the house light switched off and rats were removed from the chamber. Amount of sugar pellets eaten was measured.

Histology

At the end of behavioral testing, all animals were deeply anesthetized with an overdose of sodium pentobarbital and transcardially perfused with isotonic saline followed immediately by 10% formalin in phosphate buffer (formalin/PB). The brains were removed and stored in formalin/PB. Before sectioning, the brains were placed in a solution of 15% sucrose in formalin/PB for at least 24 h. Brains were cut in 60-μm sections, mounted and stained with cresyl violet. The placement of injector tips was determined using light microscopy.

Data analysis

All data were analyzed using SuperANOVA software. Effects of food deprivation on lever pressing were analyzed using three-factor ANOVA (deprivation state × training session × lever), with repeated measures for the within-subjects variable, training session.

Effects of stimulation of GABAergic, opioid, and dopaminergic receptors within the nucleus accumbens on instrumental learning were analyzed with three-factor ANOVA, (treatment × training session × lever). There were four levels for the treatment factor corresponding to the four different drug treatments (saline, muscimol, DAMGO, and amphetamine). Separate three-factor ANOVAS were run on data for the drug-treatment phase of the experiment, (i.e., the first five test days) and the period after drug had been discontinued.

For the chow and sugar intake studies (Fig. 3A, B), multifactorial ANOVAS were performed on the food-deprived, muscimol-, DAMGO-, and amphetamine-treated groups versus their respective saline control groups, and interactions were analyzed using simple main effects.

Results

Histology

Chartings of representative placements are presented in Fig. 1.

Food deprivation enables instrumental learning

Figure 2A shows the effect of food deprivation on acquisition of lever pressing for food reinforcement. On the second day of testing, the food-deprived rats began to

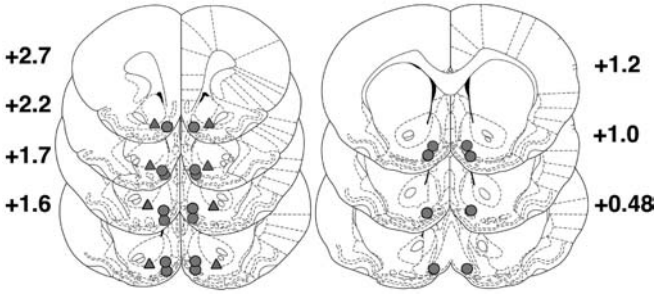

Fig. 1 Histological reconstructions of representative cannulae placements. Nucleus accumbens shell (*AcbSh*) placements are denoted by *circles*, and nucleus accumbens core (*AcbC*) placements by *triangles*. Numbers represent distance from bregma in millimeters. Adapted from the Atlas of Paxinos and Watson (1998)

show a marked preference for the correct lever that continued to increase over subsequent testing days. In contrast, the non-deprived rats did not show a preference for either lever and showed no evidence of acquisition of lever pressing. Analysis of variance on data from the first five test days revealed a significant effect of deprivation state ($F_{1,12}$=22.62, P=0.0005). There was also a significant training session × deprivation state interaction ($F_{4,48}$=3.521, P=0.0134) reflecting the fact that food-deprived and ad lib-fed rats showed similar rates of lever presses for the first training session in contrast to the marked difference seen on subsequent days. Both these effects were significant through the last five training sessions (deprivation state, $F_{1,12}$=16.524, P=0.0016, and deprivation state × training session, $F_{4,48}$=5.526, P=0.0010), reflecting the fact that ad lib-fed rats do not acquire this task within the 10- to 11-day training period, whereas the food-deprived group continued to show increased lever pressing.

Stimulation of GABAergic, opioid, or dopaminergic receptors within the nucleus accumbens does not enable instrumental learning in ad lib-fed rats

As shown in Fig. 2B, intra-AcbSh muscimol, DAMGO or amphetamine did not facilitate acquisition of lever pressing in ad lib-fed rats. Analysis of the first 5 days of training, during which drug infusions were administered prior to the testing sessions, indicated that the number of correct food-reinforced lever presses did not differ between drug-treated rats and saline controls (day × treatment interaction for muscimol: $F_{4,40}$=0.935, n.s.; DAMGO: $F_{4,48}$=0.926, n.s.; amphetamine: $F_{4,48}$=0.744, n.s.). We observed marginally significant main effects for muscimol and amphetamine treatments (main effect of treatment for muscimol: $F_{1,10}$=4.763, P=0.0540; amphetamine: $F_{1,12}$=6.438, P=0.0261). These effects were of extremely small magnitude (for example, mean correct presses per 15-min training session for ad lib-fed rats =0.567 muscimol-treated rats =4.367, food-deprived rats =43.025) and were due to slight differences only on the first two testing days, when sucrose pellets were taped to the correct levers to enhance learning. Thus, these small main effects did not reflect acquisition of operant responding in drug-treated rats.

Lever pressing for food reward was significantly different for the food-deprived rats relative to drug-treated rats (treatment × day × lever interaction for muscimol: $F_{4,48}$=3.247, P=0.0195; DAMGO: $F_{4,56}$=5.158, P=0.0013; amphetamine: $F_{4,56}$=4.955, P=0.0017), consistent with the failure to acquire lever-pressing by the drug-treated groups. Analysis of days 6–10 of the experiment, after cessation of drug treatments, revealed no delayed effects of drug-treatment on acquisition of lever pressing (main effect of treatment for muscimol: $F_{1,10}$=0.915, n.s.; DAMGO: $F_{1,12}$=2.138, n.s.; amphetamine: $F_{1,12}$=0.069, n.s.).

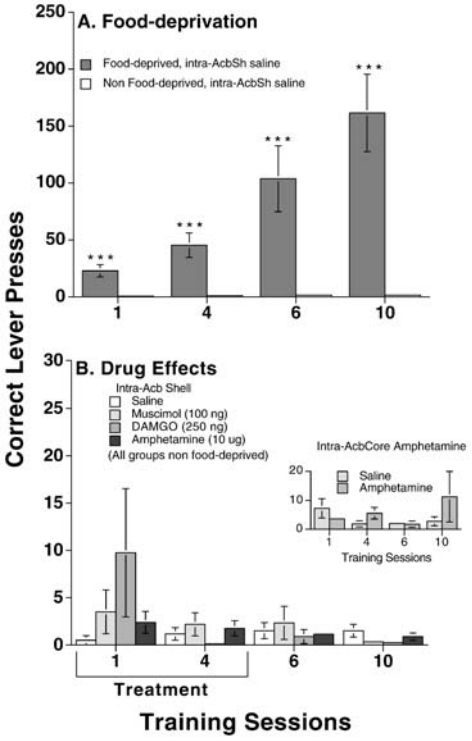

Fig. 2 A Effect of food deprivation on acquisition of lever pressing for food reinforcement in vehicle-treated rats. For the sake of clarity, four representative testing days (of a total of 12) are shown. *Error bars* represent one SEM, *P<0.05, **P<0.01, ***P<0.001. **B** Effect of muscimol (100 ng per side), D-Ala^2, *N*-me-Phe^4, Gly-ol^5-enkephalin (DAMGO; 250 ng per side), amphetamine (10 µg per side) or saline infusions into the nucleus accumbens shell (*AcbSh*) of ad lib-fed rats on acquisition of lever pressing for food reinforcement. *Error bars* represent one SEM. *Inset* Effect of saline or amphetamine infusions into nucleus accumbens core (*AcbC*) of ad lib-fed rats on acquisition of lever pressing for food reward. *Error bars* represent one SEM. Note that different scales were employed (for the sake of clarity) for the *y-axis* for **A**, **B**, and the *inset*

Similar to the effects seen with the intra-AcbSh amphetamine, intra-AcbC amphetamine did not facilitate acquisition of lever pressing in free-feeding rats. Analysis of the first 5 days of training revealed that the number of correct food-reinforced lever presses did not differ between amphetamine-treated rats and saline controls (main effect of treatment for amphetamine: $F_{1,15}$=1.438, n.s.), but differed significantly from results seen in food-deprived rats ($F_{1,15}$=26.784, P=0.0001; see Fig. 2B inset).

Effect of stimulation of GABA, opioid, or dopamine receptors within the nucleus accumbens on laboratory chow intake

To demonstrate the established effects of intra-Acb muscimol, DAMGO, and amphetamine on general laboratory chow intake, and to validate that behaviorally active drug doses were used in this study, a laboratory chow-intake probe was carried out in ad lib-fed rats

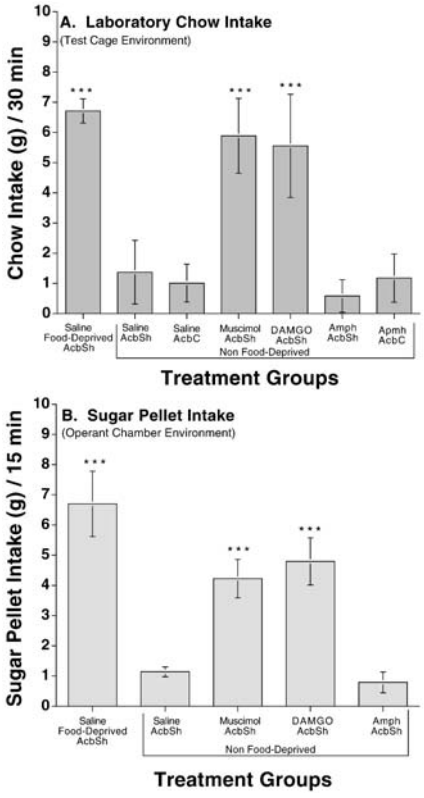

Fig. 3 A Effects of infusions of saline, muscimol, D-Ala^2, *N*-me-Phe^4, Gly-ol^5-enkephalin (DAMGO), and amphetamine into nucleus accumbens shell (*AcbSh*) or nucleus accumbens core (*AcbC*) of ad lib-fed rats on total laboratory chow intake in a 30-min testing session. Testing was done in test chambers similar to the home cage. A food-deprived, untreated group is shown for comparison. *Error bars* represent one SEM. **B** Effects of infusions of saline, muscimol, DAMGO, and amphetamine into AcbSh of ad lib-fed rats on total sugar pellet intake in a 15-min testing session in operant chambers. Effects of saline into the AcbSh of food-deprived rats is shown for comparison. *Error bars* represent one SEM

(Fig. 3A). Infusions of muscimol (F=56.067, P=0.0001) or DAMGO (F=54.941, P=0.0001) significantly increased food intake relative to saline-treated rats. Amount of food intake in these two drug-treated groups approximated levels seen in rats after 20 h food deprivation. In contrast, and in agreement with previous results, amphetamine did not significantly increase food intake (F=1.931, P=n.s.). Nevertheless, previous work has shown that this dose of amphetamine significantly elevates the break-point for food reward in a progressive ratio task (Zhang et al. 2003) indicating that this dose is behaviorally active.

Effect of stimulation of GABA, opioid, or dopamine receptors within the nucleus accumbens on sugar pellet intake

To demonstrate the established effects of intra-Acb muscimol, DAMGO, and amphetamine on general sugar pellet intake, and to validate that behaviorally active drug doses were used in this study, a sugar pellet-intake probe was carried out in ad lib-fed rats (Fig. 3B). Food-deprived rats eat significantly more sugar pellets than free-feeding rats in this paradigm (F=46.448, P=0.0001). Additionally, infusions of muscimol (F=14.310, P=0.0007) or DAMGO (F=19.253, P=0.0001) significantly increased sugar pellet intake relative to saline-treated rats. Consistent with previous results, amphetamine did not significantly increase sugar pellet intake (F=0.184, n.s.).

Discussion

The main finding of this study was that neither intra-AcbSh muscimol, DAMGO, nor amphetamine facilitated acquisition of an instrumental act (lever-pressing) to acquire food reinforcement in ad lib-fed rats, in marked contrast to the clear learning curve exhibited, as expected, by food-deprived, vehicle-treated rats. This dissociation stands in apparent contradiction to the ability of these neuropharmacological manipulations to produce other behavioral effects reminiscent of a food-deprived state, such as hyperphagia in ad lib-fed rats, in the case of muscimol and DAMGO (Stratford and Kelley 1997; Basso and Kelley 1999), or an increased break-point to obtain food-reinforcement in a progressive-ratio task, in the case of DAMGO and amphetamine (in this study rats had already acquired the task in a food-deprived state; Zhang et al. 2003). The question arises, therefore, as to how to characterize these drug-induced motivational states that mimic some, but not all, features of the state arising from 'hunger'.

Analysis of the behavioral effects of GABAergic, opioid, or dopaminergic stimulation of the nucleus accumbens reveals that these neurochemical systems subserve dissociable components of feeding. One obvious difference among these three manipulations, as validated by the present work, is that GABAergic or opiatergic stimulation of the AcbSh produces hyperphagia in a free-feeding test, whereas dopaminergic stimulation of the Acb produces little or no feeding (Stratford and Kelley 1997; Basso and Kelley 1999). The latter finding is consistent with the observation that dopamine receptor antagonism in the Acb produces little effect on food intake in hungry rats while strongly suppressing locomotion and rearing (Bakshi and Kelley 1991; Baldo et al. 2002), and supports the hypothesis that dopamine does not mediate the rewarding or hedonic aspects of the consummatory act (Wyvell and Berridge 2000). In contrast, intra-Acb opiate receptor stimulation appears closely linked to the hedonic effects arising from the consumption of the palatable foodstuffs (Pecina and Berridge 1995, 2000; Kelley et al. 2002). Stimulation of the mu-opioid receptors in the Acb selectively increases intake of sweetened solutions or high-fat foods and enhances unconditioned orofacial behaviors associated with palatability (Berridge 1996; Zhang and Kelley 1997, 2000; Zhang et al. 1998). Conversely, intra-Acb opiate receptor blockade decreases sucrose intake (Bodnar et al.

1995; Koch et al. 1995). Moreover, recent evidence indicates that long-term exposure to a highly palatable chocolate solution alters proenkephlin gene expression throughout the striatum (Kelley et al. 2000). Both intra-Acb opiate and intra-Acb dopamine stimulation augment lever pressing for sucrose pellets and increase break-point for food reinforcement in a progressive ratio task (Zhang et al. 2003). Opiate effects on break-point measure are hypothesized to result from an enhancement of the hedonic impact of ingesting the sucrose pellets, whereas dopamine's effect is thought to be due to an augmentation of the salience of stimuli previously associated with reward, and a consequent increase in motor effort organized in relation to these incentive stimuli ["liking" versus "wanting," according to the theoretical framework elaborated by Salamone (1996); Berridge and Robinson (1998); and Zhang et al. (2003)].

Like intra-AcbSh opiate receptor stimulation, GABA receptor stimulation in this structure dramatically increases chow intake, or ingestion of sucrose solution, in ad lib-fed rats (Stratford and Kelley 1997; Basso and Kelley 1999; Zhang et al. 2003). In contrast, this manipulation does not increase food-reinforced lever pressing or change break-point for food reinforcement (Zhang et al. 2003). We have hypothesized that intra-AcbSh GABA receptor stimulation, and resultant inhibition of the AcbSh output to the hypothalamus, disinhibits hypothalamic circuitry and, consequently, activates downstream targets mediating motor programs specific for food intake. This mechanism is proposed to bypass certain inputs relevant to food-seeking behavior, such as second-order stimuli associated with food reinforcement, and to directly 'switch-on' motor programs specific to ingestion. Such a mechanism would be associated with a degree of behavioral inflexibility and could account for the present finding that intra-AcbSh GABA receptor stimulation failed to enhance acquisition of a novel response to obtain food.

It is somewhat more difficult to account for the failure of the intra-Acb amphetamine or DAMGO to influence acquisition of lever pressing, particularly considering that both these manipulations markedly increase the break-point for food reinforcement in a progressive ratio task (Zhang et al. 2003). One might interpret the progressive-ratio results as indicating an increase in the reward value of the food pellets, an effect that might seem to favor the acquisition of novel responses to obtain food reinforcement. However, it is important to note that a change in break-point does not distinguish effects on motor effort directed at incentive stimuli (i.e., pressing the lever) from effects on the perceived rewarding value of the reinforcer. The theoretical paradigms described above, in which these two constructs are dissociable and differentially mediated by dopamine and opioid systems, respectively, may provide a framework with which to interpret the present results. Thus, it may be that the selective enhancement of either process in the absence of the other is insufficient to support instrumental learning. For example, amphetamine-induced enhancement of incentive-motivation and associated motor facilitation without a concomitant augmentation of the rewarding properties of food per se, while sufficient to augment the performance of an action that has already been associated with a rewarding outcome, may be insufficient to support the formation of novel action–outcome associations in ad lib-fed rats, where the outcome (i.e., food delivery) has relatively low reward value. Conversely, DAMGO would presumably enhance the hedonic impact of the few pellets earned during the initial acquisition phase of the experiment, without sufficiently increasing motivational arousal and motoric activation to overcome the low rate of responding exhibited by ad lib-fed rats. This situation may result in an insufficient density of reinforcement to support learning.

The purpose of this study was to determine whether doses of these drugs that induce modifications in food-seeking or food intake behavior also facilitate acquisition of responding for sugar pellet reward. The doses used in each of the experiments were the lowest needed to elicit hyperphagia or an increase in break point evidenced by previous dose–response studies (Stratford and Kelley 1997; Zhang et al. 1998, 2003). The latter study in particular examined established operant behavior (lever-pressing for sugar pellets) and found that doses of DAMGO and amphetamine (similar to those used in the present study) increased previously learned lever pressing. Thus, opioid and dopaminergic manipulation of the nucleus accumbens enhances responding in a previously learned task, but do not appear to facilitate acquisition of responding. However, we cannot rule out the possibility that a lower or higher dose of these compounds may have facilitated acquisition of lever-pressing.

In conclusion, while stimulation of opioid, dopamine, or GABA systems within the nucleus accumbens produce some behavioral effects associated with 'hunger,' such as the facilitation of feeding or food-seeking instrumental behaviors, these pharmacological manipulations lack the ability to facilitate the acquisition of novel behavioral responses to obtain food. This observation is consistent with the hypothesis that the neural instantiation of the motivational state associated with negative energy balance at the level of the Acb involves discrete, neurochemically coded circuits each mediating a dissociable component of the food-motivated behavior. The selective stimulation of any one of these circuits in isolation appears insufficient to recapitulate the food-deprived state in its entirety, particularly with regard to plasticity-related phenomena, such as instrumental learning, associated with hunger.

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