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Effects of muscimol, amphetamine, and DAMGO injected into the nucleus accumbens shell on food-reinforced lever pressing by undeprived rats

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ABSTRACT

Previous studies have shown that large increases in food intake in nondeprived animals can be induced by injections of both the $GABA_A$ agonist muscimol and the μ-opioid agonist DAMGO into the nucleus accumbens shell (AcbSh), while injections of the catecholamine agonist amphetamine have little effect. In the current study we examined whether injections of these drugs are able to increase food-reinforced lever pressing in nondeprived rats. Twelve subjects were trained to lever press on a continuous reinforcement schedule while food deprived and were then tested after being placed back on *ad libitum* feeding. Under these conditions, responding was markedly increased by injections of either muscimol or DAMGO, although the onset of the effects of the latter drug was delayed by 30–40 min. In contrast, amphetamine injections failed to increase reinforced lever pressing, although they did enhance responding on a non-reinforced lever, presumably reflecting alterations in behavioral activation. These results demonstrate that stimulation of $GABA_A$ and μ-opioid receptors within the AcbSh is able to promote not only food intake, but also food-directed operant behavior. In contrast, stimulation of AcbSh dopamine receptors may enhance behavioral arousal, but does not appear to specifically potentiate behaviors directed toward food procurement.

1. Introduction

A large number of studies have shown that pronounced alterations in food intake can be induced by experimental manipulations of the medial shell region of the nucleus accumbens (AcbSh) (Stratford, 2007). Activation of the AcbSh, produced either by local injections of excitatory amino acids (Stratford et al., 1998) or by electrical stimulation (Krause et al. 2010), inhibits ingestive behavior. Conversely, inactivation of the AcbSh produced by injections of inhibitory $GABA_A$ receptor agonists (Basso and Kelley, 1999; Reynolds and Berridge, 2002; Soderpalm and Berridge, 2000; Stratford and Kelley, 1997; Stratford and Wirtshafter, 2011), or of non-NMDA ionotropic excitatory amino acid antagonists (Faure et al., 2010; Maldonado-Irizarry et al., 1995; Stratford et al., 1998), induces pronounced ingestion of both solid and liquid diets. Water intake and gnawing behavior, however, are not affected by these treatments (Basso and Kelley, 1999; Stratford and Kelley, 1997; Stratford et al., 1998; Stratford and Wirtshafter, 2004), suggesting that they may have specific effects on feeding mechanisms. Ingestive behavior can also be produced by intra-AcbSh injections of μ-opioid agonists (Hanlon et al., 2004; Pecina and Berridge, 2005; Taha et al., 2009; Zhang and Kelley, 1997), although the effects of these drugs are not identical to those of muscimol (Basso and Kelley, 1999; Stratford and Wirtshafter, 2007; Wirtshafter and Stratford, 2010; Zhang and Kelley, 2002).

Very little is known about the functional mechanisms through which drug injections in the AcbSh act to produce feeding. Kelley and her colleagues have suggested that muscimol in the AcbSh may directly activate motor patterns involved in ingestion, and thus promote food intake without actually inducing a motivational state aimed at procuring food (Baldo and Kelley, 2007; Kelley et al., 2005; Meredith et al., 2008). If this were the case, one would expect that intra-AcbSh muscimol would not potentiate food-reinforced operant behavior. We have found, however, that muscimol injections are able to enhance food-reinforced progressive ratio performance by mildly deprived rats (Wirtshafter and Stratford, 2010). Although these findings suggest that inactivation of the AcbSh enhances the motivation to obtain food, it should be remembered that a variety of "nonspecific" and motor-related factors might also influence performance on progressive ratio schedules (Aberman et al., 1998; Skjoldager et al., 1993). Since performance on these schedules is believed to reflect a balance between the attractive properties of the reinforcer and the amount of time and effort which must be expended in obtaining it, it seems likely that manipulations which alter perceived effort, or willingness to exert effort, would also influence progressive ratio performance. Salamone has suggested, in fact, that the nucleus accumbens may play a major role controlling effort expenditure (Salamone et al., 2007), so it is not implausible that muscimol injections into this structure might alter performance through effects not specific to feeding. It is striking in this regard

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that although amphetamine in the AcbSh has little or no effect on food intake (Hanlon et al., 2004), injections of this compound are able to enhance food-reinforced progressive ratio responding (Wirtshafter and Stratford, 2010; Zhang et al., 2003).

Typically, animals working on a progressive ratio schedule consume only a small amount of food before responding terminates; performance on these schedules is thus limited primarily by the amount of effort that has to be expended, not by the amount of food that the animal consumes. One might thus refer to a progressive ratio schedule as an "effort-limited" task, and it is clear that performance on it might be affected by anything which alters the "activation level" or "effort tolerance" of the animal. Consider, in contrast, a paradigm in which an animal has to make only a simple response, such as a single lever press, to obtain food; it seems unlikely that effort-limitation would play a major role in this situation, especially if animals were tested in the absence of deprivation when baseline response rates would be low. If under these "non-effort limited" conditions, inactivation of the accumbens shell were to increase the motivation to obtain food, one would expect that lever pressing would be promoted. On the other hand, if muscimol worked only to activate feeding reflexes, or primarily affected some effort related variable, one would not predict that responding would be increased.

In view of these considerations, we here examined the effects of intra-AcbSh injections of muscimol and amphetamine on continuously reinforced operant responding by nondeprived rats. In order to assess the extent to which changes in responding might reflect alterations in locomotor activity, which might result in animals "inadvertently" depressing the lever, testing was conducted in two-lever operant boxes in which responding on only one lever was reinforced. Based on the conception that muscimol injections increase feeding motivation whereas amphetamine injections have a more generalized effect not directed at a particular goal object, we predicted that muscimol, but not amphetamine, would selectively enhance lever pressing on the reinforced lever. We also examined the effects of intra-AcbSh injections of DAMGO to determine the extent to which they resembled those of muscimol.

2. Methods

2.1. Animals

Subjects were 12 male Sprague–Dawley rats obtained from Charles-River Inc. (Chicago, IL) weighing approximately 300 g at the time of surgery. Animals were individually housed in plastic cages with food (Harlan 2018 Rodent Diet) and water available *ad libitum*, except as noted below.

2.2. Surgery

Rats were anesthetized with sodium pentobarbital (60 mg/kg) and bilateral 22-gauge stainless steel guide cannulae (Plastics One, Roanoke, VA) were implanted using standard, flat-skull stereotaxic techniques. The guide cannulae were aimed so as to terminate 2.0 mm dorsal to the AcbSh using the following coordinates: anteroposterior: 1.6, mediolateral: ±0.8, and dorsoventral: −6.1 (mm from bregma). The guide cannulae were held in place using denture lining material and stainless steel screws and stainless steel obturators were inserted into the lumen of each cannula to help maintain patency. Each rat was allowed to recover for at least seven days before being placed on deprivation and beginning operant training.

2.3. Apparatus

Animals were trained in one of six identical standard twin lever operant chambers (Med-Associates, St. Albans, VT) housed within sound attenuating chambers with ventilation and masking noise provided by an exhaust fan. The chambers were equipped with a click generator to provide audible feedback of food delivery, and an infrared photobeam was placed across the entrance to the food hopper to allow for the recording of times of head entries.

2.4. Operant training

After recovering from surgery, rats were placed on a restricted feeding schedule in which they were given 17-18 g of lab chow to eat each day. After one week on this schedule, animals were given two daily, 30 min magazine training sessions in the operant boxes during which reinforcers (F0021 45 mg Precision Dustless pellets, BioServe, Frenchtown, NJ) were presented at one min intervals, with a "click" being generated at the same time as food delivery. Animals were then manually shaped to lever press over one or two days, and then each day for the next five days were run for one-hour on a continuous reinforcement schedule. At the end of this training period, food was returned to the rats *ad libitum*, but they continued to receive daily sessions in the operant boxes near the middle of their light period. After at least 6 runs under nondeprived conditions, drug injections began, as described below.

2.5. Intracerebral injections

In order to make injections, rats were gently restrained, the obturators removed, and a 28-gauge stainless steel injection cannula, extending 2.0 mm beyond the ventral tip of the guide, inserted into each guide cannula. Rats then received simultaneous bilateral 0.50 µl infusions at a rate of 0.33 µl/min. using a motor-driven microsyringe connected to the injection cannulae through a length of fluid filled polyethylene tubing. After the infusions, the injection cannulae were left in place for an additional 60 s in order to minimize leakage up the tracks after which they were removed and replaced with the obturators. Animals were then immediately placed in the operant chambers. Animals were given one injection of saline several days before the start of drug testing in order to acclimate them to the procedure. Animals were tested following injections of either muscimol (molecular weight = 114.1, 50 ng/side), D-amphetamine (molecular weight = 135.2, 10 µg/side) or [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin (DAMGO, molecular weight = 513.6, 0.25 µg/side). The dose chosen have all been shown to produce effects in previous studies of operant behavior following injections in the AcbSh (Covelo et al, 2011; Wirtshafter and Stratford, 2010; Zhang et al., 2003).

2.6. Procedure

Rats received six test sessions in the operant chambers following intracranial drug injections separated from each other by at least two days during which animals continued to be run. These six sessions were divided into three groups of two injections, each consisting of a drug injection and a paired saline treatment; i.e., a separate saline control session was run in proximity to each drug test. The order of drug and saline injections was randomized within each drug condition, and the order of drug testing was randomized between subjects. Test sessions following injections of DAMGO, or its paired vehicle treatment, were 240 min. in duration, based on data suggesting that the effects of this are drug frequently delayed (Bakshi and Kelley, 1993; Taha et al., 2009; Zhang et al., 2003; Zhang and Kelley, 1997), whereas other test sessions were of 60 min duration.

2.7. Perfusion and histology

At the completion of behavioral studies, animals were deeply anesthetized with sodium pentobarbital and perfused transcardially