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Evidence for motivational effects elicited by activation of GABA-A or dopamine receptors in the nucleus accumbens shell

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ABSTRACT

Microinjections of the inhibitory GABA-A receptor agonist muscimol into the shell region of the nucleus accumbens (AcbSh) have been reported to induce large increases in food intake, but the effect of these injections on motivational processes is less clear. In the current study, bilateral injections of saline, muscimol (50 ng/side) or D-amphetamine (10 μg/side) were made into the AcbSh of rats trained to lever press on a progressive ratio schedule for food reward. Injections of both muscimol and amphetamine were found to produce a large increase in the breaking point relative to saline injections. This result suggests that inactivation of the AcbSh does not simply drive ingestive behavior, but also affects motivational processes assessed by the progressive ratio schedule. Breaking points were also increased by injections of amphetamine into the AcbSh.

1. Introduction

Evidence has accumulated for many years suggesting that the nucleus accumbens is importantly involved in a broad range of motivated behaviors, but more recently it has become apparent that the shell region of the accumbens (AcbSh) may play a more specific role in the control of feeding (Stratford, 2007). For example, injections of the inhibitory GABA-A agonist muscimol into the AcbSh induce large increases in food intake by nondeprived rats, but have no effect on the intake of water or saline solutions, or on locomotor activity in the presence of food (Basso and Kelley, 1999; Stratford and Kelley, 1997). These effects seem specific to the medial AcbSh, as similar injections into the lateral shell or the core of the accumbens have much smaller effects on feeding (Basso and Kelley, 1999). It should not be surprising that different behavioral effects can be elicited from the shell and core regions of the accumbens as the connections of these two areas are quite different. For example, of the two, only the AcbSh projects to the lateral hypothalamus (Heimer et al., 1991; Williams et al., 1977; Zahm and Brog, 1992), a region long considered to play a major role in the control of feeding, and several lines of evidence suggest that the lateral hypothalamus may indeed mediate some of the ingestive effects obtained from the AcbSh (Stratford, 2005; Stratford and Kelley, 1999; Zheng et al., 2003).

Although it is well established that muscimol injections into the AcbSh can induce feeding in sated animals, no consensus has been reached as to the appropriate functional characterization of this effect. For example, it is possible that inhibition of cells in the AcbSh might increase the perceived "palatability" of food, or induce a state similar in some respects to that produced by food deprivation. However, a very different sort of proposal has been made by Kelley and colleagues (Baldo and Kelley, 2007; Kelley et al., 2005; Meredith et al., 2008) who have suggested that inhibition of cells in the AcbSh does not affect motivational mechanisms, but rather "bypass[es] certain inputs relevant to food-seeking behavior", acting "to directly 'switch on' motor programs specific to ingestion" (Hanlon et al., 2004). Presumably such a process would simply activate feeding without promoting appetitive behavior directed at food or without influencing the reward value of the food. The principal support for this claim comes from an experiment which failed to find an effect of intra-AcbSh injections of muscimol on performance on a progressive ratio schedule (Zhang et al., 2003).

In progressive ratio (PR) schedules, the number of responses that the subject must make in order to gain reinforcement increases after each reinforcement, so that progressively more effort is required to gain each successive reward. The classical measure of performance on PR schedules is the "breaking point" (sometimes called the "break point"), the number of responses made in the final ratio completed before failing to respond for some fixed length of time. The rationale for

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this measure is that the breaking point is presumed to reflect a situation where the "anticipated" effort required to obtain the next reinforcer has become sufficiently great that the animal stops responding. Since they were first introduced by Hodos and his coworkers (Hodos, 1961; Hodos and Kalman, 1963), PR schedules have become one of the most frequently employed tests for alterations in the motivational states of animals, although they are obviously also sensitive to alterations in motor response systems (Skjoldager et al., 1993). Breaking points typically increase when the food reward is made larger or more palatable (Hodos, 1961; Hodos and Kalman, 1963; Reilly, 1999), or when animals are subjected to relatively severe deprivation conditions (Ferguson and Paule, 1997; Hodos, 1961; Jewett et al., 1995; Skjoldager et al., 1993). Breaking points are also increased in response to a number of central manipulations which promote food intake, including intra-hypothalamic injections of orexin A (Thorpe et al., 2005), intraventricular injections of neuropeptide Y (Jewett et al., 1995) or direct injections of the opiate agonist DAMGO into the nucleus accumbens (Zhang et al., 2003). Given this pattern of results, the failure of intra-AcbSh muscimol to alter PR responding is indeed striking and it is understandable that this failure would prompt the suggestion that GABAergic mechanisms in the AcbSh are specifically involved in the production of motor patterns.

Only a single paper has investigated the effects of intra-AcbSh muscimol on PR performance (Zhang et al., 2003). Given the substantial theoretical importance attributed to the results of that study, it seemed worthwhile to us to reexamine this issue using a slightly modified method which, as will be discussed below, seems to offer important advantages over that employed in the experiment of Zhang et al. (2003). For comparative purposes, and as a positive control, we also examined the response to intra-AcbSh injections of amphetamine.

2. Methods

2.1. Animals

Subjects were six male Sprague–Dawley rats obtained from Charles River (Chicago, IL) weighing approximately 300 g at the time of surgery. Animals were individually housed in plastic cages with food 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 stainless steel screws and denture lining material and stainless steel obturators were inserted into the lumens of each cannula to help maintain patency. Each rat was allowed to recover for at least seven days before deprivation and operant training began.

2.3. Apparatus

Animals were trained in identical standard twin lever operant chambers (Med-Associates, St. Albans, VT) housed within sound attenuating chambers with masking noise provided by an exhaust fan. The chambers were equipped with a click generator to provide audible feedback of food delivery and a 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, which continued for the remainder of the experiment, 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 (45 mg Precision Dustless pellets, which have a macronutrient composition similar to the maintenance diet, BioServe, Frenchtown, NJ) were presented at 1-min intervals, with a "click" being generated at the same time as food delivery. Animals were shaped to lever press over one or two days, and then placed on a continuous reinforcement schedule for two days. Rats were then given one 30-min session of training on an FR2 schedule followed the next day by one on an FR4 schedule. Subjects were then switched to PR6 schedule, which continued for the remainder of the experiment. On each day each rat was placed into an operant chamber with the house light on and both levers extended; only one lever was associated with food reward, although presses on both levers were recorded. The first response on the correct lever was followed by food reward, paired with the operation of the clicker. The number of responses required to earn each subsequent food pellet was incremented by six after each reinforcer, so that seven responses were required to earn the second pellet, 13 to earn the third, and so on. The time of each lever press was recorded, as were the times at which head entries into the food hopper occurred. Each session continued until a pause in responding of 3 min duration occurred, a cut-off value which has been used in other studies (Reilly, 1999), or 60 min elapsed, at which time the house lights were turned off, the levers retracted, and the rats removed from the chambers. The breaking point was calculated as the final ratio completed in the session. Animals run for five to six days per week and were given 16 daily training sessions on the PR6 schedule before the start of drug treatments.

2.5. Intracerebral injections

In order to make injections, rats were restrained gently, the obturators removed, and a 28-gauge stainless steel injection cannula, extending 2.0 mm beyond the ventral tip of the guide, was inserted into each guide cannula. Rats then received simultaneous bilateral 0.50 µl infusions at a rate of 0.33 µl/min by means of 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 cannula track after which they were removed and replaced with the obturators. Animals were then returned to their home cages for a period of 10 min, to allow for drug diffusion, after which they were placed in the operant boxes for their daily run. Each subject received one saline injection several days prior to the start of drug injections to acclimate them to the procedure. Beginning on day 17 of training on the PR6 schedule, animals received, in a randomized order, injections of muscimol (50 ng/side, Tocris, Ellisville, MO), D-amphetamine (10 µg/side, Sigma Chemical Co, St. Louis, MO), or the sterile, isotonic saline vehicle. Injections were separated from each other by at least two days and animals were run in the operant boxes on at least one, and frequently both, of these days.

2.6. Perfusion and histology

At the completion of behavioral studies, animals were deeply anesthetized with sodium pentobarbital and perfused transcardially with saline followed by 10% formalin. The brains were then removed and stored in formalin for several days after which cryostat sections were prepared through the injection site at a thickness of 50 µm and subsequently stained with cresyl violet.

3. Results

3.1. Histology

Examination of stained sections indicated that all of the cannulae terminated bilaterally within the ventral portion of the AcbSh (Fig. 1)

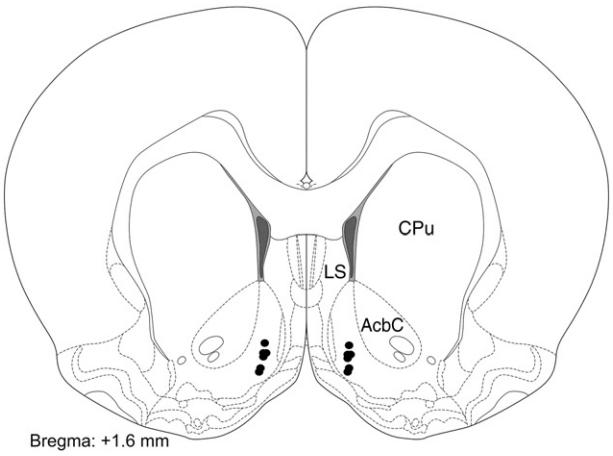

Fig. 1. Schematic representation of injection sites in the nucleus accumbens shell. AcbC: nucleus accumbens core, CPu: caudate-putamen, LS: lateral septum.

at locations very similar to those we have examined in previous studies (Stratford, 2005; Stratford and Kelley, 1997; Stratford and Wirtshafter, 2004).

3.2. Behavioral data

Compared to injections of saline, intra-AcbSh injections of either muscimol or amphetamine produced very similar increases in the breaking points of rats performing on the PR6 schedule, as shown in panel A of Fig. 2. Analysis of this data by means of a one-way analysis of variance (ANOVA) with repeated measures indicated a significant treatment condition ($F(2,10) = 5.86$, $p<0.025$) and single degree of freedom contrasts indicated that both drug groups differed significantly from the saline condition ($p<0.025$). All earned pellets were eaten by all subjects.

Numbers of presses on the reinforced lever are shown in panel B of Fig. 2, and statistical analysis of these results again indicated a significant overall effect of drug treatment ($F(5,10) = 5.35$, $p<0.05$), with more responses being made after injections of either muscimol or amphetamine, as compared to saline ($p<0.05$ in both cases). Numbers of responses on the nonreinforced lever are also shown in the lower panel of Fig. 2, where it can be seen that both muscimol and amphetamine tended to produce small increases in responding on this lever, as compared to saline injections. These differences were not statistically significant, however ($p>0.3$), and may have, at least in part, resulted from the fact that session duration was increased by the drug treatments, as described below.

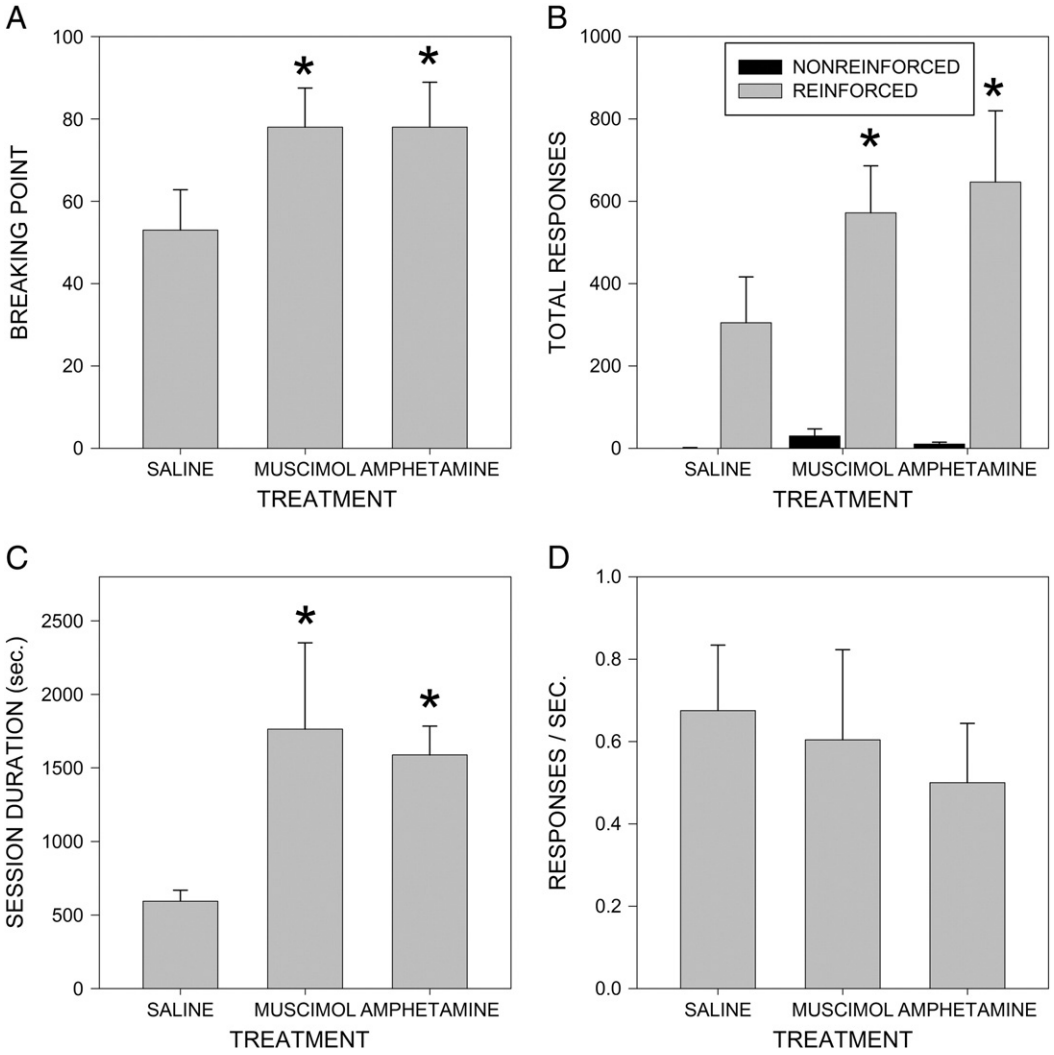

Fig. 2. Panel A: Mean breaking points of rats after injections of saline, muscimol (50 ng/side) or amphetamine (10 μg/side) into the AcbSh. * = $p<0.025$ vs. saline condition. Panel B: Mean total responses on the reinforced and nonreinforced levers after injections of saline, muscimol or amphetamine. * = $p<0.025$ vs. saline condition. Panel C: Mean session durations after injections of saline, muscimol or amphetamine into the AcbSh. * = $p\leq0.05$ vs. saline condition. Panel D: Mean response rates measured across the entire session after injections of saline, muscimol or amphetamine.

Increased numbers of lever presses could result either from animals pressing for a longer period of time, from rats responding at a higher rate, or from some combination of these two effects. Panel C of Fig. 2 shows that muscimol and amphetamine treatments both tended to produce similar increases in session duration, measured from the first lever press until the time the houselights were extinguished. Repeated measured ANOVA conducted on log transformed session duration scores indicated a significant effect of treatment condition ($F(2,10) = 5.10$, $p<0.05$) and analysis of contrasts indicated that both drug conditions differed significantly from the saline condition ($p<0.05$). Panel D of Fig. 2 shows that the mean rate of responding across the entire duration of the sessions tended to decrease slightly after drug treatment. A trend in this direction is not surprising, since drug treated animals ended up working on much higher ratios than did control subjects, but these differences were not statistically significant ($p>0.2$). Analysis of a number of other response measures including latency to the first lever press, mean latency to enter the hopper after food delivery, number of entries into the food hopper made when food was not present and post-reinforcement pause duration also failed to indicate significant differences between groups (data not shown).

4. Discussion

The results of this experiment clearly indicate that injections of both muscimol and amphetamine into the AcbSh increase the breaking point of rats responding on a PR6 schedule for food reinforcement. Responding on a nonreinforced lever was not significantly altered indicating that the effect on reinforced responding was not secondary to nonspecific effects such as alterations in general activity. These findings demonstrate that stimulation of GABA-A receptors within the AcbSh does not simply activate a motor pattern generator for feeding, but rather also alters "motivational" aspects of food reinforced behavior.

This conclusion differs drastically from that of Zhang et al. (2003) who found that progressive ratio performance was not altered by similar injections of muscimol into the AcbSh. Although there are a number of minor differences between that study and the current experiment, the most likely reason for the differing results relates to the ways in which PR schedules were administered in the two studies. We used a classical ("open-ended") PR design in which rats performed until their responding was interrupted by a pause of a predetermined length (3 min), at which time the session was terminated. This procedure resulted in relatively short duration sessions — for example, after saline injections the mean session duration, not counting the 3-min terminal segment, was about 7 min. In contrast, Zhang et al. used what has sometimes been called a "time-constrained PR schedule." All animals were run for a fixed length of time, 120 min, and the breaking point was calculated as the last ratio completed before the end of the session. Obviously the two measures of performance are not equivalent; breaking point in a classical PR schedule is defined in terms of pauses in responding, whereas breaking point in a time-constrained schedule depends solely on the number of ratios completed over the course of the session, irrespective of the duration of any pauses which might have occurred. Although it is likely that the two measures will be affected in a similar manner by various experimental manipulations, this may not always be the case. In the current context, however, it seems more important that the two techniques result in behavior being assessed over very different time periods. The available data suggest that the bulk of the feeding induced by muscimol in the AcbSh occurs in the first hour, primarily in the first 20–30 min following treatments (Basso and Kelley, 1999; Stratford and Kelley, 1997; Stratford and Wirtshafter, 2007). Assessing performance by measuring the total number of ratios completed over a two hour period may be a suboptimal way to detect an effect of muscimol, as alterations in responding occurring early in the testing period may be obscured by responding which occurs later, after the drug is no longer exerting a hyperphagic effect. It is striking that the data presented by Zhang et al. (2003) show that their saline injected rats continued to press at substantial levels throughout the entire duration of the session, while muscimol injections, at the same dose used here, tended to increase responding during the first 45 min following injections, but actually tended to suppress it during the second hour post treatment, with the result that total numbers of presses did not differ significantly between groups. It should be noted that the more an animal responds during the early stage of a time-constrained PR schedule, the higher the ratios presented to it in the latter part of the session will be. Since response rates tend to be slower on higher ratios (Hodos and Kalman, 1963; Rickard et al., 2009), it is plausible that manipulations which selectively increase response rates in the early part of a time-constrained session would actually result in reductions in rates at later times, thus tending to render the breaking point measure insensitive.

Many previous studies have implicated dopamine in the control of PR performance. For example, increases and decreases in responding have been reported after systemic injections of low doses of amphetamine (Poncelet et al., 1983; Smith et al., 1997) or of dopamine antagonists (Aberman et al., 1998), respectively, and reduced response output has also been observed after dopamine depleting lesions of the nucleus accumbens produced by local injections of 6-hydroxydopamine (Aberman et al., 1998). Our current finding of increased breaking points following injections of amphetamine into the AcbSh is consistent with the data obtained using a time-constrained design (Zhang et al., 2003), and suggests that the AcbSh, in particular, may play a role in PR performance.

In contrast to muscimol, injections of amphetamine into the AcbSh have no effect on food intake (Hanlon et al., 2004); it is thus striking that the two drugs produce similar effects on PR performance. This pattern of results can be explained in two ways. First, amphetamine and muscimol may differentially affect neural circuitry so as to produce distinct functional effects with partially overlapping behavioral manifestations. For example, amphetamine injections might reduce the perception of "work-related response costs" (Aberman et al., 1998) associated with lever pressing, whereas muscimol injections might potentiate the reinforcing properties of food. Both of these effects might be expected to increase lever pressing, but only the latter would be expected to increase intake. Obviously it is a requirement of this type of approach that amphetamine and muscimol affect *distinct* functional mechanisms. For example, injections of both of these compounds into the nucleus accumbens have been proposed to increase "wanting" of food (Reynolds and Berridge, 2002; Wybell and Berridge, 2000), but to have explanatory value, such a proposal would have to explain why increased "wanting" is associated with increased feeding after muscimol, but not after amphetamine, injections. Alternatively, amphetamine and muscimol may produce effects on multiple neural mechanisms which overlap partially, but not completely. For example, both amphetamine and muscimol might exert similar effects on one population of cells in the AcbSh, resulting in an increased breaking point, whereas muscimol, but not amphetamine, might additionally influence another group of cells through which changes in feeding behavior are produced. Clearly, further experimentation is necessary to address these issues.

In summary, the current study demonstrates that injections of muscimol into the AcbSh produce changes in performance on a PR schedule which are very similar to those seen after amphetamine. This finding indicates that the effect of muscimol cannot be adequately characterized as a simple disinhibition of feeding pattern generators, and suggests that inhibition of AcbSh neurons must influence motivational factors as well.

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