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Influence of bombesin on thresholds for feeding and reward in the rat

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Abstract. Bombesin's purported role in satiety mechanisms prompted this investigation of its effects on thresholds for stimulation-induced feeding and self-stimulation in the rat. Single electrodes were implanted in the lateral hypothalamus and the ability of each electrode to support self-stimulation and stimulation-induced feeding was evaluated at four current levels between 80 and 320 μA. The frequency thresholds associated with each current value were assessed following four intraperitoneal doses of bombesin, 2, 4, 8, and 16 μg/kg, as well as a saline dose. Bombesin increased the thresholds for stimulation-induced feeding at doses known to reduce food intake without influencing self-stimulation thresholds. From these findings we conclude that (1) the effects of peripheral bombesin on stimulation-induced feeding are analogous to its effects on normal feeding and (2) the data provide additional evidence for a pharmacological dissociation between stimulation-induced feeding and reward.

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INTRODUCTION

While it is well-known that electrical stimulation of the lateral hypothalamus (LH) elicits both stimulation--induced feeding (SIF) and self-stimulation (SS), until recently, the arguments as to whether the neural circuitries underlying the two behaviors are anatomically distinct were not firmly substantiated (Hoebel and Teitelbaum 1962, Margules and Olds 1962, Wise 1974). Lately, the development of techniques for inferring the electrophysiological properties of neurons activated during stimulation (Yeomans 1975, Shizgal et al. 1980, Bielajew and Shizgal 1982a,b, Durivage and Miliaressis 1987) has yielded data more in favor of the notion that the same directly stimulated substrate supports feeding and reward (Hawkins et al. 1983, Gratton and Wise 1988a,b). For example, there is significant overlap in the estimates of refractory period for SS and SIF (Hawkins et al. 1983, Gratton and Wise 1988a), and results of the collision test for assessing the nature of the functional connectivity between the LH and ventral tegmental areas (Shizgal et al. 1980, Bielajew and Shizgal 1982b) have suggested that fibers with remarkably similar conduction velocities subserve both SS and SIF (Gratton and Wise 1988b).

The findings from a variety of pharmacological studies lend indirect support to this idea. For example, both systemically administered 9-tetrahydrocannabinol and centrally injected morphine into the ventral tegmental area similarly facilitate SS and SIF elicited from the anterior medial forebrain bundle (Jenck et al. 1986, 1987, Gardner et al. 1988, Rompré and Wise 1989, Trojniar and Wise 1991), while intraperitoneal pimozide disrupts both SIF and SS (Streather and Bozarth 1987, Hunt and Atrens 1992).

It is rarer to observe a pharmacological dissociation of the two behaviors. However, a few studies of this nature have been reported. For example, frequency thresholds for SIF and SS obtained through the same LH electrode are decreased (facilitation) and increased (inhibition), respectively, following intraperitoneal picrotoxin (Porrino and Coons 1980) while central injections of cholecystokinin produce a different pattern - no effect on SS frequency thresholds and an inhibitory one on SIF (Konkle et al. 1999). These reports do not necessarily contradict the theory that the directly stimulated fibers underlying SS and SIF are identical. A recent study reported that increases in current differentially affect SS and SIF; the SS rate-frequency curves are moved leftward while the asymptotic portion of the corresponding SIF curves increase (Waraczynski and Kaplan 1990). The authors interpreted the dissimilar parametric profiles to suggest that SS and SIF are characterized by different postsynaptic integrators. Likewise, pharmacological studies that demonstrate a dissociation between SS and SIF can be said to describe a property of the circuitry beyond the first stage or directly stimulated substrate.

The aim of the present study was to examine the effects of a compound known to influence natural feeding, on SIF and SS using threshold measures (Gallistel et al. 1981, Liebman 1983, Miliaressis et al. 1986). Bombesin, a putative satiety peptide, was chosen because immunoreactivity and in situ hybridization studies have shown that bombesin-like peptides and their receptors appear in the gut and the central nervous system of mammals (Erspamer and Melchiorri 1975, Zoeller et al. 1989, Battey and Wada 1991) and peripherally administered bombesin has been shown to suppress food deprivation-induced feeding (Gibbs et al. 1979), stress-induced feeding (Morley and Levine 1981), and sham feeding (Martin and Gibbs 1980) and to induce a sequence of behaviors typically associated with satiety (Gibbs et al. 1980). Furthermore, endogenous bombesin-like peptides appear to be physiologically important in the modulation of food intake (Kateb and Merali 1992, Merali et al. 1993).

We were specifically interested in addressing two questions. First, does bombesin affect SIF in the same way as it is known to alter normal ingestion and second, are such effects specific to SIF and not SS.

METHODS

Subjects

Five male Long-Evans rats (Charles River Laboratories) were housed individually in plastic cages with *ad libitum* access to food and water. They were maintained on a 12 h light and 12 h dark cycle with lights on at 7.00 h. At weights of 300-340 g, the animals were prepared for stereotaxic surgery using a combination of sodium pentobarbital anesthesia (65 mg/kg i.p.) and xylazine (0.05 mL i.m.). With the head oriented in a flat-skull position, four subjects were implanted with bilateral monopolar stimulating electrodes aimed at the LH; the fifth subject received a single LH electrode. The coordinates, based on the Paxinos and Watson (1986) atlas, were 2.6 mm posterior to bregma, 1.7 mm lateral to the mid-saggittal suture, and 8.2 mm ventral to the skull surface reading at bregma.

Apparatus

The monopolar electrodes were fashioned from 250 μm, stainless steel wire and insulated with Epoxylite to the flattened tips. A wire soldered to a gold amphenol pin and wrapped around four stainless steel skull screws served as the current return. The entire electrode assembly was anchored to the skull screws with dental acrylic.

All tests were conducted in a wood and Plexiglas box measuring 28 cm x 38 cm x 44 cm. A rodent lever protruded from a side wall approximately 3 cm above the floor. During SIF tests, access to the lever was blocked by a piece of wood and the floor of the chamber was covered with rat chow pellets.

A constant-current amplifier (Mundl 1980) and an integrated circuit pulse generator built in-house supplied the electrical stimulation. The current was continuously monitored on an oscilloscope by reading the voltage drop across a 1 kΩ precision resistor in series with the rat. During SS tests, each depression of the lever produced a 500 ms train of rectangular, monophasic, cathodal pulses of 100 μs duration; during SIF tests, the trains of stimulation alternated between a 20 s on and 20 s off schedule. Current and frequency were varied as described below.

Drugs

Bombesin was obtained from Peninsula Laboratories (Belmont, California). Freeze dried aliquots were dissolved in 0.9% saline in varying concentrations such that the injection volumes across doses were identical (1 ml/kg). The vehicle injections consisted of 0.9% saline.

Procedure

SIF SCREENING

The presence of SIF was evaluated at three current levels - 50, 100, and 150 μA, and each current was tested at three frequencies - 20, 32, and 63 Hz. The screening procedure began with a 20 s trial of a combination of current (50 μA) and frequency (20 Hz) equivalent to the lowest level of stimulation. The frequency was then increased to 32 and 63 Hz for the subsequent two trials after which the current was increased and presented again with the same frequency values. This procedure was continued until the response at all current levels had been assessed. Each current-frequency combination was tested twice within a screening session.

A site was considered positive for SIF if feeding was initiated during the 20 s on phase and terminated at onset of the 20 s off phase. At least one electrode per subject was found to support SIF during the first screening session. Once the current-frequency combination that produced SIF was determined, the current was kept constant and the frequency was decreased in 0.05 common log unit steps until SIF was not observed for two successive frequencies. The threshold was defined as the first frequency associated with an absence of SIF.

SS SCREENING

Lever pressing for electrical stimulation was evaluated using standard operant shaping techniques. Once the behavior was deemed reliable, meaning that the animals readily pressed the lever without prompting, subjects were trained to respond during a 60 s discrete trial. Each trial was preceded by 5 trains of priming stimulation delivered 1 s apart. The value of the priming stimulation was identical to that of the stimulation parameters available in the succeeding trial and was intended to signal the beginning of a new trial. Once attention to the primes was acquired (initiation of lever pressing within 12 s following the delivery of the priming stimulation), the current was held constant and an ascending frequency series was introduced. Beginning at a value that produced no response, the frequency was increased by 0.10 common log units at the start of each 60 s trial until the rate exceeded the criterion response rate. The threshold was defined as the frequency at which 35 responses/min was obtained and was interpolated from the rate-frequency function. This index for threshold was chosen to avoid eliciting seizures in animals, a condition that has been shown to occur from stimulation at this site when rats respond at their maximum rates.

STABILIZATION

When the SS and SIF frequency thresholds generated at the stabilization current did not vary by more than 0.10 common log units within a session, preliminary current-frequency trade-off functions were collected at four currents - 80, 125, 200, and 320 μA. The within session stability criterion was defined as a difference of no more than 0.10 common log units in the frequency thresholds collected at 200 μA at both the beginning and end of each

session. Drug tests began when the SS and SIF frequency thresholds associated with each current met the above criterion.

DRUG TESTS

Both SS and SIF were tested during the same session which lasted approximately one hour. Three intraperitoneal doses of bombesin as well as a saline dose were initially assessed: saline, 2, 4, and 8 μg/kg. The order of doses was determined using a 4 x 4 Latin Square design with the premise that four replications of the current-frequency trade-off function for each dose would be sufficient to obtain a consistent result. SIF and SS tests were interdigitated so that two sessions began with SIF tests and two with SS tests. The four currents were presented randomly and were independent of the other behavior's current schedule. At the beginning of each session, the subject was administered an intraperitoneal injection of the assigned dose of bombesin; stimulation tests began 10 min later. Animals were tested daily for 16 days. At the end of this protocol, three subjects were evaluted with an additional intraperitoneal dose of 16 μg/kg bombesin, administered every second day for a total of four sessions; two control (saline) injection tests were conducted during the non-drug days.

HISTOLOGY

When all behavioral tests were completed, the subjects were perfused intracardially with a solution of 0.9% saline followed by 10% formalin. The brains were removed and stored, for at least one week, in 10% formalin after which the 40 μm sections were collected and stained with cresyl violet. The location of the electrode tips was based on the Paxinos and Watson (1986) atlas.

Statistical analyses

The frequency thresholds were analyzed using an ANOVA design with four repeated factors - behavior (SS, SIF), dose (saline, 2, 4, and 8 μg/kg bombesin), current level (80, 125, 200, and 320 μA), and the four repetitions (Statistica 98). The highest dose tested (16 μg/kg) was excluded from the analysis since it was not part of the original design and delivered to only a subset of the five animals. Greenhouse-Geisser correction for violations of sphericity was applied where appropriate - factors with more than two levels (Howell 1997); the Greenhouse-Geisser epsilon values are reported with the F values rather than the adjusted degrees of freedom.

RESULTS

Figure 1 shows the location of the five electrodes, all of which were verified to be within the boundaries of the lateral hypothalamus/ medial forebrain bundle.

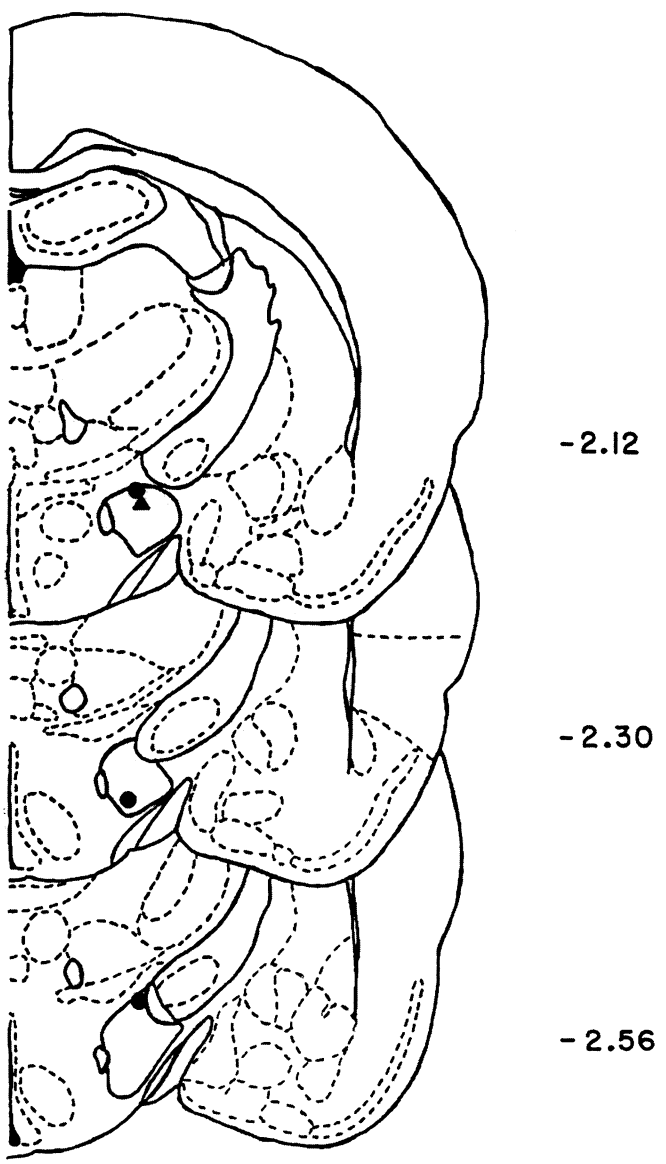

Fig. 1. Tracings from the Paxinos and Watson (1986) atlas plates that best match the location of the electrode tips. The distance (mm) behind bregma of each plate is listed on the right; note that the filled circle associated with the middle plate (-2.30) represents the placements of two animals.

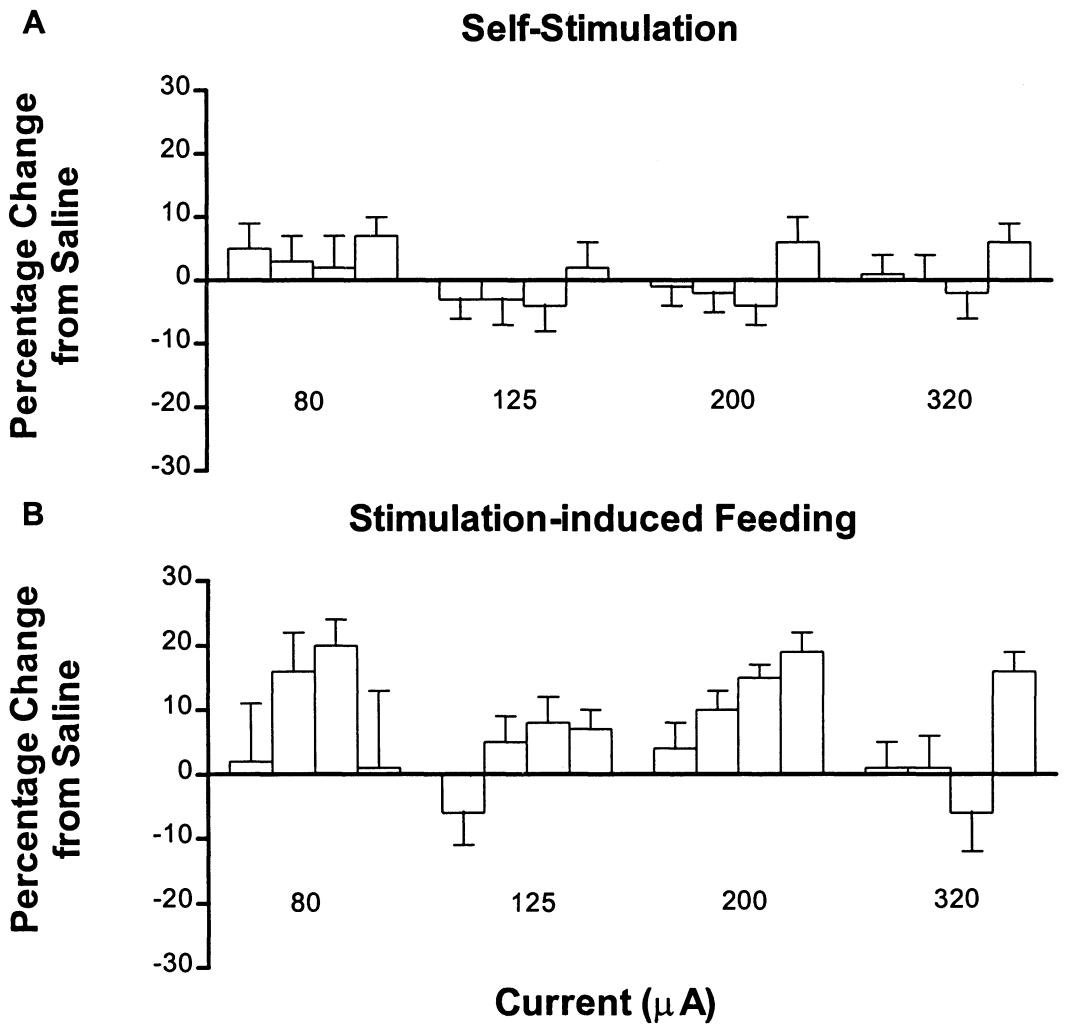

Fig. 2. A, summary of SS data. Mean change in frequency threshold expressed as a percentage of the average saline frequency threshold, plotted as 0% on the ordinate. The results are grouped according to current (μA), the value of which is indicated below each group. Within each current group from left to right, the four bars and associated standard error of the mean represent the threshold changes following 2, 4, 8, and 16 μg/kg bombesin (i.p.). B, summary of SIF data expressed as in 2A.

The effects of the four doses (2, 4, 8, and 16 μg) of bombesin on SS and SIF, plotted as threshold changes, are illustrated in Fig. 2A and B. The data are expressed as percentage changes from saline (represented as the 0% point on the ordinate); an increase in this value indicates that a higher frequency was required to sustain a criterion level of responding (either feeding or bar-pressing) and thus, is interpretted as a suppression of the behavior. Hence, the opposite pattern, a decrease in the percentage change from saline, is understood as an augmentation of the behavior. The results of the omnibus analysis yielded a significant effect of behavior ($F_{1,4}$ = 22.40; P<0.01), current ($F_{3,12}$ = 102.50; P<0.001; ε = 0.04), and their interaction ($F_{3,12}$ = 19.48; P<0.001). These are expected given the difference in baseline thresholds between SS and SIF (see Table I). The other main effects, repetition and dose, were found to be non-significant. Of primary interest to us was the significant interaction between behavior and dose ($F_{3,12}$ = 5.01; P<0.05). To examine this further, we evaluated the behaviors separately *via* an interaction contrast and confirmed the pattern suggested by Figure 2, that SIF thresholds rise, in an almost linear manner, with increasing doses of bombesin ($F_{3,12}$ = 4.40; P<0.05) while SS thresholds remain unaltered. No other higher order interaction with the exception of the one combining all factors ($F_{27,108}$ = 1.69; P<0.05) was detected.

TABLE I

Range of frequency thresholds across animals for each behavior

	SS	SIF
80 μA	68-286 Hz	41-97 Hz
125 μA	42-135 Hz	24-34 Hz
200 μA	28-69 Hz	14-34 Hz
320 μA	19-34 Hz	11-15 Hz

The range of frequency thresholds across animals for each current and behavior is shown in Table I. It is intended to illustrate the differences in the frequencies needed to elicit each behavior, which was always lower in the case of SIF, at any one current.

DISCUSSION

As in the case of spontaneous or food-deprivation induced feeding (Gibbs et al. 1979, Merali et al. 1993), bombesin was found to suppress SIF in the current study. In contrast, bombesin failed to influence SS thresholds, suggesting that bombesin-induced anorexia is not due to generalized anhedonia and adds further evidence to the position supporting a functional dissociation between SIF and SS (Porrino and Coons 1980, Bielajew and Bushnik 1994).

The difference in the range of thresholds across animals is reported in Table I. The trade-off between current and frequency for each behavior that is suggested from these values corroborates our earlier finding (Bielajew and Bushnik, 1994) and that of Waraczynski and Kaplan (1990), which is that the trade-offs representing SIF data are consistently shallower than those for SS; it is believed that this feature characterizes differences in the integrative properties of the two behaviors.

Bombesin has been reported to be ineffective in producing a decrease in food intake in food-deprived rats at an intraperitoneal dose of 2 μg/kg (Laferriere et al. 1992) whereas peripheral doses of 4, 8, and 16 μg/kg have been shown to dose-dependently reduce food intake (Ladenheim and Ritter 1991). The same pattern was generally observed in SIF in this study. However, the suppression of normal feeding by bombesin is typically a larger effect than the one observed here on SIF.

There are a number of reasons why SIF may be more modestly affected by bombesin than is the case with normal feeding. First, although SIF is generally responsive to the same challenges that affect natural feeding (Tenen and Miller 1964, Devor et al. 1970, Hollister 1971, Carr and Simon 1984, Jenck et al. 1986, 1987, Trojniar and Wise 1991, Gosnell and Krahn 1993), the elicitation of feeding by electrical means bypasses the usual contribution of sensory mechanisms to normal ingestion, all of which may be relevant to the production of satiety.

Second, a concern regarding the specific action of bombesin is whether its effect is exerted through the induction of satiety or the production of malaise (Deutsch 1980, Gibbs and Smith 1980, Deutsch and Parsons 1981, McCoy et al. 1990). If bombesin suppresses feeding due to a general discomfort, the electrically induced stimulus to feed and the relatively short intervals during which feeding is typically elicited (20 s) may not be as revealing or as sensitive to this effect. From the literature, however, it seems that the contribution of malaise may become a factor only at the highest dose of bombesin (16 μg/kg i.p.) used in this study. Bombesin has been shown to produce a conditioned taste aversion at an intraperitoneal dose of 16 μg/kg (Deutsch and Parsons 1981); however, at lower doses still capable of producing satiety (4 μg/kg i.p.), a conditioned taste aversion has not been observed (Kulkosky et al. 1981). Similarly, while defensive burying of a milk spout that had been previously paired with bombesin doses of 4, 16, or 32 μg/kg (i.p.) has been reported, the lowest dose of bombesin induced far less of this behavior than the higher ones (Bowers et al. 1983). Thus, the modest suppression of SIF observed at 4 and 8 μg/kg of bombesin in this study is probably not due to the production of malaise. Furthermore, in SS tests, no changes in response rates, a valid indicator of performance effects (Gallistel et al. 1981, Liebman 1983, Miliaressis et al. 1986), were observed at any dose of bombesin.

The third possibility that might explain why we failed to observe a stronger suppression of SIF following doses of bombesin that are known to induce satiety may lie in our measurement procedure. In this study, we noted the simple presence or absence of SIF during the 20 s stimulation trial which may be less sensitive than, for example, the amount of food eaten. In fact, it has been observed that peripheral administration of bombesin appears to reduce normal food intake in two ways - one, through decreasing the size of a meal and two, through increasing the inter-meal interval (Gibbs et al. 1979, King 1991).

Our simple measure of SIF as an index of bombesin's effect on feeding may be inadequate to assess all facets of the drug's actions.

This study adds an extra dimension to this area by evaluating a peptide that is strongly implicated in the peripheral and central induction of satiation following natural feeding. In addition, it contributes to the body of literature which has demonstrated a behavioral dissociation of feeding and reward and supports the idea that while the directly stimulated fibers have very similar characteristics (Gratton and Wise 1988a,b) and therefore the two behaviors may result from activation of the same fibers, there is a significant separation of the substrates underlying SS and SIF downstream from the site of stimulation.

Thus, we have demonstrated that bombesin-induced suppression of food intake is a relatively specific effect and not due to a generalized anhedonia. The suppression of SIF and unaltered SS thresholds at doses of bombesin known to induce satiety suggests a dissociation between two motivational states.

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