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Bombesin decreases yawning in a high-yawning subline of Sprague–Dawley rats

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Abstract

This study analysed the effect of the intracerebroventricular administration of bombesin (BN) at doses of 0.001, 0.005, 0.1 and 1.0 μg/2 μl on yawning, grooming and other behavioral correlates in two inbred strains of male rats. These were selected for high-yawning (HY) and low-yawning (LY) frequency, a difference that correlates with novelty-induced grooming. Grooming increased with BN in a strain-specific manner, and yawning decreased in HY rats. Principal component analysis (PCA) showed that rats' behaviors changed from yawning to grooming with BN. Such change differed between the strains. While the first principal component was dominated by grooming in both strains, the second principal component was dominated by stretching and penile erections in HY rats, and by scratching in LY rats. While LY rats spent more time in scratching both within and outside grooming bouts, HY rats tended to favour the latter category. An increment in mean duration of grooming bouts characterized the effect of the highest dose. These findings show that BN inhibits yawning and increases grooming, suggesting that this peptide enhances the initial response to novel environments. The study shows the importance of combining studies on inbred strains with appropriate multivariate methods to separate drug-induced behavioral patterns.

Keywords: Inbred strains; Rats; Stress; Penile erection; Principal component analysis; Scratching; Stretching; Yawning; Grooming

1. Introduction

The effects of the central administration of bombesin (BN) have been documented for a variety of behaviors (Kulkosky et al., 1982; Cowan et al., 1985). Some of the most conspicuous are vigorous scratching (Brown et al., 1977), excessive grooming and wet-dog shaking (Gmerek and Cowan, 1981). BN also stimulates dose-dependent elevations in plasma adrenocorticotrophic hormone (ACTH), corticosterone, catecholamines and glucose (Brown et al., 1979, 1988; Gunion et al., 1989; Plamondon and Merali, 1993, 1997; Malendowicz and Nussdorfer, 1995). These findings have led to suggest that BN-like peptides play a role in the mediation of the stress response (Kent et al., 1998). The behavioral reactions to mild stress, however, include not only excessive grooming but also other behaviors such as yawning (Delius, 1967, 1988; Dourish and Cooper, 1990). Yet, there are few reports on the relation between BN and yawning (Kulkosky et al., 1988) which could indicate whether BN has different behavioral correlates from other peptides involved in the stress response. Indeed, peptides such as ACTH, whose link to stress is well established (Akil and Moreno, 1995), elicit grooming and yawning (Gispen and Isaacson, 1981; Argiolas and Melis, 1998). The lack of such studies is partly because yawning occurs spontaneously at a very low frequency, making it difficult to appreciate little variations that may otherwise, indicate meaningful changes.

We have in our laboratory two strains of Sprague–Dawley rats that were selectively bred for high-yawning (HY) and low-yawning (LY) frequency (Urbá-Holmgren et al., 1990). The strains also differ in other behaviors. For example, HY rats circulate in an open field arena and groom in a novel environment more than LY rats (Moyaho et al., 1995; Eguíbar and Moyaho, 1997). The fine structure of water-induced grooming is also different between both strains: LY rats show sequences that are more stereotyped than those of HY rats (Moyaho et al., 1995). In addition, HY rats yawn more than LY rats after the administration of cholinesterase inhibitors and dopa-

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mine receptor agonists (Urbá-Holmgren et al., 1993). Thus, the unusual characteristics of these strains make it interesting to study the behavioral correlates of BN administration in these rats. This might contribute to our understanding of the potential role of the BN-related peptides in the stress response.

The present study assessed the effects of centrally administered BN on yawning and grooming in HY rats. The study included the analysis of other behaviors that commonly correlate with yawning or grooming. Because of the number of behaviors recorded, we analysed the data with multivariate methods as recommended by Ståhle and Wold (1988) to avoid misinterpretation of results. LY strain was also used for comparative purposes.

2. Materials and methods

2.1. Animals

We used 30 HY (316.77±6.24 g) and 30 LY (332.57±5.46 g) male rats bred in our own colony. After weaning (30 days), they were housed (four rats a cage, 46×32×20 cm) with free access to food (PMI, USA) and tap water. The colony room environment was maintained at 21±1 °C and under a 12-h light–dark cycle (lights on at 0700 h). Experiments were conducted in accordance with protocols approved by the Mexican rules on animal experimentation and welfare for experimental animals.

2.2. Surgery and drugs

Rats were anaesthetized with chloral hydrate (0.3 g/kg). Then, a stainless-steel guide cannula (22 gauge, Plastics One, USA) was stereotaxically implanted in the left lateral ventricle (AP: −0.6 mm, ML: −1.4 mm, DV: −3.2) of each rat according to the atlas of Paxinos and Watson (1998). The cannula was then secured with cranio powder (Plastics One), and stainless-steel stylets were inserted to maintain patency. Rats were allowed 1 week to recover from surgery. During this time, each rat was handled daily for subsequent intracerebroventricular injections.

On days of experiments, the rats were brought to the experimental room. To inject BN or saline solution (see below), a 28-gauge injector attached to tygon tube was inserted through the indwelling cannula. The distal end of the tubing was attached to a 5-μl Hamilton syringe. BN (Sigma-Aldrich, USA) was dissolved in 0.9% saline; doses of 0.001, 0.005, 0.1 and 1.0 μg/rat were used. Two microliters were injected over 60 s. Control animals received an equivalent volume of saline. We did not cover the intermediate doses because there is evidence that no substantial behavioral changes occur with them (Kulkosky et al., 1982, 1988). In addition, the study was not intended for determining dose–response curves, because several behaviors were analysed and possibly many are incompatible, making the interpretation of dose–response functions difficult.

After experimentation, the animals were injected through the guide cannula with 3 μl of methylene blue

Table 1
Means ± S.E. for HY variables for controls and BN-treated groups

	GE	TG	YS	PE	ST	BS	GI	TGI	GD	TGD	TG/GE	TGI/GI	TGD/GD
Saline, n=6													
Mean	24.83	351.8	13.17	0.33	1.17	1.17	12.33	107.5	2.33	23.5	16.4	7.47	6.14
S.E.M.	4.17	82.6	3.66	0.21	0.65	0.54	3.34	36.2	0.96	11.3	4.17	2.54	2.37
BN 0.001, n=6[a]													
Mean	21.5	452	9.67	0.33	1.33	3.17	6.5	77.7	2.83	40.8	25.03	14.61	12.53
S.E.M.	7.83	102	0.84	0.21	0.42	1.01	1.82	19.5	0.7	12.6	4.2	4.07	4
BN 0.005, n=6[a]													
Mean	8.83	282.8	9.33	0.5	0.83	3.33	2.83	54.3	2.83	54.8	32.32	19.94	16.01
S.E.M.	1.56	64	2.08	0.22	0.4	0.56	0.48	14.3	1.01	17.9	4.76	3.87	4.72
BN 0.1, n=5[a,b]													
Mean	103	3762	0.2	1.4	4.2	15	41	928	61.2	4665	37.01	25.23	54.9
S.E.M.	6.67	257	0.2	0.25	0.8	4.94	3.96	164	13.1	3556	3.14	7.41	33
BN 1.0, n=6[a]													
Mean	108.5	5687	0.17	0.33	1.17	20.83	59.7	1125	109.17	2403	61.7	20.85	21.94
S.E.M.	16.6	249	0.17	0.21	0.65	5.21	12	137	8.45	430	12.8	2.97	3.52
ANOVA, $F(4,24)$	27.56	228.92	8.82	4.01	4.92	7.89	17.15	32.93	58.82	2.15	6.14	2.53	1.96
P value<	.001	.001	.001	.05	.006	.001	.001	.001	.001	.2	.003	.07	.2

[a] Indicates significant differences from control rats, tested by T^2. The F values are for descriptive purposes. GE: grooming episodes; TG: time spent in grooming; YS: yawning; PE: penile erections; ST: stretching; BS: body shaking; GI: grooming-independent scratching; TGI: time spent in grooming-independent scratching; GD: grooming-dependent scratching; TGD: time spent in grooming-dependent scratching.

[b] To have sufficient degrees of freedom, one variable, PE, was left out of the comparison with the corresponding saline group.

Table 2
Means ± S.E. for LY variables for controls and BN-treated groups

	GE	TG	YS	PE	ST	BS	GI	TGI	GD	TGD	TG/GE	TGI/GI	TGD/GD
Saline, n=6													
Mean	13.67	393	2.83	0.17	0.17	2.67	9.33	56.5	4.33	116.7	29.2	9.48	13.73
S.E.M.	2.58	122	2.06	0.17	0.17	1.33	3.98	25.6	2.29	60.2	10.5	2.19	6.59
BN 0.001, n=6[a]													
Mean	5.67	127	2.33	0	1.17	2.17	1.83	13	0.83	19.3	27	4.47	7.19
S.E.M.	1.76	55.5	0.62	0	0.4	0.87	0.6	5.15	0.54	14.6	14.1	1.68	5.01
BN 0.005, n=6[a]													
Mean	5.67	153	1.18	0.67	0.33	1	1.83	20.5	1	5.83	26.7	5.02	3.08
S.E.M.	1.41	38	0.65	0.33	0.21	0.45	1.14	15.9	0.52	2.97	3.27	2.64	1.5
BN 0.1, n=5[a,b]													
Mean	109.2	4176	0.4	0.2	2.2	9.6	55.4	973	64	1459	38.93	17.74	23.19
S.E.M.	7.68	244	0.25	0.2	1.5	3.17	4.91	101	9.85	179	3.43	1.62	1.53
BN 1.0, n=6[a]													
Mean	74.17	5909.3	0	0.33	2.67	29.5	40.2	673	135.5	2801	83.04	18.49	23.12
S.E.M.	6.77	73.3	0	0.21	0.56	3.11	10.4	136	19.2	179	7.52	2.08	3.61
ANOVA, $F(4,24)$	101.68	533.72	1.37	1.42	2.81	35.9	18.88	33.4	38.14	126.63	7.21	10.14	4.49
P value<	.001	.001	.3	.3	.05	.001	.001	.001	.001	.001	.002	.001	.009

[a] Indicates significant differences from control rats, tested by T^2. The F values are for descriptive purposes. Abbreviations as in Table 1.
[b] To have sufficient degrees of freedom, one variable, PE, was left out of the comparison with the corresponding saline group.

dye, and immediately anaesthetized with an overdose of sodium pentobarbital (50 mg/kg). Rat brains were then dissected, and a visual inspection of the dye-stained cerebroventricular system served to verify the correct placement of the cannula.

2.3. Experimental procedure

Following injection, the rats were put singly in acrylic cages (23×32×20 cm) and observed for 120 min. By a continuous sampling procedure, an observer recorded grooming episodes, which included either of the following components (Gispen and Isaacson, 1981): head washing (vibrating movements of the fore paws in front of the snout and licking of the same paws followed by strokes along the snout, and by semicircular movements over the top of the head), body grooming (licking of body fur), genital grooming (licking of genital area), paw licking (licking of fore-paws and hind-paws) and scratching (scratching of the body with the limbs); interruptions greater than 5 s determined separate episodes. In addition, scratches were divided into those occurring within a grooming sequence and those occurring alone. Yawning, stretching, penile erections and body shaking (rapid and vigorous movements involving the musculature around the spinal axis) were recorded as well. Besides, duration of grooming and scratching bouts was included. The observer knew the treatment condition, but the fact that several behaviors were scored and that there were no directional hypotheses reduced the possibility of affecting the results. All experiments were conducted between 0900 and 1400 h.

2.4. Statistical analysis

Because of the high number of behaviors studied, we used multivariate analysis of variance (MANOVA) and Hotellings T^2 test for hypothesis testing (Morrison, 1976). All variables but ratios (mean durations) were included in this analysis. Although MANOVAs gave us valuable information about the effect of BN on rats' behavior, it did not tell us how each behavior contributed to the total variance. The use of univariate methods is not recommended for this purpose because Type 1 error increases due to the large number of recorded responses. This study included 13 behaviors, so that 48.7% ($1-.95^2$) of them might have been false positives if we had based statistical

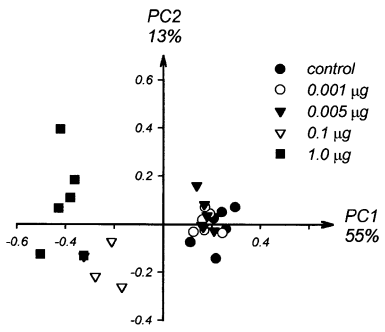

Fig. 1. Component scores of HY rats for each dose of BN. PC1 and PC2 are the first and the second principal components, respectively. One subject corresponding to the 0.1-μg group was discarded from the analysis because of being an outlier.

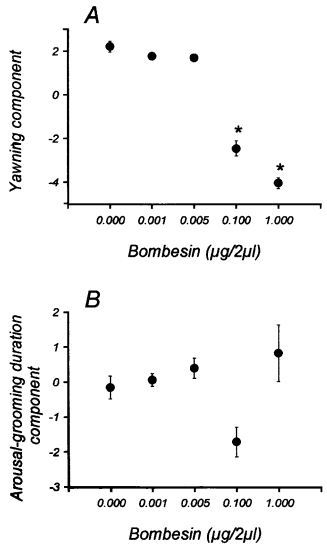

Fig. 2. Dose–response curve for BN on the first (A) and second (B) principal components for HY rats. Values are means ± S.E. * Indicates $P < .05$ compared to saline controls using Dunnett's multiple comparison test.

analysis on univariate methods. Principal component analysis (PCA) summarizes most of the variation in a multivariate system in fewer variables (Morrison, 1976) and gets around the problem of the dependence structure when the responses are many and from the same animal. In addition, PCA allows us to have a whole picture of the relationships among the studied behaviors and among the subjects' scores. Therefore, PCA was used and the components scores analysed for group differences (Ståhle, 1992; Ståhle and Ungerstedt, 1986) using one-way analysis of variance and Dunnett's test (Iles, 1993). In this case, all variables were included. To test for outliers, a Jackknife distance procedure was applied (Miller, 1974), and a graphical procedure, 'elbow criterion' (van der Heijden et al., 1990), was used to determine the number of components to be analyzed. Only P values below .05 were accepted as significant.

Table 3
Component loadings of HY rats' variables

Variables	PC1	PC2
GE[a]	−.325	−.225
TG	−.364	.103
YS	.283	.157
PE	−.070	−.426
ST	−.101	−.575
BS	−.305	−.055
GI	−.312	−.164
TGI	−.341	−.171
GD	−.349	.144
TGD	−.329	.286
TG/GE	−.244	.433
TGI/GI	−.158	−.031
TGD/GD	−.212	.229

[a] Abbreviations as in Table 1.

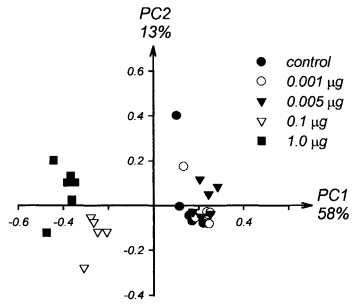

Fig. 3. Component scores of LY rats for each dose of BN. PC1 and PC2 are the first and the second principal components, respectively.

3. Results

Two subjects, one from each strain, were discarded from analyses because their scores after BN administration were low, although the cannulae were placed correctly. The fact that both rats were injected with BN solution from the same vial suggests that the sample was bad. That vial was not used for more rats because the corresponding group was complete.

BN significantly affected behaviors of both strains of rats: MANOVAs, $F(40,54) = 113.702$, $P < .005$, and $F(40,54) = 75.606$, $P < .005$ for HY and LY rats, respectively. All effects due to doses were statistically significant relative to HY and LY controls (Tables 1 and 2).

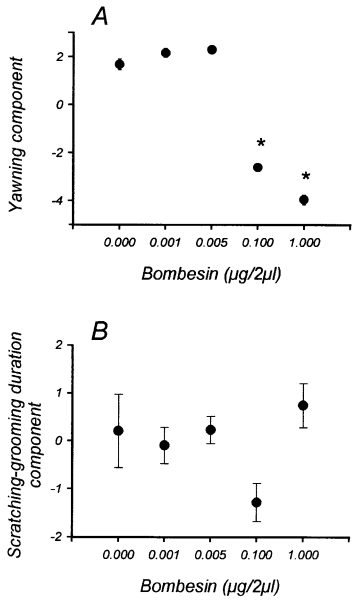

Fig. 4. Dose–response curve for BN on the first (A) and second (B) principal components for LY rats. Values are means ± S.E. * Indicates $P < .05$ compared to saline controls using Dunnett's multiple comparison test.

3.1. PCA on HY variables

Components PC1 and PC2 accounted for 55% and 13% of the total variation, respectively. PC1 separated controls and two treated groups (0.001 and 0.005 μg) from the groups with the highest doses of BN (Fig. 1). These had significantly lower scores (Fig. 2A) [$F(4,23) = 170.28$, $P < .005$] due to yawning, which had the positive loading on this component (Table 3), and hence indicate a decrease of this behavior across consecutive doses (Fig. 1). PC2 separated animals given 0.1 μg from those given 1.0 μg (Fig. 1), though neither group had scores significantly different from those of the control group (Fig. 2B); yet the variation across groups was significant [$F(4,23) = 3.38$, $P < .05$]. PC2 was bipolar: on the positive side, mean grooming duration had the highest loading (Table 3) and corresponded to the subjects given 1.0 μg of BN (see Table 1 and Fig. 1). On the negative side, stretching and penile erections had the highest loadings (Table 3) and corresponded to 0.1 μg of BN (see Table 1 and Fig. 1). Hence, PC2 may be considered as an arousal-grooming duration component (Fig. 2B).

3.2. PCA on LY variables

PC1 and PC2 explained 58% and 13% of the total variation, respectively. PC1 separated the control group, along with the groups that received 0.001 and 0.005 μg of BN, from those that received 0.1 and 1.0 μg (Fig. 3). Animals of these latter groups had significantly lower scores (Fig. 4A) [$F(4,24) = 233.90$, $P < .005$]. Yawning had the only positive loading on this component (Table 4) and therefore its decrease reflects a change from low to high doses (Fig. 3). PC2 divided the groups into that given 0.1 μg of BN and that given 1.0 μg (Fig. 3). Yet, neither of these groups had scores significantly different from those of the control group (Fig. 4B) [$F(4,24) = 2.14$, $P > .05$]. Mean grooming duration, yawning and penile erection had the highest positive loadings for PC2 (Table 4), while grooming-independent scratching had the highest negative loading (Table 4). Therefore, animals either spent more time in grooming, yawning and penile erection or in scratching (Fig. 4B). These alternatives correspond to the groups given 1.0 and 0.1 μg (see Table 2 and Fig. 3).

Table 4
Component loadings of LY rats' variables

Variables	PC1	PC2
GE[a]	−.325	−.241
TG	−.361	.037
YS	.114	.440
PE	−.003	.423
ST	−.203	−.178
BS	−.308	.199
GI	−.299	−.316
TGI	−.316	−.270
GD	−.319	.183
TGD	−.342	.144
TG/GE	−.252	.485
TGI/GI	−.285	−.024
TGD/GD	−.250	.185

[a] Abbreviations as in Table 1.

4. Discussion

This is the first study that reports a significant inhibition of yawning by the central administration of BN. The result could not be only attributed to a peculiarity of HY rats, for PCA showed a diminution of yawning even in LY rats. Although earlier studies had described the behavioral effects of BN and its difference from other peptides with stimulating effects on grooming, they scarcely mentioned yawning (Kulkosky et al., 1982; Gmerek and Cowan, 1983). This probably was because the spontaneous frequency of this behavior was too low for detecting a significant decrease. In addition, those data were analysed with univariate methods, which are not sensitive, as PCA is, to qualitative changes of behavior. PCA showed the dynamic effect of BN on HY and LY rats' behavior.

BN-treated rats yawned 2–3 h after the beginning of the experiments (data not shown). The frequency of response did not seem to differ from that of control animals, so that BN administration displaced yawning. This finding suggests that BN affects the length of the initial response to novel environments. In this respect, BN differs from other peptides such as oxytocin and ACTH which equally increase grooming and yawning (Ferrari et al., 1963; Gispen et al., 1975), and therefore the entire response to novel environments. Grooming and yawning, however, are not exclusively connected with novelty. Grooming may serve several functions: from cleaning the fur to the spread of chemical substances or as a displacement activity (for a review, see Spruijt et al., 1992). Yawning, apart from its relation to stress, has been associated with transitions between sleep and waking, with threats and conflict, and with boredom and sleepiness (Provine et al., 1987; Baenninger, 1997). Therefore, the effect of BN on grooming and yawning could be linked with other functions. Stress, on the other hand, may involve the activation of a feed-forward system to mobilize the central nervous system (Koob, 1999). Thus, the behavioral responses to stress may not be straightforward so that HY rats might groom vigorously because of skin irritation. Our findings, though, are more consistent with the view that grooming is a way to reduce arousal (Gispen and Isaacson, 1981), for BN administration correlates with elevations in stress-related substances (Kent et al., 1998, 2001).

PCA showed that BN produced a qualitative change from yawning to grooming that varied in a strain-specific manner. While in HY rats penile erections and stretching of the body dominated part of the variation explained by PC2, in LY rats the corresponding variation, although of lower load, was

due to the frequency and duration of grooming-independent scratching. Therefore BN at certain doses seems to favour a kind of 'arousal' component in HY rats, whereas in LY rats predominates a response more like that reported by previous studies (Brown et al., 1977). Besides confirming a strain-dependent effect, this finding is remarkable because given the positive correlation between yawning and penile erection in HY rats (Holmgren et al., 1985), it would have been expected both responses to change in the same direction. This raises the possibility that BN-like peptides may be involved in the functional association between yawning and penile erection. It is timely to mention that the separation between animals receiving 0.1 μg and those challenged with 1.0 μg is more apparent in HY rats than in LY rats. Yet, the application of other doses between 0.005 μg and 0.1 μg would have not changed so much the picture, as they would have lain in the centre of the principal component ordinates (see Figs. 1 and 3), and hence with little contribution to the components.

The variation due to PC3 (data not shown) in HY rats arose from scratching that occurred independent of grooming episodes, whereas in LY rats it resulted from scratching within grooming bouts. Accordingly, BN did not affect scratching duration within grooming bouts in HY rats (Table 1), so that this peptide seems to discriminate between apparently two types of scratching. Interestingly, this behavior occurs more frequently in HY than in LY rats after exposure to a novel environment. Although scratching was not divided in that study (Eguíbar and Moyaho, 1997), there is the possibility that the time sampling method used for recording it (Gispen and Isaacson, 1981) had not distinguished between categories. In other words, that part of the scores corresponded to scratches occurring separated from grooming sequences. This presumption and the present data suggest that there could be two subsystems of scratching. The differential effect of the central administration of BN on them indicates that it is likely that they depend on different neurochemical mechanisms and neural substrates. Although previous evidence suggests that vigorous scratching after BN does not reflect a direct response to skin irritation (Gmerek and Cowan, 1983), there remains the possibility that scratching independent of grooming episodes does result from changes in skin sensation as has been suggested by previous studies (Gmerek and Cowan, 1981; Cowan et al., 1985). Perhaps, scratches independent of grooming sequences depend directly on a spinal command, while those within grooming are modulated by higher brain centres. Indeed, it is known that such centres may inhibit the activity of the generator for scratching (Gelfand et al., 1988).

Since some of the behavioral effects of BN seem to depend on intact muscarinic cholinergic activity (Kulkosky et al., 1988), a plausible explanation of the differential effects of this peptide on HY and LY rats' behaviors is that the former strain might have a higher cholinergic tone than the latter, as has been suggested (Urbá-Holmgren et al., 1993). However, preliminary results have also indicated that the dopaminergic transmission may be involved in HY and LY rats behavioral differences. Therefore, both cholinergic and dopaminergic systems may contribute to the differences between HY and LY rats. Alternatively, the differential effects of BN on HY and LY rats might reflect differences in sensitivity of the two subtypes of receptors (Battey and Wada, 1991) to BN. Further studies are necessary to tease apart these possibilities.

In summary, the inhibition of yawning with BN enhances the initial response to novel environments by increasing grooming. The findings support the suggestion that BN-like peptides in the central nervous system of mammals might modulate the neurochemical correlates of the restorative behavioral actions to face stress situations. BN also appears to differentiate between two subsystems of scratching. If so, these findings would be potentially relevant to further study what the function of these subsystems is.

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