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Characterization of Bombesin-Induced Behavioral Changes in the Rat: Investigation of the Neuroanatomical Loci and Role of Dopaminergic System(s) in the Mediation of the Behavioral Effects of Bombesin

Thesis submitted to the School of Graduate Studies as partial fulfillment of the degree of Doctor of Philosophy (Psychology, Neuroscience Specialization)

by

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I dedicate my thesis to Susie, Stephen, Paul, and Tom.

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Curriculum Studorium

Sheila Johnston (née Kaiser) was born in 1945, in Picton, Ontario. She obtained a BA (psychology) from University of Toronto, in 1967, and a BA (honors psychology) from the University of Ottawa, in 1982.

ABSTRACT

The purpose of this thesis research was to characterize the behavioral effects of bombesin (BN) after central administration and to investigate the neuroanatomical loci and the role of dopaminergic system(s) in the mediation of the behavioral effects of BN.

In Phase 1 the time-course and dose-effect of BN (0.0001-1.0 ug/5 ul) administered intracerebroventricularly (ICV) were characterized. Bombesin quite potently stimulated locomotion, floor activity and rearing in rats. The duration of this response was dose-related. Also, the results demonstrated that neuroleptics (fluphenazine and haloperidol, 0.1-2.5 mg/kg, interperitoneally (IP)) could effectively antagonize the behavioral effects of BN.

In Phase 2 we demonstrated that fluphenazine only at the dose of 0.1 mg/kg (IP) specifically blocked the locomotor, floor activity and rearing effects of BN (1 ug), without affecting baseline behavior. In addition, rats with the dopaminergic pathways lesioned with 6-hydroxydopamine (250 ug/10 ul ICV), were administered BN (0.001-1.0 ug, ICV). The behavioral response of dopaminergic lesioned rats was significantly attenuated.

In Phase 3 we investigated the behavioral effects of BN microinjected ICV or at selected rat brain sites endowed with a high density of BN binding sites including the

nucleus tractus solitarius (NTS), the hippocampus (CA4), nucleus accumbens (NA), the fundus striati (FST), the central nucleus of the amygdala (CE), and the anterior olfactory nucleus (AON). Grooming elements were monitored by an observer while, simultaneously, the frequencies of locomotion, floor activity and rearing were recorded by a Z-80 microprocessor controlled infrared beam grid system over 60 min. Different behavioral responses to BN (1 ug) were most pronounced site specifically at the NA and NTS. At the NA the locomotor stimulatory effect of BN was most pronounced, but not grooming, whereas at the NTS grooming was most potently stimulated, but not locomotion. The effect of BN ICV, where both locomotion and grooming were stimulated, may have resulted from diffusion of BN to various loci such as the NA and NTS.

In Phase 4 the behavioral effects of BN (0.0001-1.0 ug/0.5ul) microinjected centrally at the NTS, the NA and peripherally (1-8 ug/kg; IP) were characterized. The role of dopaminergic system(s) in BN-induced behavioral effects was also investigated. The behavioral effects monitored included locomotor activities, grooming and satiety, using two paradigms. In the first paradigm (the paradigm in Phase 3) locomotor activities and grooming were monitored over 60 min. In the second paradigm grooming and eating behaviors were monitored simultaneously in 5 hr-food deprived rats trained to take part of their daily food intake over 20 min. The results of Phase 3 were replicated demonstrating that

BN-induced locomotor stimulation in rats was specific to administration at the NA and grooming stimulation was specific to the NTS. In addition results demonstrated that BN-induction of satiety was specific to administration at the NTS and not the NA. At the NA, BN-induced behavior appeared to be significantly mediated through the dopaminergic system(s) whereas, at the NTS, it did not appear to be. The BN-induced grooming profile appeared to be paradigm independent although the baseline grooming profile varied significantly according to paradigm. Intra NTS, a dissociation of BN-induced grooming and reduction in eating was evident on the basis of time-course and dose-effect. The similarity of the time-course of BN-induced satiety intra NTS and IP suggested that BN at NTS may mediate a physiological satiety signal. Since BN induced satiety 10^4 X more potently and 20% more efficaciously intra NTS than IP, the NTS may be a critical site for BN-induced satiety.

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INTRODUCTION

Peptides are Ubiquitous

Peptides are chains of amino acids consisting of two or more amino acids that are connected by a covalent peptide bond between the carboxyl group of one amino acid and the amino group of another; the formation of each such connection is accompanied by the loss of one molecule of water (Luttinger et al, 1984). Recent improvements in the abilities to isolate, characterize and synthesize peptides, and the recent discovery of new techniques for the quantitation and localization of peptides and their receptors have resulted in an exponential increase in the number of known peptides (or neuropeptides) within the central nervous system (CNS).

A major conceptual shift in neuroscience has been wrought by the realization that neuropeptides such as bombesin (BN), whose number exceeds 50 are presently known to modulate brain function. Originally neuropeptides were studied in the context of hormones, gut peptides or growth factors (Pert et al, 1985; Halasz, 1985).

In addition to neuropeptide receptors occurring in the brain, gut and neuroendocrine system, neuropeptide receptors occur on mobile cells of the immune system. Monocytes can chemotax to numerous neuropeptides (Ruff et al, 1985a); such

as substance P, (Ruff et al, 1985c); and opioids, (Ruff et al, 1985b) via processes shown by structure-activity analysis to be mediated by distinct receptors. These receptors are indistinguishable from those found in the brain (Pert et al, 1985; Moody et al, 1987).

Peptides date back in origin to the unicellular organism; opioid peptides (LeRoith et al, 1982) and insulin have been identified in unicellular organisms (LeRoith et al, 1980). Bacteria and protozoa produce peptide hormones that are bioactive and immunologically comparable to those found in mammalian tissue such as insulin (LeRoith et al, 1980, 1981), relaxin (Schwabe et al, 1983), somatostatin (Hoskins, 1978), adrenocorticotrophic hormone (ACTH) and B-endorphin (LeRoith et al, 1982), and a choriogonadotropin (CG)-like factor similar to human CG B (Maruo et al, 1979). The conservation of peptide sequences indicates that specific regions of a peptide were recognized by an equally conserved region of the receptor which sustained their intimate compatibility throughout evolution.

Probably, the specific peptide sequences that are conserved throughout evolution were associated with biological activities such as growth and cellular metabolism. These activities are essential for survival of species as divergent as unicellular organisms, amphibians and mammals (Lazarus et al, 1985).

Thus, perhaps in the beginning there was a genoma and from that started the biological "big bang" (Angelucci,

1985). Evolution would consist more in the multiplicity of forms in which cells can aggregate to form organisms, rather than in the progressive acquiring of new specialized cellular properties (Angelucci, 1985). Biochemistry would at most, contribute to the upsurge of homologies in messenger molecules and their receptors, with full conservation of transduction mechanisms such as cyclic nucleotides, calcium ions and phosphoinositides (Angelucci, 1985). In regard to the "peptide language" the messenger peptide would convey a signal, the meaning of which is understood not for intrinsic content, which would not exist, but according to the logic of the recipient. The message they convey would be understood in relation to the specific bio-organizational situation; thus, the peptide would appear as a general promoter more than a specific activator of responses (Angelucci, 1985).

Peptide hormones are derived from high molecular weight polypeptide precursors, termed prohormones (Griffiths and McDermott, 1984). The initial processing of the hormone from the precursor appears to be governed by the presence of paired dibasic amino acid residues as illustrated in the ACTH/B-endorphin prohormone (Eipper and Mains, 1980). An endopeptidase has recently been found which specifically cleaves these bonds (Julius et al, 1984; Mizuno and Matsuo, 1984). Other endo- and exopeptidases may act on both the prohormone and the liberated peptide in order to produce a bioactive substance. In the case of the peptide ligand, the

conserved region often represents only a small portion of the total mass, the remainder of which is frequently more variable. For instance only the C-terminal hepta portion of BN, whose prohormone is a protein of 148 amino acids (Spindel et al, 1984), contains the bioactive determinants (Erspamer and Melchiorri, 1973). Thus, the bioactive C-terminal heptaportion of BN and its receptor are highly conserved across the species (presently investigated) from insects (Veenstra and Yanaihara, 1984) to man (Moody et al, 1987).

Peptide signal specificity resides in their receptors (distinct classes of recognition molecules), rather than the close juxtaposition occurring at classical synapses (Pert et al, 1985; Herkenham and McLean, 1986). This mismatch between receptor and neurotransmitter localization is not unique to peptides; it is evident for muscarinic cholinergic receptors, B-adrenergic receptors and Gamma-aminobutyric acid receptors just to name a few (Kuhar et al, 1986; Herkenham and McLean, 1986). One example of a mismatch, as illustrated in Figures 1 and 2, is the high density of BN receptors in regions of the hippocampus, frontal cortex and striatum but low density of BN-like peptides in these areas. Furthermore, very few if any, neuronal cell bodies which synthesize BN, or neuronal fibers and terminals have been found in the above three areas (Moody et al, 1987; Moody and Pert, 1979; Moody et al, 1981c; Roth et al, 1982; Panula et al, 1982; 1984). Thus, there is a mismatch in the

distribution of BN-like peptides, neurons and receptors in some areas of the brain.

The movement of peptides to relatively distant sites within the central nervous system (CNS), is feasible since 20% of the total brain volume is taken up by extracellular spaces and the fluid within them (Schmitt, 1984). Current research on the relationship of the parenchymal extracellular fluid (ECF) to the cerebral spinal fluid (CSF) with which it is in continuity, suggests evidence of bulk flow and active pumping mechanisms (Oldfield et al, 1985; Rennels et al, 1985). Furthermore, most degradative enzymes that inactivate transmitters are either membrane-bound or confined to the cell cytoplasm; the CSF and ECF are nearly devoid of such enzymatic activity (Herkenham and McLean, 1986; Schmitt, 1984; McKelvy, 1983). Thus it is reasonable to postulate that peptides released at a nucleus in one area of the brain could have an action in CNS structure(s) several micrometers away, within a few minutes of release. The effects may continue over a long period of time, since B-endorphin and many other neuropeptides may be stable for many hours in the CSF (Sagar et al, 1984).

A number of brain loci, especially in limbic areas are endowed with many types of neuropeptide receptors suggesting a convergence of information at these "nodes" (Pert et al, 1985). One example of a nodal area where cells have receptors for several peptides is the "mediansoms" in the median eminence (Fuxe et al, 1985). Neuropeptides and their

receptors may join the brain, glands, gut and immune system in a network of communication, within the entire organism. Pert (1986) has hypothesized a system where these neuropeptides are the keys to the biochemistry of emotion, that is that they provide the physiologic basis for the emotions. Each neuropeptide may bias information processing uniquely when occupying receptors at nodal points within the organism. If so, then each neuropeptide may evoke a unique "tone" or mood. According to this hypothesis, it would no longer be necessary to divide the organism into brain and body since neuropeptides transmit mood throughout the organism as an integrated whole. For instance angiotensin microinjected in the brain causes drinking, probably alters consciousness to make us feel thirsty and in co-ordination with this, receptors at the kidney for angiotensin when activated result in the conservation of water. Moreover, this overall integration of behavior seems consistent with survival (Pert, 1986).

In line with this role of neuropeptides as the biochemical basis for emotion, Pert and her colleagues, in mapping neuropeptide receptor locations have noted that for virtually all the senses for which we know the entry area, the location is always a nodal point for neuropeptide receptors such as the dorsal horn of the spinal cord and the nucleus tractus solitarius (Pert et al, 1985; Pert, 1986). Many neuropeptides (BN, substance P, neurotensin, cholecystokinin, somatostatin, avian pancreatic peptide,

enkephalin and neurophysin II, to name a few) are located in these areas where modulation of sensory transmission does occur. Such areas include lamina II and III of the dorsal horn and the various nuclei of visceral and taste pathways from the periphery to sensory cortex (O,Donohue et al, 1984; Fuxe et al, 1983; Mantyh and Hunt, 1984; Kalia et al, 1984; Hokfelt et al, 1984). Pert (1986) has theorized that the immaterial substrate mind is the energetic controller of the neurochemical network.

In summary, neuropeptides and their receptors are evident in the brain, gut, glands and the immune system and may form a network of communication within the entire organism. Furthermore, neuropeptides and their receptors are ubiquitous and highly conserved throughout evolution from unicellular organisms to man.

The Discovery of Nonmammalian Peptides

Around 1940, the biogenic amine "explosion" produced a growing interest in biologically active, naturally occurring substances. In the isolated organ preparation inaugurated by Magnus in Utrecht, it was possible to test with high specificity and sensitivity biologically active extracts of natural origin with reduced needs of the difficult, low-yield purification methods of that time (Melchiorri, 1985). Eledoisin action was first noted by Erspamer during the bioassay of biogenic amines in extracts of posterior

salivary glands of the Mediterranean octopod Eledone moschata (1949). Finally, thirteen years later (1962), elodoisin was recognized as a peptide and its primary structure established. The biological activity of elodoisin soon proved to be very similar to that described by von Euler and Gaddum for their substance P. Between 1962 and 1969 Erspamer's group tested 100 more eledoisin analogues finding that the C terminal pentapeptide represented the minimum amino acid sequence essential for the biological activity of eledoisin. In the following 10 years Erspamer and his colleagues traced, isolated and sequenced three peptides of amphibian skin, physalaemin (1964), caerulein (Anastasi et al, 1967) and BN (Anastasi et al, 1971) and several other peptides of the BN family (Table 1) such as litorin and ranatensin (Anastasi et al, 1975). The latter two families of peptides led to the identification of the amino acid sequences of two mammalian analogues, cholecystokinin and gastrin releasing peptide (GRP-27) (McDonald et al, 1979). A BN-like peptide has been also identified (Melchiorri, 1980), isolated and sequenced (McDonald et al, 1980) in chicken proventriculus. More recently two ten amino acid peptides of the BN family were isolated from the spinal cord based on their ability to stimulate rat uterus smooth muscle and named neuromedin B and C (Minamino et al, 1983; 1984). However neuromedin C was previously identified by Reeve et al, (1983) from the muscle of the canine small intestine, and named GRP-10. In

addition, other peptides have been identified in cockroaches (proctolin, Brown and Starratt, 1975) locusts (Stone et al, 1976) artropods, chicken pancreas (avarian pancreatic peptide, Kimmel et al, 1975) and 100 species of amphibia (6 new tachykinins, two bradykinin-like peptides and an angiotensin-like peptide, and two new peptides dermorphin and sauvagine) (Erspamer, 1981; Erspamer et al, 1981). This is not an exhaustive list; discovery of peptides continues in all organisms from unicellular organisms to man.

The BN family of peptides can be divided into two or possibly three or four subfamilies: the BN/alytesin subfamily, the litorin/ranatensin subfamily, the phyllolitorin subfamily and the rohdei-litorin subfamily (Cei, 1985). One basis for the division of the BN family into subfamilies involves differences in the amino acid sequences at the bioactive "C" terminal of these peptides. Bombesin and alytesin, possess a very similar sequence of 14 amino acid residues, with the C terminal tripeptide His-Leu-Met-NH_2 (Table 1). They have been found in the skin of the Archaeobatrachian, Eurasiatic discoglossid genera Bombina and Alytes (Erspamer et al, 1972). The peptides of the litorin/ranatensin subfamily, are characterized by the C terminal tripeptide His-Phe-Met-NH_2. They have been found in the skin of several species of pelodryadids, myobatrachids as well as from American and Australasian ranids (Anastasi et al, 1975). The candidate phyllolitorin subfamily, is constituted by two members, phyllolitorin and [Leu ^{8}]

phyllolitorin, characterized by the unusual C terminal tripeptide Ser-Phe(Leu)-Met-NH_2 (Cei, 1985). This substitution of Ser for His is not a simple single base change in mRNA code, but implies a more complex amino acid replacement, involving a two base change (Erspamer et al, 1985). The two phyllolitorin peptides have been isolated from the skin of the South American Phyllomedusa sauvagei. The phyllolitorins are not present in the skin of Phyllomedusa rohdei, which contains a nonapeptide, rohdei-litorin. Rohdei-litorin shares with neuromedin B the entire C-terminal octapeptide and presents, again like neuromedin B and ranatensin-C, a Thr residue substituted for the usual Val residue at position 5 from the C-terminus. This substitution involves a two base change in mRNA code (Erspamer et al, 1985; Cei, 1985).

Table 1. The BN Family of Peptides

BN	pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
Litorin	pGlu-Gln-Trp-Ala-Val-Gly-His-Phe-Met-NH_2
Phyllolitorin	pGlu-Leu-Trp-Ala-Val-Gly-Ser-Leu-Met-NH_2
Rohdei-litorin	pGlu-Leu-Trp-Ala-Thr-Gly-His-Phe-Met-NH_2
GRP-27	Ala-Pro-Val-Ser-Val-Gly-Gly-Gly-Thr-Val-Leu-Ala-Lys-Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
Neuromedin C (GRP-10)	Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
Neuromedin B	Gly-Asn-Leu-Trp-Ala-Thr-Gly-His-Phe-Met-NH_2

Initial studies on the pharmacological actions of BN include: hypertensive action in the dog; potent stimulant action on the rat uterus, the rat and guinea-pig colon and the cat ileum; remarkable stimulant action on the gastric secretion in the chicken and the dog; stimulant action on the transport of Cl^- ions from the serosal to the mucosal side of the isolated gastric mucosa of amphibians; hyperglycemic action in the rat and the dog; and increase in immunoreactive insulin levels in the peripheral blood of the dog (Anastasi et al, 1971).

Since the initial discovery of BN, research on BN has been carried out in a very broad spectrum involving all

fields of neuroscience. Studies indicated that frog skin peptides such as BN are structurally similar to endogenous peptides present in the mammalian CNS and periphery (McDonald et al, 1979). The mammalian equivalent of BN, gastrin releasing peptide (GRP-27), a heptacosa peptide was isolated from porcine nonantral stomach tissue and purified by gel filtration and high pressure liquid chromatography. It was named for its ability to stimulate gastrin secretion from dog stomach. Subsequently, two, ten amino acid peptides, were isolated from porcine spinal cord, named neuromedin B and neuromedin C, and pharmacologically verified by their ability to stimulate rat uterus smooth muscle preparations (Minamino et al, 1983, 1984). At the same time, Reeve et al, (1983) isolated and sequenced three canine BN-like peptides containing 27, 23, 10 amino acid residues from 820 g of intestinal muscle and tested for stimulation of contraction of canine gastric antral smooth muscle and for stimulation of gastric release from isolated, perfused rat stomach. Canine BN-like peptides represent another example of mammalian neuropeptides existing in more than one biologically active molecular form. Neuromedin C and canine GRP-10 are identical decapeptide sequences, thus these two names are interchangeable (Bunnett et al, 1985). In addition to neuromedin B and C, Minamino et al, (1985) have isolated a neuromedin B-30 and neuromedin B-32, from porcine spinal cord, by utilizing bioassay for the effects on the contractiblity on smooth-muscle preparations coupled

with high resolution high performance chromotography. Recently, isolation and high performance liquid chromatography analysis of bombesin-like-peptide (BLI) in bovine adrenal medulla revealed the presence of four molecular forms, coeluting with GRP-27, or neuromedin B, or GRP-18-27 (LeMaire et al, 1986). Biochemical studies of small cell lung cancer biopsy specimens indicated that GRP-10 or neuromedin C was present there (Wood et al, 1981; Erisman et al, 1982).

Furthermore, the gene for BN-like peptides was recently cloned from human bronchial carcinoid tumor (Spindel et al, 1984). The cloning was made possible by the high level of BN-like peptides detected in human bronchial carcinoid tumor (2-40 pmol/g wet tissue) (Wood et al, 1981). Near the N-terminal of the 148 amino acid precursor GRP-27 was identified. Trypsin-like enzymes may metabolize the precursor to peptides such as GRP-27 or neuromedin C, in addition, these fragments must be metabolized by post transitional processing enzymes so that the C terminal is amidated (Moody et al, 1987). The function and characterization of the remaining amino acid residues of the prohormone remain unknown.

Although the list of GRP and related fragments continues to grow, the C-terminal of these peptides, which is required for biological activity is highly conserved. However the N-terminal which is not essential for activity may differ by as much as 4, 5, or 9 amino acids for canine, human and

chicken GRP (respectively) in comparison to porcine GRP (Moody et al, 1987).

Furthermore, in comparison to all BN-like fragments, BN appears, thus far, to be one of most active fragments (Broccardo et al, 1975; Rivier and Brown, 1978; Brown et al, 1977b; Marki et al, 1981; Girard et al, 1983; Girard et al, 1984; O,Donohue et al, 1984; Bunnett et al, 1985; Mayer et al, 1986; Greeley et al, 1986; Moody et al, 1987). The purity of commercial preparations of peptides varies greatly, as can the rate of degradation; thus, verification of administered peptide doses by amino acid analysis and radioimmunoassay must be undertaken to determine relative potencies of BN-like peptides. Without such monitoring, significant errors may be introduced by nonspecific losses of basic peptides during storage and handling (Mayer et al, 1982, 1986). The immediate behavioral effects of BN, GRP-27 and neuromedin B are similar following intrathecal administration. However, the time course of behavioral activation following BN administration is much longer than the mammalian analogues of BN. Therefore, this may cause an error in the estimation of potency of the analogues if the time course is not carefully controlled after drug administration. Furthermore, because of its particular structure, BN is much more resistant to degradation than the mammalian counterparts. Consequently few precautions need to be considered in relation to experimental procedures as far as administering several animals over 20 min with one (BN)

drug solution. On the other hand GRP-27, GRP-10, or neuromedin B, may be completely inactive by the time the last animal is injected (O,Donohue et al, 1984; Bishop et al, 1986). Mayer et al, (1986), using the forementioned verification procedures demonstrated that three forms of natural canine GRP, synthetic GRP-10, and synthetic porcine GRP-27 were similar in potency to synthetic amphibian BN on stimulation of spontaneously occurring contractions of canine circular antral muscle, in vitro. Due to the methodological difficulties, many researchers of structure activity have reports in the literature, where they did not use the above procedures. Thus their results may require further verification.

Generally, the data suggest that the 8, 10, 12, 13, and 14 positions of BN are essential for high receptor binding and biological potency whereas the first six amino acids at the N-terminal are not essential (Broccardo et al, 1975; Rivier and Brown, 1978; Brown et al, 1977b; Marki et al, 1981; Girard et al, 1983; Girard et al, 1984; Bunnett et al, 1985; Mayer et al, 1986; Greeley et al, 1986; Moody et al, 1987). Furthermore substitution of proline for glycine in position 11 which disrupts the a-helix structure of proteins, reduced the potency of BN four orders of magnitude, thus BN may have a secondary or tertiary structure such that it is folded when it binds to its receptors (Moody et al, 1987).

Distribution of BN-Like Peptides in the Brain

Bombesin-like peptides have been detected by radioimmunoassay and immunocytochemical techniqes using antisera which recognize the C-terminal of BN or gastrin releasing peptide (GRP-27). The antisera cross react with endogenous BN-like peptides present in the mammalian central nervous system and periphery such as GRP-27 and neuromedin C but not neuromedin B. The concentration of BN-like peptides in vertebrate tissue range from 10 pmol/g wet weight in the intestine to 1-8 pmol/g in the adrenal, brain, pituitary and spinal cord (Moody et al, 1987).

Within the CNS, BLI is distributed unevenly in discrete brain loci, with over 30-fold variation (Brown et al, 1978; Moody and Pert, 1979; Moody et al, 1981a). Bombesin-like immunoreactivity has been detected in the brain of rats, dogs, and sheep (Walsh et al, 1979; Brown et al, 1978; Walsh and Wong, 1979, Moody and Pert, 1979; Soveny and Hansky, 1980; Villarreal and Brown, 1978; Soveny et al, 1984; Panula et al, 1982, 1984). The endogenous localizaton in rat brain is highest at the nucleus substantia gelatinosa trigemini and nucleus tractus solitarius (5.9-4.7 fmol/ug protein) of the medulla; moderately high concentrations of BLI exist in the inter-peduncular nucleus and central gray of the midbrain (2.41-1.45 fmol/ug protein). In the diencephalon, the arcuate nucleus (2.3 fmol/ug protein), as well as the hypothalamus, generally, show moderately high BLI (1.59-0.90

fmol/ug protein). In the telencephalon moderately high BLI is evident in the central and medial amygdaloid nucleus (1.44-1.22 fmol/ug protein) and the interstitial nucleus of the stria terminalis, ventral (1.16 fmol/ug protein). Other areas such as the cingulate cortex, hippocampus, septum, nucleus accumbens, olfactory tubercle and caudate nucleus have low BLI (0.89-0.22 fmol/ug protein) (Moody et al 1981a). The distribution pattern of BLI in the rat CNS is illustrated in Figure 1.

The regional distribution of BLI in the human brain showed highest concentrations in hypothalamus, septal nuclei, nucleus accumbens, globus pallidus, amygdala, periaqueductal grey, and substantia nigra. The medulla was not reported on (Ghatei et al, 1984a). In the human, rat and guinea-pig pituitary, high levels of BLI are present. In human pituitary, highest densities were evident in the anterior pituitary (4.6 pmol/g wet tissue) and the stalk (8.1 pmol/g wet tissue) (Major et al, 1983). Measureable quantities of BLI have also been reported in human cerebrospinal fluid, (Yamada et al, 1981).

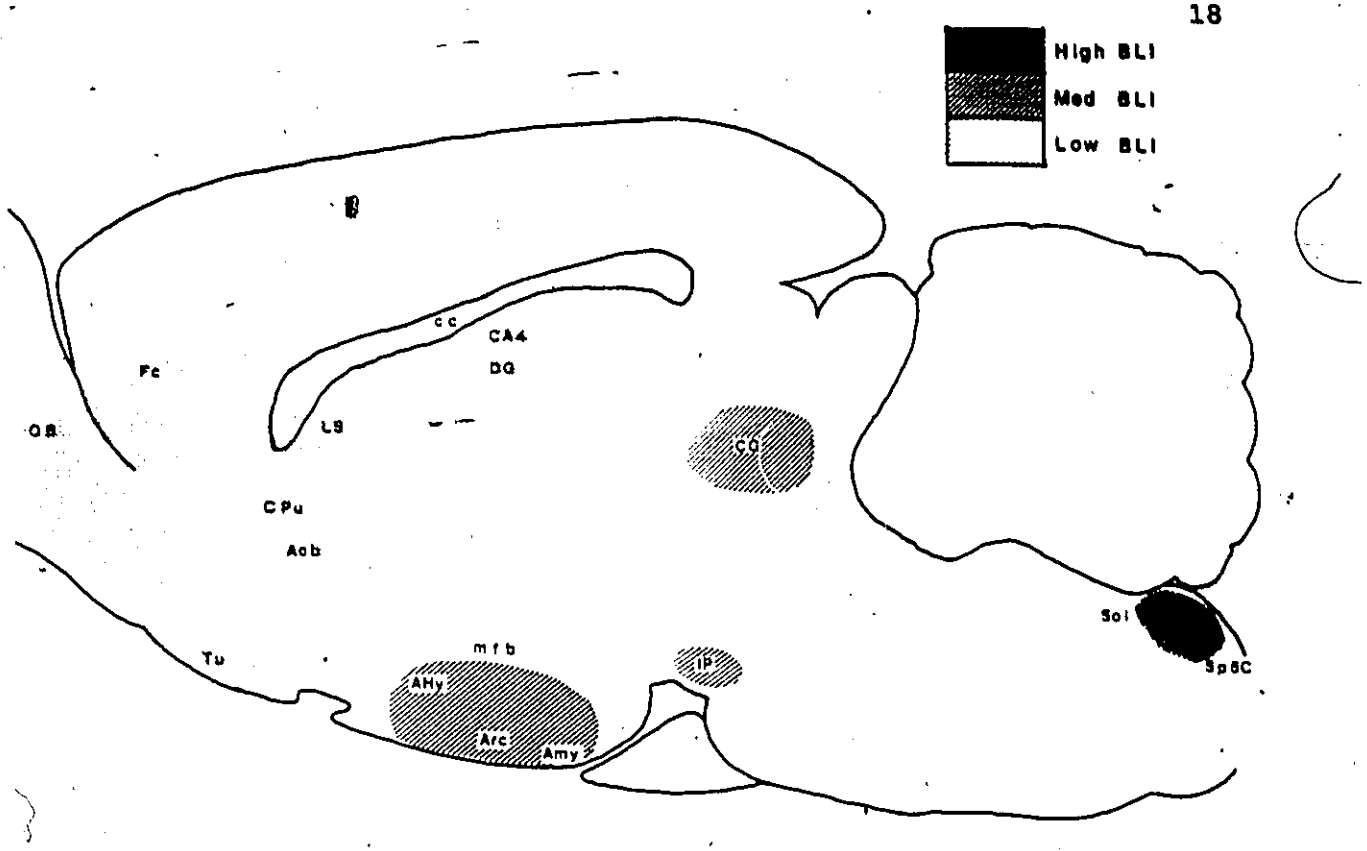

Figure 1: The localization of BN-like immunoreactivity (BLI), in the rat brain (saggital view: adapted from Paxinos and Watson, 1982).

Abbreviations: Figures 1-3, 9-14.

10 dorsal motor nucleus of the vagus

12 hypoglossal nucleus

3V third ventricle

4V fourth ventricle

A 8,9,10,12,15, dopamine cell bodies

aca anterior commissure, anterior

Acb accumbens nucleus

Acc anterior cingulate cortex

ACo anterior cortical amygdaloid nucleus

AHi amygdalohippocampal area

AOP anterior olfactory nucleus posterior

Amy amygdala

AP area postrema

Arc arcuate hyopthalamic nucleus

BL basolateral amygdaloid nucleus

BLV basolateral amygdaloid nucleus, ventral

BM basomedial amygdaloid nucleus

CA2 field CA2 of Ammon's horn

CA3 field CA3 of Ammon's horn

CA4 field CA4 of Ammon's horn

cc corpus callosum

CC central canal

Ce or CE central amygdaloid nucleus

CG central grey

Cl claustrum

CM central median thalamic nucleus

Cop copula of the pyramis

CPu caudate putamen

cu cuneate fasciculus

Cu cuneate nucleus

DA dorsal hypothalamic area

DG dentate gyrus

Dr dorsal raphe nucleus

E ependyma and subependymal layer

Ec entorhinal cortex

En endopiriform nucleus

f fornix

fmi forceps minor corpus callosum

Fr frontal cortex

G gelatinosus nucleus thalamus

GP globus pallidus

Gr gracile nucleus

Hb habenular nucleus

Hif hippocampal fissure

ic internal capsule

IP interpedunclar nucleus

La lateral amygdaloid nucleus

LC locus coeruleus

LH lateral hypothalamus

lo lateral olfactory tract

LS lateral septal area

LV lateral ventricle

MD mediodorsal thalamic nucleus

ME medial eminence

mfb median forebrain bundle

OB olfactory bulb

opt optic tract

Pe periventricular hypothalamic nucleus

PLCo posterolateral cortical amygdaloid nucleus

PMCo posteromedial cortical amygdaloid nucleus

PO primary olfactory cortex

py pyramidal tract

Re reuniens thalamic nucleus

RF rhinal fissure

SI sublenticular substantia innominata

sm stria medularis thalamuss

Sol nucleus solitary tract

SOR supraoptic hypothalamic nucleus retrochiasmic

sox supraoptic decussation

sp5 spinal tract of the trigeminal nerve

Sp5C nucleus of the spinal tract of the trigeminal nerve, caudal part

st stria terminalis

Tu olfactory tubercle

VMH ventromedial hypothalamic nucleus

Y dopamine terminal areas

In the spinal cord of the rat, BLI is low, however, the concentration is 8-fold greater in the dorsal than ventral horns (Moody et al 1981c), suggesting a functional role in the transmission of sensory information. This sensory role is further supported by the very high BLI in the nucleus tractus solitarius, a visceral sensory nucleus, and the substantia gelatinosa trigemini an area important for pain and temperature sensitivity (Carpenter, 1978, 1985; Moody et al, 1981a). Moreover, BLI has been located in rat primary

sensory ganglia cell bodies (Panula et al, 1983; Fuxe et al, 1983).

Histochemical localization of BLI in the rat brain reveals cell bodies in the telencephalon restricted to stria terminalis and lateral septal nucleus. In the diencephalon, cell bodies were abundant in the paraventricular nucleus. Soma were present in the anterior and medial parvocellular part, only. These cells were ovoid with long varicose processes. A low number of perikarya were located in the medial preoptic nucleus and the suprachiasmatic nucleus. A moderate number of cells were found in the anterior hypothalamic nucleus and in the central gray. Abundant perikarya were also found in nucleus tractus solitarius and the nucleus reticularis gigantocellularis (Chronwall et al, 1985; Panula et al, 1982; Panula et al, 1984).

A high density of nerve fibers was found in the central amygdaloid nucleus, the lateral amygdaloid nucleus, interstitial nucleus of the stria terminalis, and medial preoptic and anterior hypothalamic nuclei. Occasionally, axons were found in the septal area. The superchiasmatic nucleus contained very high fiber density, especially in the central parts. Occasional fibers were found in the thalamus. Extended fibers occupied the area between the substantia nigra (or dorsally the ventral nucleus of the thalamus) and medial lemniscus forming a chevron of high density BLI. The nucleus tractus solitarius and nucleus reticularis

gigantocellularis had low numbers of fibers (Chronwall et al, 1985; Panula et al, 1982).

The existence of BLI in rat sensory ganglia and spinal cord was confirmed using immunocytochemistry, gel filtration chromatography and high performance liquid chromatography combined with radioimmunoassay (Panula et al, 1983; Fuxe et al, 1983). Bombesin-containing neuronal processes were demonstrated in laminae I and II of the dorsal horn of the cat, rat, and mouse spinal cord by immunocytochemistry and radioimmunoassay. Bombesin-binding sites were also localized in superficial laminae of the dorsal horn (O'Donohue et al, 1984). Pharmacological actions of BN injected into the spinal cord indicative of sensory stimulation (biting and scratching) were demonstrated (O'Donohue et al, 1984; Bishop et al, 1986).

The subcellular distribution studies indicate that BN-like peptides may be localized to synaptosomes in the presynaptic nerve endings in close juxtaposition to postsynaptic BN receptors and are released by depolarizing stimuli in a Ca^{++}-dependent manner (Moody et al, 1980, 1981b). Potassium (75 mM) stimulated a 3-fold increase in the rate of release of BN-like peptides in a Ca^{++}-dependent manner from rat spinal cord slices; as well, veratridine (100 uM) stimulated release of BN-like peptides in a Ca^{++}-dependent manner and this release is blocked by tetrodotoxin (1 uM). Thus, BN-like peptides may function as neuroregulators and/or neurotransmitters in the CNS.

Distribution of BN-Like Peptides in the Periphery

Bombesin-like immunoreactivity is present in mammals in certain ganglia, the gastrointestinal tract, pancreas, lung, female rat genito-urinary tract and adrenal medulla.

Schultzberg (1983) using immunocytochemical techniques has demonstrated BLI in prevertebral sympathetic ganglia of the rat and guinea-pig in nerve fibers of the inferior mesenteric and caeliac-superior mesenteric ganglia. In addition, BLI is present on a subpopulation of small ganglia and fibers of the rat superior cervical ganglia (Helen et al, 1984b). Similarly, human paravertebral sympathetic ganglia show BLI (Helen et al, 1984a).

Bombesin-like peptides are found in gastrointestinal tract of teleost fish (Langer et al, 1979; Van Noorden and Falkmer, 1980), in various frog species (Lechago et al, 1978), in turkeys, pigeons, and chickens (Erspamer et al, 1979). Walsh et al, (1979) reported that, in the rat, high levels were present in the fundus and colon (50 pmol/g wet tissue) and moderate levels in the antrum, duodenum, ileum and jejunum (14-19 pmol/g wet tissue). Studies of distribution and projection of neurons with BLI in the guinea-pig small intestine suggest BN-like peptides may be synthesized in intrinsic neurons present in myenteric plexus (Costa et al, 1981). These neurons project rostrally and innervate the circular muscle as well as the celiac ganglia

(Costa et al, 1981; Buffa et al, 1982). In human gastrointestinal tract, Price et al, (1984) using radioimmunoassay for BN, have found highest levels of BLI in the fundus, antrum, pylorus and pancreas with lower levels in the duodenum, jejunum, terminal ileum and colon. Most recently, Greeley et al, (1986) quantitated and characterized the variants of BLI in the alimentary canal in the mucosal and muscular layers of the rat, rabbit, hawk, owl, dog, monkey and human. Throughout the entire gastrointestinal tract of all species studied, BLI was present. Gel chromatography showed that BLI corresponded to GRP-27, BN or GRP-10.

In the pancreas of man, pig, calf, rat and guinea pig, BLI is present in moderate density (Ghatei et al, 1984b). In the rat, immunocytochemical studies demonstrated BLI in nerves in the exocrine pancreas, and small quantities in the pancreatic islets as well (Jensen et al, 1978).

In female rat genito-urinary tract using radioimmunoassay, BLI has been detected, with highest concentration in the vagina, and moderate concentrations in the uterus and bladder (Ghatei et al, 1985). The BLI nerve fibres were localized by immunocytochemistry in the smooth muscle layer, around blood vessels and in the submucosa of the vagina and bladder.

More recently, radioimmunoassay of bovine adrenal medulla indicated relatively high levels of BLI (27 pmol/g tissue) (LeMaire et al, 1986).

Bombesin-like peptides are present in endocrine cells of rat, fetal lung but not in the adult lung (Wharton et al, 1978). In human fetuses and neonates BLI is present in the respiratory tract and may play a role in the differentiation of the lung (Ghatei et al, 1983). Recently BLI has been found in human and guinea pig alveolar macrophages of the healthy lung (Wiedermann et al, 1986).

High levels of BLI are present in small cell lung cancer biopsy specimens (10-750 pmol/g wet tissue) (Moody et al, 1981b; Wood et al, 1981; Erisman, 1982).

Distribution of BN Receptors in the Brain

In the rat brain homogenate (10 mg wet tissue) receptor binding studies indicated that iodinated tyrosine (^{125} I-Tyr^4) BN bound with high affinity (Kd = 4 nM) to a single class of sites in specific areas of the rat brain, spinal cord and gut. This binding is reported to be specific, saturable and reversible (Moody et al, 1978; 1987). Bombesin-like peptides may bind to a membrane protein of subunit molecular weight 78,000 daltons. The receptor density ranges from 2000-5000 per cell (Moody et al, 1987; Moody et al, 1985).

Radioreceptor assays for BN, in the rat brain, show that the highest concentrations of receptors appear to be associated with limbic forebrain and midbrain structures (Moody et al, 1979; Pert et al, 1980). Pert et al (1980)

reported highest levels of BN binding in the amygdala (18.6 fmol/mg protein), hypothalamus (17.8 fmol/mg protein), frontal cortex, olfactory nuclei, and nucleus accumbens (15.4 fmol/mg protein), dorsal hippocampus (13.6 fmol/mg protein), periaqueductal gray (11.1 fmol/mg protein), and corpus striatum (9.1 fmol/mg protein). The distribution pattern of these receptor densities is illustrated in Figure 2.

In more recent studies, highest grain densities for BN binding sites (as determined by in vitro, light microscopic autoradiography) were found, as above, in limbic areas, namely: the nucleus accumbens, olfactory bulb, olfactory tubercle, medial and anterior cortical amygdaloid nuclei, outer molecular layer of the dentate gyrus, and area CA4 of the hippocampus and the subiculum, stria terminalis, and the paraventricular, periventricular and suprachiasmatic nuclei of the hypothalamus, and dorsomedial and rhomboid thalamus. In addition highest grain densities were found in the nucleus tractus solitarius and the substantia gelatinosa. Moderate grain densities are found in deep layers of the parietal cortex, deep layers of the neocortex, rhinal cortex, caudate putamen, stria terminalis, locus ceruleus, parabrachial nucleus and facial nucleus other parts of the hippocampus, and basomedial and central amygdaloid nucleus (Wolf et al, 1983; Wolf and Moody, 1985; Zarbin et al, 1985).

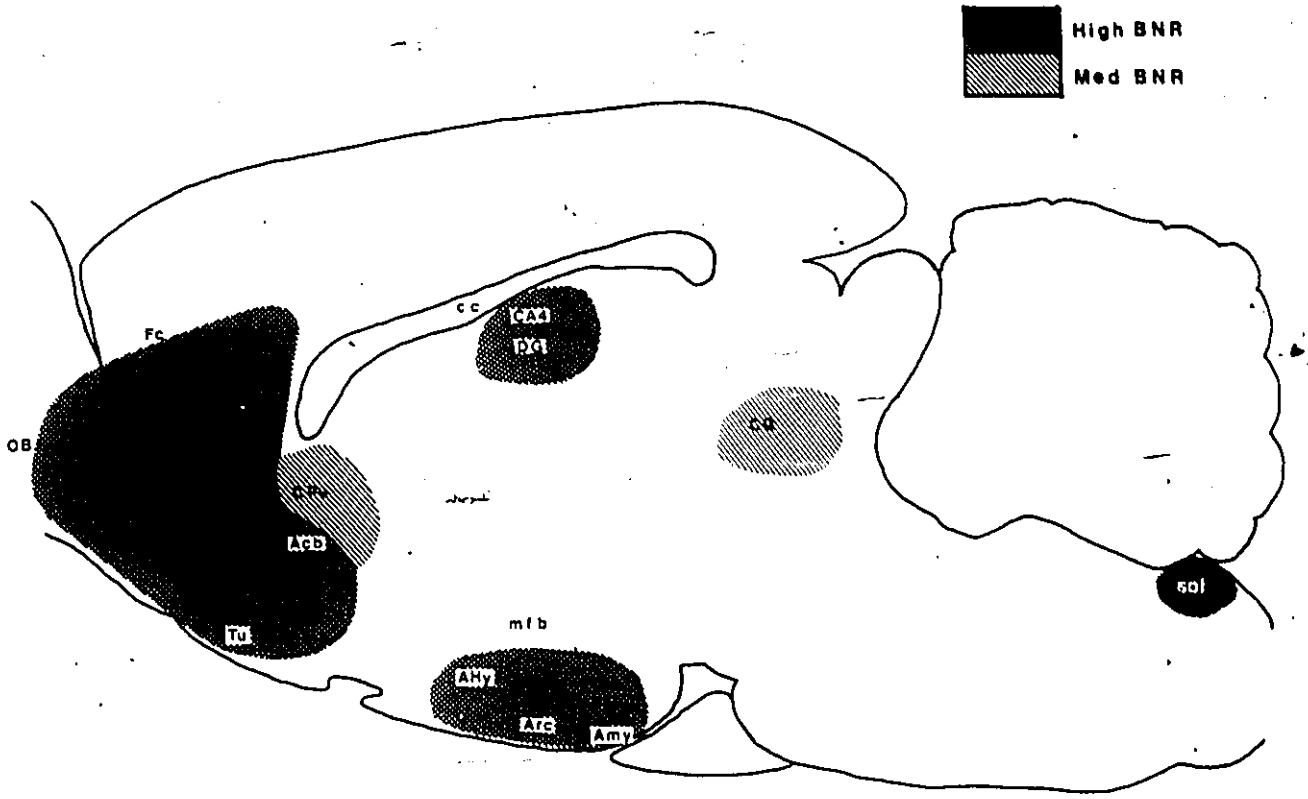

Figure 2: The localization of BN binding sites (BNR) in the rat brain (saggital view) adapted from Paxinos and Watson, (1982).

In addition, receptors for BN-like peptides are present on pituitary cells of the rat (Westendorf and Schonbrunn, 1983). The discrete regional distribution of binding suggests that endogenous BN-like peptides may function as important regulatory agents in certain brain loci.

Distribution of BN Receptors in the Periphery

Bombesin receptors in the gastrointestinal tract are localized to the fundus, gastric antrum, duodenum and ileum of the rat (Moran et al, 1985). Bombesin binding was limited to the neural plexus and was not found in the mucosal or muscular layers. In the guinea-pig, receptors for BN are present in pancreatic acini (Jensen et al, 1978). Additionally, BN has been found to bind with specific receptors on normal human pancreatic membranes (Scemama et al, 1986). As well, BN binds with high affinity to small cell lung cancer cells. Binding was reversible, saturable and specific (Moody et al, 1985).

In all instances the C-terminal of BN/GRP is important for high affinity binding of BN-like peptides to their receptors. There is no evidence, at this time, for more than one type of receptor for BN-like peptides (Moody et al, 1987).

Effects of BN Administered Centrally

Central administration (ventricularily) of BN induces many changes such as hyperglycemia, hypothermia in cold exposed rats, alteration of pituitary secretions, decrease in gastric secretion, increases in locomotion and grooming in rats and suppression of feeding behavior.

Hyperglycemia

Central injection of nanogram doses of BN in conscious rats results in a 2-fold elevation of plasma glucose that is independent of ambient temperature (Brown et al, 1977b). This hyperglycemic effect is accompanied by a 10-fold increase in plasma epinephrine (Walsh. 1982; Brown et al, 1979), an increase in plasma glucagon and a relative decrease in plasma insulin. The hyperglycemic response to central administration of BN is prevented by adrenalectomy. These results are consistent with a primary effect of BN in the brain that outflows to the adrenal gland, with hyperglycemia resulting from increased secretion of adrenal medullary epinephrine (Fisher and Brown, 1984; Brown et al, 1977b; Brown et al, 1979). Specifically, BN microinjected in the dorsal hypothalamus, in conscious dogs caused rises in blood sugar and a specific rapid rise in plasma epinephrine (Brown, 1983). Lateral hypothalamic lesions or transections

blocked the BN induced hyperglycemia in rats (Gunion et al, 1984). The hyperglycemic effect of BN was antagonized by peripheral or central administration of somatostatin analogs (Francesconi and Mager, 1981; Brown et al, 1979). This central effect of BN does not appear to be due to activation of adrenergic or cholinergic pathways (Brown, 1981b).

Furthermore, Okubo et al (1985) demonstrated BN and GRP-27 stimulated a dose-related release of epinephrine from isolated rat adrenal gland. A half maximal effect of BN was observed at 1.2×10^{-9} M and a maximal release of epinephrine at 1×10^{-6} M. Bombesin also significantly stimulated the release of norepinephrine at concentrations above 1×10^{-7} M. Bombesin-induced catecholamine releases were not inhibited by hexamethonium or atropine. In addition, BN had additive effects on the nicotine-induced epinephrine and norepinephrine release. More recently, LeMaire et al, (1986) have demonstrated very high levels of BLI in bovine adrenal medulla. These data strongly suggest that BN-like peptide(s) play a physiological role as one of the important regulators in catecholamine secretion in the adrenal gland.

Poikilothermic Effects

Bombesin is one of the most potent substances known to influence thermoregulation through a CNS site of action (Brown et al, 1977c). Bombesin-induced hypothermia in cold-

exposed rats is associated with a reduction of metabolic rate relative to the ambient temperature (Brown, 1982). Bombesin treated rats display no increases in oxygen consumption rate when placed in a cold environment in contrast to controls, hence, regulatory heat production is inhibited following BN administration and cold exposure leads to hypothermia (Brown, 1982). Bombesin may prevent cold-induced elevations of oxygen consumption through a CNS action on cardiac function, since BN administered ICV produces dose-related suppressions of cold-induced elevations of heart rate and oxygen consumption (Fisher et al, 1985). The CNS-selective somatostatin analog ODT8-SS, injected ICV, reversed the effects of BN on heart rate and oxygen consumption during cold exposure, but atropine methyl nitrate administered intravenously did not (Fisher et al, 1985). The antagonism of ODT8-SS and TRH on BN-induced hypothermic effects is physiological since ODT8-SS and TRH are not BN-receptor antagonists (Moody et al, 1987). The direct injection of BN into the anterior hypothalamic preoptic area results in potent hypothermic effects in cold-exposed rats suggesting that this is a specific site for this effect of BN (Pittman et al, 1980; Wunder et al, 1980). Tolerance has been shown to develop to the BN-induced hypothermia when rats were infused with BN (0.18 ug/hr; ICV) (Cowan et al, 1985).

In contrast, BN has little or no effect on body temperature in rats maintained at thermoneutrality and

elicits hyperthermia in heat-stressed rats (Tache et al, 1980).

Pituitary Hormone Secretion

In male steroid-primed, urethane-anesthetized rats, intracisternal injections of BN (3-10 ug) stimulated growth hormone(GH) and prolactin release (Rivier et al, 1978). However, in conscious male rats, BN (500 ng - 1ug) injected ICV decreased basal GH levels and induced a long lasting suppression of episodic GH release as measured by serial blood sampling. In conscious male rats, BN injected ICV or into the preoptic anterior hypothalamus induced a long lasting inhibition of plasma prolactin levels (Tache et al, 1979b). Moreover, BN administered intracisternally was able to antagonize the rise in plasma prolactin levels induced by dopamine antagonists injected intracisternally or IP. This BN antagonism of dopamine-antagonist-induced rise in prolactin does not occur at the dopamine receptor (Collu et al, 1983; Moody et al, 1987).

Kentroti and McCann, (1985), report the injection of porcine GRP-27 (2 ug to 10 ng; ICV) in ovariectomized female rats resulted in a significant decrease in plasma GH levels, lasting 90 min. The minimal effective dose of GRP-27 was 10 ng (3.6 pmol); however, the GH-releasing action of the human pancreatic growth hormone-releasing factor (GRF)(intravenous injection 0.1 ug/kg) was blocked by 5 ng (1.8 pmol) GRP-27.

Also, all GH pulses were abolished over the 90 min. However, GRP-27 has a direct stimulatory effect on GH release on dispersed pituitary cells perfused with medium (Westendorf and Schonbrunn, 1982, 1983; Kentroti and McCann, 1985). Kentroti and McCann (1985) suggest that GRP-27 may act on periventricular structures to release somatostatin, which reduces GH release. In agreement with this hypothesis, it has been demonstrated that the inhibitory effect of ICV administered GRP-27 on plasma GH levels was significantly attenuated by administration of antisomatostatin serum (Yasuhiro et al, 1984). Also, Abe et al (1981) found that in anesthetized rats BN (0.2 ug; ICV) increased immunoreactive somatostatin concentrations in hypothalamo-hypophyseal portal blood, but not in jugular blood. Thus, it appears likely that the mechanism for this effect of GRP-27 to alter GH secretion is via the paraventricular nucleus where GRP-27 (Roth et al, 1982) increases somatostatin release into portal vessels where somatostatin passes down the portal vessels and blocks the release of GH from somatotrophs (Kentroti and McCann, 1985). Further studies may reveal a physiological role for this BN-like peptide in the release of GH.

Gastric Secretion

Bombesin or GRP-27 (pmol range) injected into the cerebrospinal fluid, inhibited basal gastric secretion and

significantly or completely suppressed acid secretion stimulated by various secretagogues or surgical interventions in rats (Tache et al, 1980), cats (Aronchick et al, 1983) and dogs (Pappas et al, 1984). From these studies it appears that the antisecretory effect of BN-like peptides delivered in the CSF can be observed irrespectively of the animal preparation or type of gastric stimuli (Tache and Gunion, 1985). Peptide action is dose-dependent, long lasting, and structurally specific.

Several lines of evidence indicate that BN-induced inhibition of gastric acid secretion is not only localized in forebrain hypothalamic structures. Electrolytic lesions of the lateral, ventromedial or the paraventricular nucleus or knife cuts around the lateral hypothalamus did not modify gastric response to intracisternal BN (Tache et al, 1982; Gunion et al, 1984; Tache et al, 1984). Brainstem mechanisms alone are sufficient to mediate this peptide action since midbrain transection did not alter the dose-dependent inhibitory effect of intracisternal BN on gastric secretion (Gunion and Tache, 1984). Central action of BN to inhibit stimulated gastric secretion has not been shown to be reversed by central catecholamine, serotonin, or opiate receptor antagonists (Tache and Collu, 1982). Neural transmission from the brain to the stomach of the BN inhibitory effect is not vagally or adrenomedullarily mediated. However, transection of the spinal cord up to the 7th cervical levels and blockade of a_2-adrenergic receptors

reversed the rise in gastric pH induced by intracisternal BN, suggesting a mediation in part through the sympathetic nervous system (Lesiege and Tache, 1983). However, chlorisondamine, a ganglionic blocking agent known (0.5 mg/kg given intravenously) to completely block sympathetic and parasympathetic outflow (Taylor, 1980; Fisher et al, 1982; Lenz et al, 1985), did not block inhibition of gastric acid secretion induced by BN or GRP-27. This indicates that this effect of BN and GRP-27 is not mediated by the autonomic nervous system (Lenz et al, 1986).

Recent research on the structure activity of BN-like peptides injected centrally to reduce gastric acid secretion in rats demonstrated that GRP-27 is almost as potent as BN whereas litorin is only 85% as potent as BN. Neuromedin B and C were inactive (Guglietta et al, 1985). However, as in other comparative studies, time course is an important factor as well as purity of the drug, when carrying out comparative studies. Since no mention of control for either factor is evident in the methodology final conclusion on structure activity must await more stringent control of both time to degradation and purity of peptide.

Grooming

The most striking behavioral activity induced by nanogram doses of BN administered ICV or intrathecally is increased grooming with a predominant scratching which

continues for up to 2.5 hr. Similar effects have been observed in mice, guinea pigs, rabbits and monkeys (Katz, 1980; Cowan et al, 1985). Tolerance did not develop to the effects of BN on grooming (Gmerek and Cowan, 1982; Kulkosky et al, 1982b; Van Wimersma et al, 1985a). Bombesin-induced grooming was blocked by benzomorphan, diazepam or haloperidol administration, but these drugs may act as physiological, not BN receptor antagonists (Crawley and Moody, 1983; Van Wimersma Griedanus et al, 1985b; Moody et al, 1987). Naloxone was reported by Gmerek and Cowan (1983) not to block BN induced grooming at behaviorally non-depressant doses, whereas, Van Wimersma Greidanus et al (1985b) report that naloxone does depress BN-induced grooming, although at the doses used naloxone did significantly suppress the baseline total grooming score.

Bombesin induced grooming is qualitatively different from that associated with ACTH, the prototypic groom-inducing agent (Gispen et al 1975; Dunn et al, 1981; Katz, 1980). The grooming behavior elicited by ACTH and other lipotropin-derived peptides consists of yawning and stretching followed by head to tail licking, and are all naloxone reversible. Tolerance develops to ACTH-induced grooming but not to BN-induced grooming (Kulkosky et al, 1982b; Gmerek and Cowan, 1983). No cross tolerance between BN and ACTH has been reported to develop (Van Wimersma Griedanus, 1985a). The BN-induced behavior consists mostly of facial grooming. In addition to normal facial washing, an

atypical form of grooming consisting of extensive scratching of the neck and upper body quadrant with the hind paws is evident (Katz, 1980; Kulkosky et al, 1982a,b; Van Wimersma Greidanus, 1985b). A recent study indicates that BN-induced grooming occurs through central but not intravenous administration of BN. Furthermore, this CNS initiated grooming is independent of the pituitary-adrenal axis (Gmerek and Cowan, 1983).

Locomotion

Central administration of BN (10 ng-1 ug) increases locomotor activities and exploration in a dose-dependent fashion, over 60-90 min (Kulkosky et al, 1982a,b; Gibbs et al, 1982; Pert et al, 1980). Bombesin-induced stimulation of locomotor activity does not occur following peripheral administration (Gibbs et al, 1982).

Satiety

Peripheral or central administration (ICV) of BN, induces reduction of eating, however, only the peripheral administration of BN is accompanied by the normal satiety sequence of behavior: grooming and exploration occur for up to 6 min after the meal followed by rest (Gibbs et al, 1979). The central administration of BN results in feeding inhibition accompanied by increased grooming and locomotion

lasting 60-90 min, as well as inhibitions of water intake (Kulkosky et al, 1982a,b; Gibbs et al, 1982). Thus, although BN (ICV) produced large, dose-related inhibitions of food intake with a threshold dose of 100 ng, the effect was not behaviorally specific. Injection of BN bilaterally into the lateral hypothalamus specifically reduced food intake and did not cause a disruption of the characteristic sequence of satiety related behaviors. Willis et al (1984) showed that 100 ng of BN injected bilaterally at the paraventricular nucleus of rats inhibited food intake, specifically as well. However, in both studies reduction of food intake was small (20-25%). Thus the specific central site of BN-induced satiety remains to be discovered.

Gastrointestinal Transport

Bombesin administered ICV (0.0003-0.1 ug/mouse), intrathecal (0.003-0.3 ug/mouse) and IP (3-750 ug/mouse) significantly delayed gastrointestinal transit in a dose related fashion. Bombesin ICV was 13.5 times more potent than BN intrathecally and 3406 times more potent than BN IP. Colonic bead expulsion was significantly delayed in a similar dose dependent way by BN ICV (0.0003-1.0 ug/mouse) and intrathecal (0.003-1.0 ug/mouse) injection. Bombesin, IP, at the doses tested (0.3-250 ug/mouse) was ineffective. Bombesin, ICV, was 1.54 times more potent than BN intrathecally in inhibition of colonic bead expulsion (Kolso

et al, 1986a). Porreca et al, (1983) have demonstrated that BN ICV delays gastric emptying, inhibits small intestinal transit and transiently increases large bowel transit in rats. Centrally administered BN acts at a supraspinal site to produce inhibition of gastrointestinal transit (Koslo et al, 1986a). Hypophysectomy as well as adrenalectomy eliminated the gastrointestinal transit effects of BN ICV (Gmerek and Cowan, 1984) and intrathecal BN (Koslo et al, 1986b) in the rat. These studies emphasize the possible role of the pituitary-adrenal axis in the manifestation of centrally administered BN-induced gastrointestinal transit.

Peripheral Effects of BN

Certain effects of centrally administered BN do not occur when BN is administered peripherally, such as locomotion and grooming stimulation. Other central effects of BN are the inverse of the peripheral effect such as effects on gastric secretion, which are inhibited by central administration and stimulated by peripheral administration. Other actions of BN, such as induction of satiety occur after peripheral or central administration. Thus, the separation of the effects of centrally and peripherally administered BN defies simple hypotheses, at present.

As reviewed below, the peripheral effects of BN include contraction of smooth muscle, regulation of gastric exocrine and endocrine function, and pancreatic exocrine and

endocrine function. As well, peripheral BN may also act as a growth factor, and may induce satiety.

Contraction of Smooth Muscle

Initial studies on the pharmacological actions of BN revealed the following: hypertensive action in the dog; potent stimulant action on the rat uterus, the rat and guinea-pig colon and the cat ileum (Anastasi et al, 1971). In these tissues, contractile responses were not blocked by cholinergic or catecholaminergic antagonists. In contrast the contractile response of guinea pig ileum was strongly inhibited by atropine implying stimulation of a cholinergic mechanism. In the dog, intrarterial injections of GRP-27 or BN inhibited ongoing contractile activity in the small intestine of anesthetized dogs, however, pretreatment with atropine increased the ED_{50} for BN inhibition of intestinal contractions (Fox and McDonald, 1984). In addition to the cholinergic involvement in BN-induced effects in pig ileum and dog intestine smooth muscle action, somatostatin has been reported to inhibit BN-induced effects on migrating myoelectric complexes in the small intestine of the rat (Al-Saffar, 1984). Furthermore, substance P appears to mediate the effect of BN at the feline lower esophageal sphincter (Reynolds et al, 1986). These studies on contractibility have for the most part been carried out on tissue with intrinsic innervation. With this approach it remains

uncertain whether the peptides act directly on the smooth muscle cells or indirectly by affecting neuronal elements in the gut. One way to eliminate this uncertainity is to use a preparation of enzymatically dissociated smooth muscle cells thus obviating the possibility of neuronally mediated effects (Lassignal et al, 1986). Smooth muscle cells freshly dissociated from the stomach of Bufo marinus showed contractions to BN, substance P, and cholecystokinin-octapeptide, whereas vasoactive intestinal peptide, secretin and dopamine inhibited acetylcholine-induced contractions. Furthermore, acetylcholine, BN, substance P, and cholecystokinin-octapeptide, were found in some instances to act on the same cell, suggesting that receptors for these agents exist on one and the same cell (Lassignal et al, 1986). This, suggests that there may be a the direct action of both neuropeptides and acetylcholine on muscle cell contraction. Further experimentation of this type on mammals including man is necessary to ascertain if smooth muscle cells in other species have similar receptors.

In human, a 30 min infusion of 5-10 ng/kg/min of BN causes an increase in lower esophageal sphincter pressure by a non-gastrin/pancreatic polypeptide mediated mechanism (Corazziari et al, 1982), delay of solid meal gastric emptying (Scarpignato et al, 1982), inhibition of basal mechanical activity of the duodenum and jejunum (Corazziari et al, 1974b), and stimulation of gall bladder contraction and emptying (Corazziari et al, 1974a).

Bombesin has been found to inhibit gastric emptying in unanesthetized rats after central administration (0.1-1.0 ug) whereas at a dose of 20 ug/kg IP, BN failed to alter gastric emptying. This suggests that central BN receptors are responsible for the observed inhibition of gastric emptying. Subdiaphragmatic vagotomy completely abolished the inhibition of gastric emptying induced by central BN administration (Porreca and Burks, 1983). Burks et al, (1985), state that sensory nerve endings in the gastric mucosa and muscularis responding to stretch and chemical stimuli project to the CNS over vagal and splanchic afferent pathways (Paintal, 1973). The vagal afferents terminate primarily on cells in the nucleus of the tractus solitarius (Gwyn et al, 1979). Furthermore, they demonstrated that the peptide-induced, increase in gastric wall tension during the contraction phase is accompanied by an increase in the discharge frequency of brainstem neurons per unit change in wall tension. They have recorded brainstem neuronal responses to neuropeptide-induced gastric changes which are vagally mediated and are thus abolished by vagotomy. Cell bodies in the nucleus tractus solitarius project directly to preganglionic cell groups of motor nuclei in the brain stem, especially the dorsal motor nucleus of the vagus and the nucleus ambiguus (Sawchenko, 1983). Unit activity recorded from the dorsal motor nucleus of the vagus showed an increase in discharge frequency during sustained gastric distension (Ewart and Wingate, 1982). Moreover, retrograde

transport of horseradish peroxidase from the stomach to the brain indicates that motor neurons project from the dorsals motor nucleus of the vagus to the gastric corpus and antrum (Scharoun et al, 1984). The nucleus ambiguus, projects parasympathetic innervation to the stomach. Gamma-aminobutyric acid (GABA) may function as a CNS neurotransmitter in the nucleus ambiguus, exerting tonic inhibitory influences on gastric motility since blockade of the central GABA receptors results in stimulation (by disinhibition) of both antral and pyloric contractions in the stomach (Williford et al, 1981).

Thus, a sensory-CNS-motor loop between gut-brain-gut, has been outlined, for gastric emptying, where brain-gut peptides such as BN may modulate sensory activity in the gut. Peptides may be neuromodulators at the synapse of first order visceral afferent fibers, and may be neuromediators of the neurons which project to motor nuclei in the brain stem. These motor neurons then project back to the stomach. Further integration of information on the interrelationship between systems such as the one just outlined for gastric emptying, are essential for a better understanding of the physiological role of brain-gut peptides.

Gastric Exocrine and Endocrine Secretions

The peripheral effects of BN on gastric function have been explored in the rat, where BN stimulated release of

gastrin but not acid secretion, and in dogs and humans where, stimulation of gastrin release was associated with stimulation of acid secretion (Walsh, 1982). In human, BN infusion caused an increase of gastric acid secretion which was 50% lower than that induced by maximal stimulation with pentagastrin, and was in part, inhibited by atropine (Delle Fave et al; 1985; Delle Fave et al, 1980). Since the rise in gastric acid secretion follows a prompt increase of gastrin release, it is assumed that gastric secretion induced by BN is gastrin mediated. In dogs, intravenous infusion of BN produced potent stimulation of both gastrin release and gastric acid secretion. The gastrin response was abolished by antrectomy (Bertaccini et al, 1974; Impicciatore et al, 1974), whereas vagotomy led to increased gastrin response but unaltered acid response to BN (Hirschowitz and Gibson, 1978). In humans, BN-induced gastrin release was significantly lower after antrectomy, however the peak gastrin responses to BN (5 ng/kg-1/min-1) infused over 30 min were significantly over baseline, suggesting that BN acts not only on antral G cells but on all gastrin cells in the gastrointestinal tract.

Recent in vitro studies on vascularly perfused rat stomach indicated that BN and acetylcholine are the main intramural neural regulators of gastrin and somatostatin secretion. Acetycholine acts predominantly to decrease paracrine secretion of somatostatin, thus, eliminating the continuous restraint of somatostatin on gastrin secretion

and enabling BN to exert its potent stimulatory effect on gastrin secretion (Schubert et al, 1985). These results in the rat may be applicable to similar studies in the vascularly perfused pig antrum (Holst et al, 1983) and on isolated human antral glands (Richelsen et al, 1983).

Effects on Pancreatic Exocrine and Endocrine Secretion

In humans, BN stimulated the release of insulin, glucagon (Bruzzone et al, 1983), and pancreatic polypeptide(de Magistris et al, 1981). In isolated cell culture, the direct effect of BN on islet of Langerhans cells, insulin secretion has been confirmed (Swope and Schonbrun, 1984). Bombesin induced secretion of glucagon from the endocrine pancreas but also from the L cells of the intestine (Bruzzone, 1983).

In humans, BN stimulated secretion of pancreatic enzymes and caused increase of bicarbonate concentration in duodenal juice. As well, BN augmented the blood concentration of amylase, lipase and trypsin enzymes originating in the pancreas (Delle Fave et al, 1985). The mechanism of action of BN in stimulating exocrine secretion in mammals appeared to be species specific (Pearson et al, 1984; Jensen et al, 1978). With rat and mouse pancreas BN evoked membrane depolarization (Iwatsuki and Petersen, 1978) resulting in exocrine secretion. In the pig pancreas, however, findings failed to show that BN was an important

peptide neurotransmitter (Pearson et al, 1984). The mechanisn of action of BN in stimulating pancreatic exocrine secretion in humans remains to be clarified.

Satiety

Peripherally administered BN and GRP-27 produced potent dose-related and specific reductions of food intake at test meals in rats (Gibbs, 1985). In addition, BN produced satiety in genetically obese and lean mice (McLaughlin and Baile, 1981), in baboons (Woods et al, 1983) and in humans (Muurahainen et al, 1983), without any reported side effects.

The mechanism for this effect is unknown, but the action of BN is not blocked by adrenalectomy (Gibbs et al, 1981), hypophysectomy (Stukey et al, 1982), complete subdiaphragmatic vagotomy (Smith et al, 1981), celiac ganglionectomy (Gibbs, 1985), spinal cord section at the level of the 6th thoracic vertebra (Stukey et al, 1982), and area postrema or ventromedial hypothalamic lesions (Gibbs, 1985). Recently Stuckey et al (1985), reported that a total afferent disconnection of the gastrointestinal tract from the brain blocked the inhibitory effect of BN on meal size. They interpret this as suggesting that systemically-administered BN may have a peripheral site of action when it reduces food intake at a meal.

Growth Factor

Bombesin-like peptides are present in endocrine cells of the rat fetal but not adult lung (Wharton et al, 1978; Moody et al, 1984), as well, BN-like peptides are an order of magnitude higher in human fetal lung than adult lung (Cutz et al, 1981, Track and Cutz, 1982). These BN-like peptides were characterized and found to be GRP-27 and neuromedin C (Moody et al, 1987). In human neonates with acute respiratory distress syndrome there were lower levels of BN-like peptides throughout all regions of the respiratory tract compared to 24-28 week fetuses or full-term infants. Thus, BN-like peptides may play a critical role in the differentiation of the lung.

In addition to its action on lung maturation, BN has been shown to induce hyperplasia and DNA synthesis in the rat pancreas (Sagor et al, 1982; Lhoste et al, 1985). This effect is not mediated indirectly by the release of endogenous gastrin or cholecystokinin, but appears to be mediated by BN directly. Also, BN increased the crypt cell production rate and consequently increased gastrin secretion in the small intestine of rats after intestinal resection (Lehy et al, 1983).

As well as functioning as a mitogen in normal tissue, BN has also been shown to function as an autocrine growth factor in small cell lung cancer cells derived from cells of bronchial mucosa (Cuttitta et al, 1985). Biochemical studies

indicated that the BN-like peptide(s) in these cancer cells is GRP-27 and/or neuromedin C (Wood et al,1981; Erisman et al, 1982).

Summary

Bombesin appears to have major functions in growth, body metabolism, digestion, satiety, and respiration. It appears to function in the limbic, endocrine, exocrine and immune systems. One might hypothesize, as was stated in the introduction, that BN is a basic protein, whose gene structure has been conserved throughout evolution and is associated with biological activities such as growth and metabolism which are essential for survival of living organisms (Lazarus et al, 1985). It appears that the C terminal of BN-like peptides is highly conserved and necessary for biological activity and their is only one class of BN receptors for all biological effects analysed so far (Moody et al, 1987). Thus, as suggested by Angelucci (1985), the BN message is understood not for intrinsic content, which does not exist, but according to the logic of the recipient structure.

Interaction of Dopamine and BN In the CNS

Little is known about the precise neuroanatomical loci of the behavioral effects of BN or the neurochemical substratum of its mediation.

Support for the contention of dopaminergic involvement arises from recent experimental results which demonstrate that although BN is not capable of modifying the binding characteristics of [^{3}H]-spiroperidol when injected "in vivo" or added "in vitro" to hypothalamic or striatal dopaminergic receptors, BN administered intracisternally is able to antagonize the rise in prolactin levels induced by dopamine receptor antagonists injected either intracisternally or intraperitoneally (IP) (Collu et al, 1983). Thus the above results suggest that BN, centrally administered, does not mediate its pharmacological effects at the dopamine receptor, but may mediate its effects through dopaminergic neurons at terminal areas, either presynaptically or at the synapse, by facilitating the release of dopamine. Bombesin may have an action similar to that which amphetamine has on facilitating the release of dopamine synaptically to stimulate increased locomotor activity (Feigenbaum et al, 1983).

The similarity of behavioral and pharmacological effects of dopamine and BN and the shared anatomical locus of dopamine, dopamine terminals, and BN receptors further

supports the possibility that BN may mediate its effects through modulating the release of dopamine. The mesencephalic dopamine pathways (A8, A9, A10) are implicated in the mediation of increased locomotor activity, particularly at the terminal area in the nucleus accumbens (Kelly et al, 1975; Roberts et al, 1975; Pijnenburg and VanRossum, 1973; Fink and Smith, 1980). At the nucleus accumbens there is a very high concentration of BN receptors, (Pert et al, 1980), as well as, very high concentration of dopamine within the area rich in dopamine terminals (Versteeg et al, 1976; Lindvall et Bjorklund, 1978, 1983; Lindvall and Steveni, 1978; Bjorklund and Lindvall, 1986).

Dopamine and BN both suppress prolactin release (Collu et al, 1983). The locus of this pharmacological action of dopamine has been identified as the site of A12, dopamine receptors in the median eminence (Collu, 1981). Dopamine receptors and very high levels of dopamine are present in the median eminence (Lindvall and Bjorklund 1978, 1983; Versteeg et al, 1976). Furthermore, there are very high levels of BN receptors in the hypothalamus (Pert,et al, 1980).

Lately, several researchers have postulated that afferent dopamine terminals in limbic structures (i.e. amygdala and nucleus accumbens) may "gate" the output of these structures (Chronister et al, 1981; DeFrance et al, 1981; Mogenson and Yim, 1981; Roberts et al, 1982). It is of interest to note, as listed in Table 2 and illustrated in Figures 2, and 3, that high density BN binding sites occur in limbic areas where there are dopaminergic terminals, namely: frontal cortex, olfactory bulb, nucleus accumbens, olfactory tubercle, amygdala, dorsal hippocampus, and hypothalamus (Lindvall and Bjorklund, 1978, 1983; Bjorklund and Lindvall, 1986; Lindvall and Steveni, 1978; Versteeg et al, 1976; Pert et al, 1980; Wolf et al, 1983). Consequently, these behavioral, pharmacological and anatomical data suggest that dopamine may mediate BN-induced effects within limbic structures.

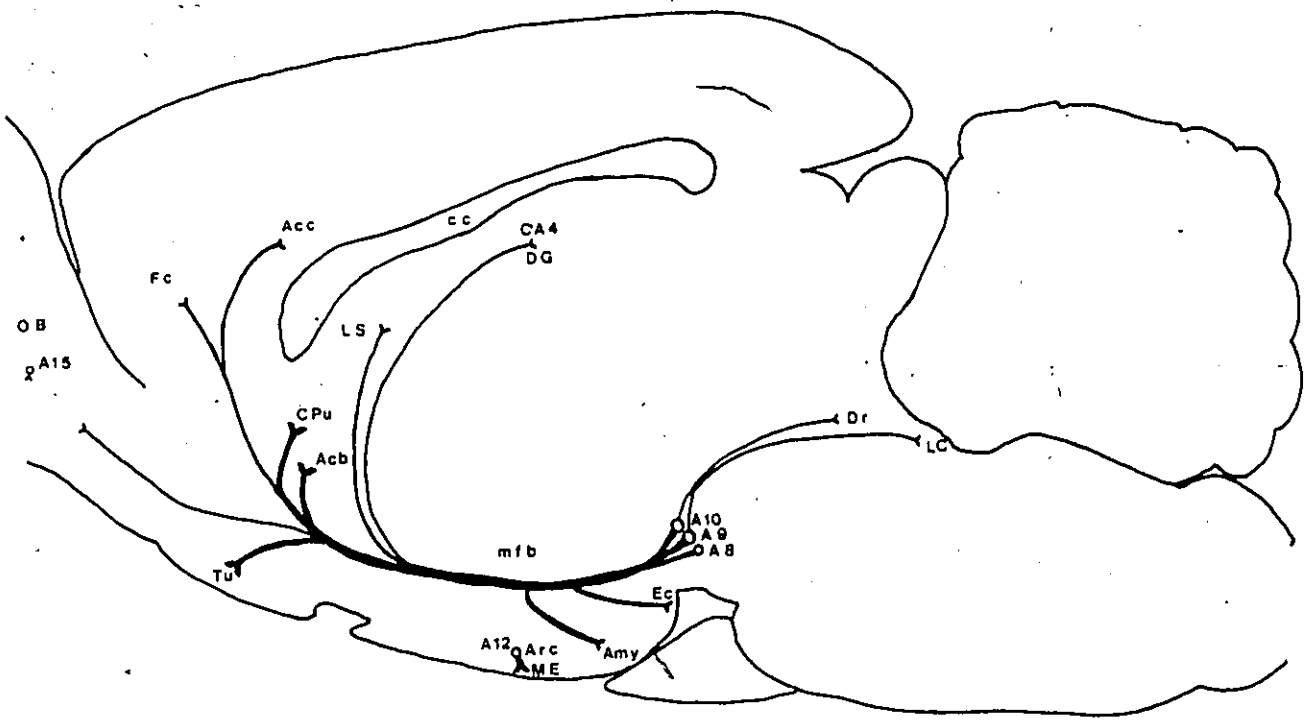

Figure 3: Principle organization of the cerebral dopamine cell bodies (o) and terminal regions (Y), in the rat brain (saggital view; adapted from Paxinos and Watson, 1982).

.t.Table 2. Comparison of BN-like immunoreactivity (BLI), BN-binding sites, dopamine (DA) terminals, and DA in various brain regions of the rat.

Site	BLI (f mol/ ug protein) (1)	BN Binding (f mol/mg) (2)	Origin of DA cells (3)	DA (pg/ug protein) (4)
Frontal Cortex	?	15.4	A9,10	1.04
Olfactory Bulb	?	15.4	A9,10,15	8.19
Nucleus Accumbens	0.56	15.4	A8,9,10	87.17
Central Amygdaloid Nucleus	1.44	18.6	A9,10	15.01
Dorsal Hippocampus	0.22	13.6	A9,10	0.36

(1) Moody et al, 1981a; (2) Pert et al, 1980; (3) Lindvall and Bjorklund, 1983; (4) Versteeg et al, 1976.

'?' no measurement available for the structure.

OVERALL OBJECTIVES

PHASE 1. Preliminary experiments were undertaken to investigate the behavioral effects (locomotion, floor activity and rearing) of BN when injected in the lateral ventricle, which served to indicate the general effect of BN within the CNS. As well, preliminary experiments investigated the effects of dopaminergic antagonists on these BN-induced behaviors.

PHASE 2. These experiments investigated further whether these BN-induced behavioral effects (locomotion, floor activity and rearing) were altered specifically by the dopaminergic antagonist flupenazine at dose(s) of antagonist which did not effect baseline behavior. Additionally we investigated whether these BN-induced behavioral effects (locomotion, floor activity and rearing) were altered by a specific lesion of dopamine neurons.

PHASE 3. After these initial studies, the objective of this thesis research was to attempt to delineate the neuroanatomical site(s) within the rat brain where BN (ICV) may have induced behavioral its effects (locomotion, floor activity, rearing and/or grooming elements). BN was microinjected at six neuroanatomical site(s) endowed with high density of BN-binding within the rat brain and ICV, and the behavioral effects monitored.

PHASE 4. The final phase experiments were undertaken to elucidate whether some of the behavioral effects (locomotion, floor activity, rearing, grooming elements and/or satiety) of BN occur concomitantly and site specifically. Two paradigms were used, namely, a "home" paradigm where locomotor activities and grooming were monitored and a "satiety" paradigm where eating and grooming behaviors were monitored. Grooming was monitored in two different paradigms to investigate whether BN-induced grooming was paradigm dependent. As well, the experiments investigated whether neurochemical differences exist in the mechanisms subserving BN-induced behavioral effects with particular emphasis on the possible modulation of BN-induced behaviors through dopaminergic neurotransmission.

CHAPTER 2

PHASE 1: BOMBESIN-INDUCED BEHAVIORAL CHANGES; ANTAGONISM BY NEUROLEPTICS

Objectives Experiments 1.1-1.3

The specific objectives of this Phase were: 1) to characterize the time-course and dose-effect of BN on the behavioral profile of rats, and 2) to elucidate whether neuroleptic drugs (dopamine receptor blockers) antagonize the behavioral effects of BN.

Animals

Male Sprague-Dawley rats (St. Constant, Quebec) were housed individually with free access to food (Master Laboratory Chow) and water. The environment was maintained at 24^{o}C, at 60% relative humidity and with 12 hr of light (6 a.m. to 6 p.m.).

Ventricular Cannulation

Rats under sodium pentobarbitol (50 mg/kg, IP) anesthesia were stereotaxically implanted with stainless-steel guide cannulae (plastic products Co., Roanoke, Virginia) in the lateral ventricle, using coordinates

derived from Pellegrino et al, 1979. The cannula assembly was cemented with dental acrylic to jeweler's screws placed in the calvarium. Stainless-steel obturators were in the guide cannulae at all times except during injections. The incisions were closed and the animals allowed a minimum of 5 days postoperative recovery period prior to the commencement of experiments. At the end of every experiment, cannulae placements were verified using dye substitution followed by standard histological procedures.

Behavioral Monitoring

Behavioral monitoring was conducted by modification of the procedure described by Merali and Toth (1982). Each behavioral observation chamber consisted of an inner clear polycarbonate cage (43 x 23 x 15 cm; identical to the rat's home cage) and an outer frame housing an array of 9 strategically placed infrared lightbeams (IR beams). An array of 5 beams located on a plane 3.8 cm above the floor transected the floor into six equal sections. Two of these IR beams projected longitudinally, parallel to the floor, and were spaced 7.65 cm from the respective side walls. The other 3 (of the 5) IR beams projected transversely on the same plane, and were spaced 11.4 cm apart. This arrangement of IR beams generated a grid with 6 points at which the beams crossed (co-ordinates). Each time an animal moved from one co-ordinate to an adjacent one, the 'locomotion count'

was incremented by 1. The actual distance traversed by an animal was computed by multiplying the 'locomotion count' by the average distance between co-ordinates (13.7 cm). In contrast, the 'Floor Activity' represented the total number of times any of these 5 IR beams were interrupted, and reflected the horizontal and lateral displacement of the animal, at this plane. A curtain of 4 longitudinal IR beams, 17.8 cm above and parallel to the floor (spaced 4.5 cm apart), served to detect the rearing activity of each animal. A custom designed Z-80 microprocessor based controller performed most of the timing and scoring functions. The system consisted of 12 chambers and each of the IR beam was sampled once every second. Simultaneous observations by human raters were conducted from an adjacent room, through a one-way mirror.

Experiment 1.1

Twelve animals equipped with ventricular cannulae were randomly divided into control (n=6) or treatment groups (n=6). During the same time on every alternate day, animals were distributed into individual experimental chambers and allowed 1 hr acclimatization period. Immediately following this, control rats were injected with saline (5 ul over 90 sec, ICV) whereas the treatment group (ascending series) received the following doses of BN (0.001, 0.01, 0.1, or 1.0 ug BN/5 ul, ICV., over 90 sec). All animals were returned to

their behavioral chambers and data collection initiated 15 min later, for the subsequent 23 hr. This procedure was repeated every alternate day, with sequentially increasing doses of BN, until all doses were tested.

Experiment 1.2

The above experiment was repeated on a separate group of animals except that these animals received BN in a descending order (descending series), that is the treatment group received the highest dose (1.0 ug) first and the lowest dose (0.001 ug) last. This procedure was adopted to investigate (and control for) the potential effects of the sequence of BN administration, on the behavioral outcome.

Experiment 1.3

The objective of this experiment was to elucidate whether neuroleptic drugs (fluphenazine and haloperidol) could antagonize the behavioral effects of BN.

Twenty-two rats were randomly divided into two groups: control (n=11) and BN (n=11). According to the split-plot factorial design, every alternate day, the control rats were treated with fluphenazine (0.0, 0.1, 0.25, 0.5 or 1.0 mg/kg, i.p.) followed 30 min. later by saline (5 ul, ICV). The BN animals received fluphenazine (0.0, 0.1, 0.25, 0.5 or 1.0 mg/kg) followed 30 min. later by BN (1ug/5ul, ICV). The

above experiment was repeated in a separate group of 8 animals using a different neuroleptic: control rats (n=4) received haloperidol (0.0, 0.1, 1.0 or 2.5 mg/kg) followed by saline, whereas the BN group (n=4) received the same neuroleptic treatment followed by BN (1 ug/5ul, ICV).

Results Experiments 1.1 and 1.2

Three way analysis of variance (repeated over time and dose), revealed no significant effect of sequence of BN administration, on locomotor, rearing or floor activity. Thus, the data for each behavior from the ascending and descending series were pooled, for each of the BN doses, and analysed using two way analysis of variance with repeated measures (correction by the Greenhouse-Geisser procedure). Post-hoc multiple comparisons were done using the Tukey test (Keppel, 1973).

The interaction between the effects of time and dose was significant $F(1,11) = 4.9$, $p<0.05$. This interaction was mainly attributable to the differential effects of the BN doses on the duration of the locomotor response. Data presented in Figure 4, demonstrate that BN at the lowest dose (0.001 ug) induced a small but significant increase in the distance traversed by the rats. This effect was detectable only during the first half hour following BN administration. With a higher dose (0.01 ug) the magnitude of the locomotor response, but not its duration, was further

increased. Further increases in the BN dosage to 0.1 and 1.0 ug, produced a more prolonged locomotor stimulation that was detectable for 60 and 90 min, respectively.

Similarly, BN produced dose- and time-related changes in the floor activity $F(1,11) = 17.4$, $p<0.01$. As can be seen in Figure 5, at doses of 0.001 or 0.01 ug BN, floor activity which appears to reflect 'excessive' grooming lasted for about 30 min. With the 0.1 ug dose, 'excessive' grooming persisted for about 90 min. And with the 1.0 ug dose, BN effects on floor activity could be detected for up to 150 min.

It is of interest to note that with the 0.1 and 1.0 ug doses, BN effects on floor activity were more prolonged than those on locomotor activity.

Bombesin also increased the frequency of rearing activity in the rats, in a time-dependent manner $F(1,11) = 31.3$, $p<0.01$. As can be seen in Figure 6, BN at a dose of 0.001 ug significantly increased the number of times the animals reared in the first 30 min. The magnitude of this response was further enhanced by the 0.01 ug dose. At doses greater than 0.1 ug, the duration of rearing frequency was extended, in a dose-related manner.

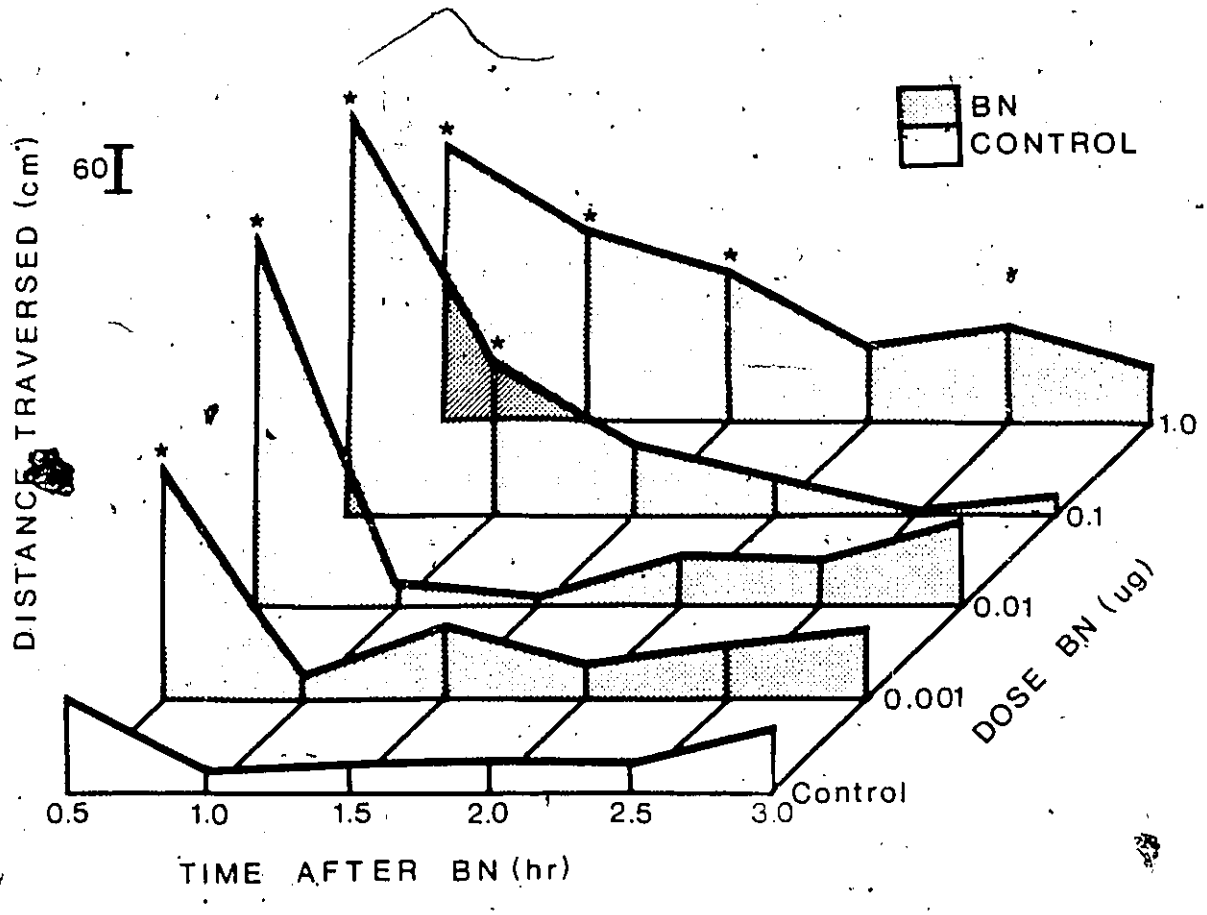

Figure 4: Time- and dose-related effects of BN on locomotor activity of rats. Tridimensional figure: On the ordinate: the magnitude of the quantified response (mean for 30 min.); on the horizontal abscissa: time after BN administration (hr); on the oblique abscissa: dose of BN administered (ug). The profile of vehicle-treated controls is represented by the unshaded area and those of BN-treated groups are represented by the subsequent shaded areas. For each session, n=12. *Significantly different with respect to the appropriate control value at $p<0.05$.

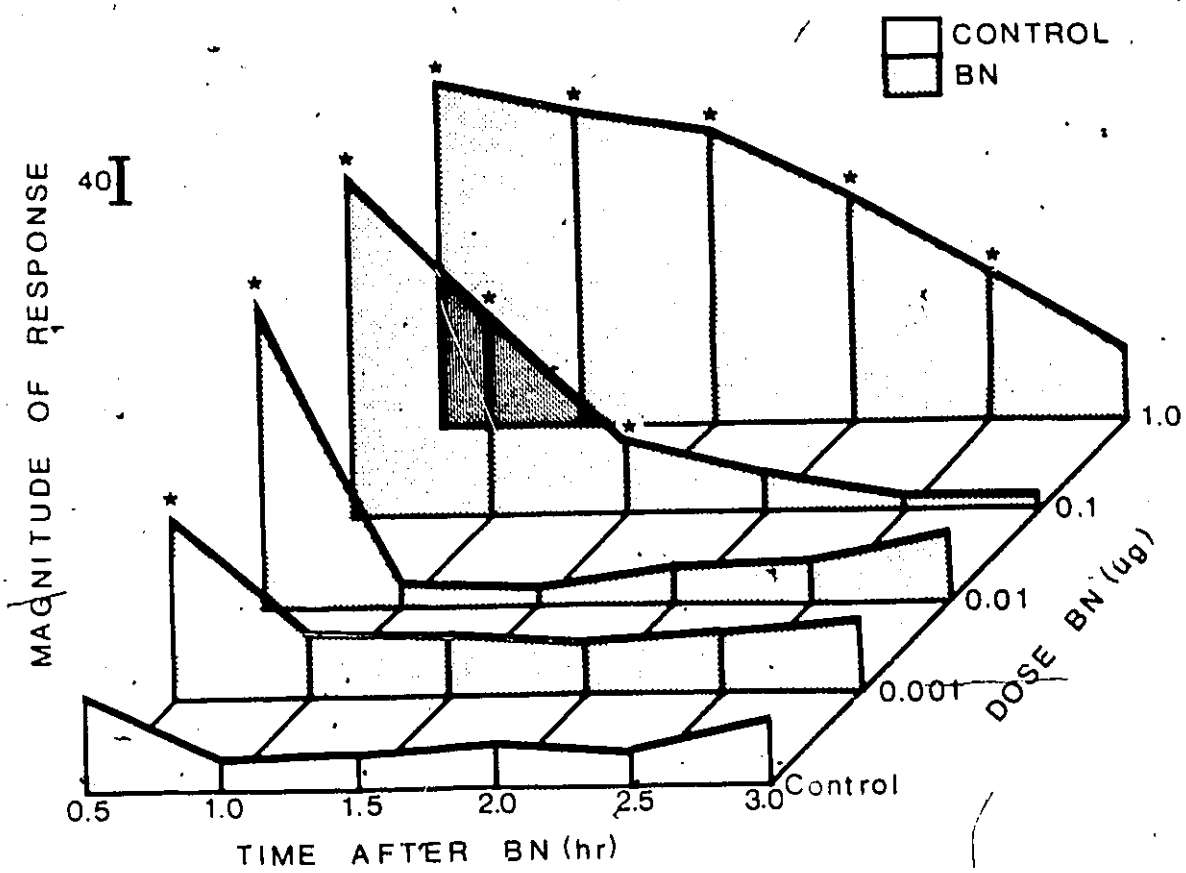

Figure 5: Time- and dose-related effects of BN on floor activity (grooming) of rats. *Significantly different with respect to appropriate control value at $p<0.05$. For further information see legend to Figure 4.

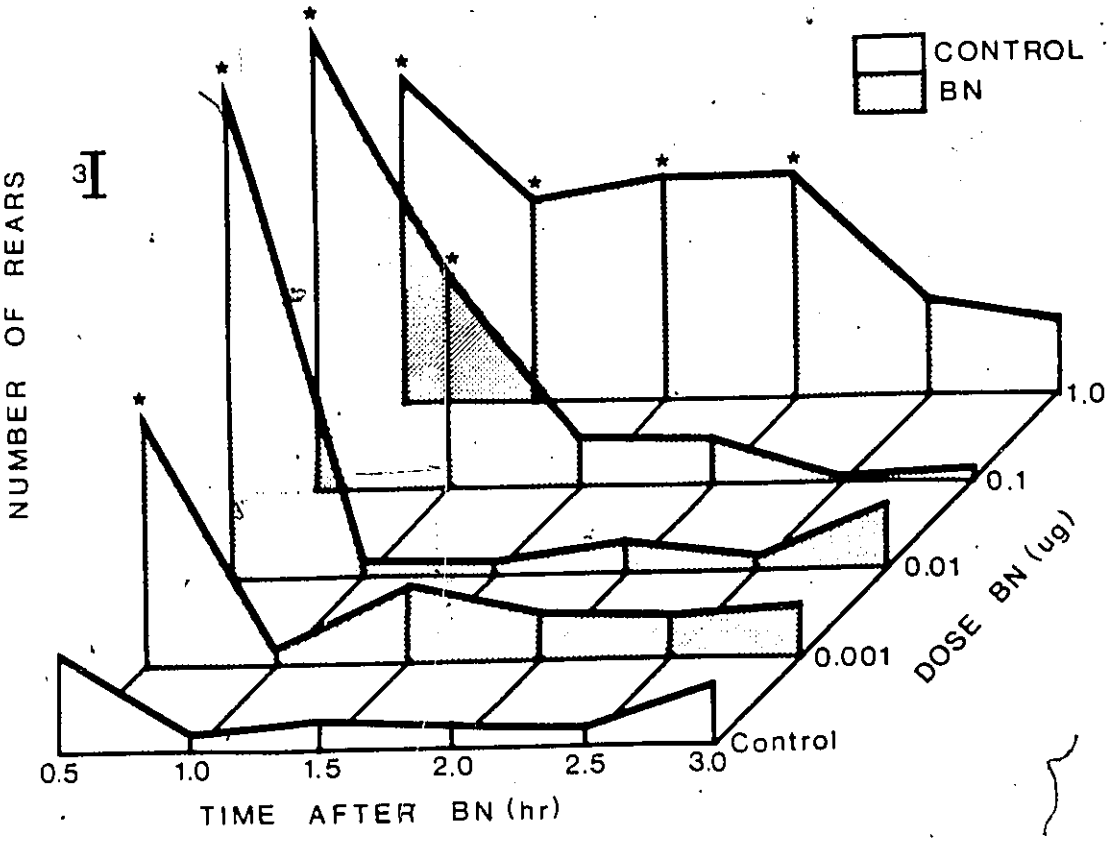

Figure 6: Time- and dose-related effects of BN on the frequency of rearing activity of rats. *Significantly different with respect to the appropriate control value at $p < 0.05$. For further information see the legend to Figure 4.

Results Experiment 1.3

The objective of this experiment was to elucidate whether neuroleptic drugs could antagonize the behavioral effects of BN. In the case of locomotor activity, two-way analysis of variance with repeated measures (correction by Greenhouse-Geisser procedure), revealed a significant group (control or BN) x treatment (fluphenazine doses) interaction $F(1,20) = 8.2$, $p<0.01$. The analysis of the simple main effects, revealed that the interaction was mainly attributable to the differential response of the control ($F(1,20) = 2.9$, N.S.) vs BN ($F(1,20) = 35.2$, $p<0.001$) groups to the various treatment conditions. The data on floor activity were subjected to similar analysis. The interaction effect between groups (control or BN) and treatment (fluphenazine doses) was significant ($F(1,20) = 37.4$, $p<0.001$). The analysis of the simple-main effects revealed that as in the case of locomotion, the interaction was largely attributable to the differential response of control ($F(1,20) = 2.5$, N.S.) vs the BN ($F(1,20) = 103.3$, $p<0.001$) groups. Haloperidol data were subjected to similar analyses followed by multiple post-hoc comparisons, using the Tukey test. The results are summarized in Table 3, below. Pretreatment of animals with either fluphenazine or haloperidol markedly attenuated or completely blocked the behavioral effects of BN (1.0 ug), depending upon the

neuroleptic dose. As illustrated in Table 3, fluphenazine alone at a dose of 0.1 mg/kg, failed to significantly alter the locomotor or floor activity, although there was an evident decrease in these behaviors. However, at that dose, fluphenazine significantly suppressed the effects of 1 ug BN, on locomotor and floor activity. Fluphenazine at higher doses completely blocked the behavioral effects of BN. However, fluphenazine at a dose of 0.25 mg/kg or greater also significantly suppressed the normal locomotor and floor activity. Similarly, haloperidol at a dose of 0.1 mg/kg blocked the behavioral effects of 1.0 ug BN, without significantly altering the normal locomotor and floor activity by itself. At higher doses (1.0 or 2.5 mg/kg), however, although there was a complete blockade of BN effects, the normal behavioral effects were also significantly suppressed.

Discussion

The data from these experiments demonstrated that BN, when administered intraventricularly, quite potently stimulated locomotor, floor activity and rearing behaviors in rats. The duration of this response was dose-related and was not influenced by the sequence of BN doses administered. The behavioral effects were prompt in onset and could be detected for up to 2.5 hr, depending upon the dosage of BN (0.001 to 1.0 ug) used, and the behavioral parameter

monitored. The effect of BN on floor activity was more pronounced relative to its effect on locomotion or rearing, and persisted for the longest period. Increased locomotion elicited by BN has also been noted by other investigators in mice (Katz and Roth, 1979), and rats (Brown et al, 1978; Brown and Vale, 1980; Pert et al, 1980; Gmerek and Cowan, 1983; Kulkosky et al, 1982a, b).

Although BN has powerful behavioral effects, little is known about the locus (or loci) of action, or the underlying neural correlates of these effects. Since explorative locomotor behavior in the rat is believed to be dependent in part to the dopaminergic system(s), we were prompted to investigate whether BN mediated its motoric effects through the dopaminergic system(s). Our results demonstrated that neuroleptics (fluphenazine or haloperidol) could effectively antagonize the behavioral effects of BN. However, the neuroleptic-induced suppression of 'normal' behaviors was, with the exception of the lowest doses, statistically significant. It is of interest that this suppression could partially be reversed by BN in the case of lower doses of fluphenazine (0.001 to 0.5 mg/kg) but not with the high dose of fluphenazine. This trend was not apparent in the case of haloperidol and the phenomenon can not be readily explained. Nonetheless, there is enough evidence to indicate that BN may be mediating its behavioral effects, at least in part, through the dopaminergic system(s).

While the significance of the characteristic behavioral syndrome induced by BN (or other peptides) is not yet fully understood, it remains possible that it reflects increased arousal or stress response. This contention is attractive because centrally administered BN also results in a dose-dependent rise in plasma epinephrine (but not norepinephrine, dopamine or corticosterone) (Brown et al, 1979; Brown and Vale, 1980). The selective rise in epinephrine would indicate that this response is not merely a non specific response to stress. Furthermore, it is interesting to note that intra-cerebroventricular administration of BN has been reported to alter the response of rats to cold-restraint stress; BN abolished prolactin rise, increased the hyperglycemic and hypothermic response, and reduced the incidence of gastric lesions, associated with cold-restraint stress (Tache et al, 1979). These data suggest that BN-like peptides may have a physiological role in stress response. In conclusion, our data demonstrate the powerful dose- and time-related effects of BN on locomotor, floor activity and rearing activity of rats. Furthermore, our results indicate that BN may be mediating these effects, at least in part, through the modulation of dopaminergic system(s).

Table 3. Effect of Neuroleptics (Fluphenazine and Haloperidol) on BN-Induced Behavioral Changes

Group	Locomotion (cm traversed)	Floor Activity (# beams broken)
Control	295 ± 13 (100)	173 ± 35 (100)
BN(1.0 ug ICV)	819 ± 139 (278)*	851 ± 60 (492)*
Flu (0.1)	151 ± 34 (51)+	104 ± 19 (60)+
Flu (0.1) + BN	344 ± 91(117)+	328 ± 59(190)+*
Flu (0.25)	80 ± 18 (27)+*	67 ± 15 (39)+
Flu (0.25)+ BN	124 ± 26 (42)+	112 ± 24 (65)+
Flu (0.5)	69 ± 15 (23)+*	49 ± 8 (28)+*
Flu (0.5) + BN	243 ± 59 (82)+	201 ± 32 (116)+
Flu (1.0)	77 ± 17 (26)+*	40 ± 10 (23)+*
Flu (1.0) + BN	89 ± 21 (30)+*	77 ± 15 (45)+*
Hal (0.1)	117 ± 45 (40)+	96 ± 19 (55)+
Hal (0.1) + BN	62 ± 16 (21)+*	94 ± 25 (54)+
Hal (1.0)	45 ± 3 (15)+*	26 ± 8 (15)+*
Hal (1.0) + BN	43 ± 8 (15)+*	23 ± 4 (13)+*
Hal (2.5)	24 ± 10 (8)+*	17 ± 10 (10)+*
Hal (2.5) + BN	34 ± 11 (12)+*	9 ± 3 (5)+*

Each value represents the mean ± standard error of the mean (sem) of the behavior monitored over 90 min for each group. Data are also expressed in percentages (in parentheses) taking the values of controls as 100%. * significantly different with respect to controls at $p<0.05$. + significantly different with respect to BN group at $p<0.05$.

CHAPTER 3

PHASE 2: ROLE OF DOPAMINERGIC SYSTEM(S) IN MEDIATION OF THE BEHAVIORAL EFFECTS OF BOMBESIN

Since the Phase 1 Experiments appeared to support the contention that centrally administered BN may mediate its effects through modulation of dopaminergic neurotransmission, additional experiments were undertaken to further test this contention.

Objectives Experiments 2.1- 2.2.

The objectives of the next set of experiments were (1) to further delineate the doses at which fluphenazine antagonizes the behavioral effects of BN, without suppressing the normal behaviors, and (2) to determine if lesioning dopamine neurons alters the behavioral response to BN.

Experiment 2.1

This experiment was designed to delineate the dose range over which fluphenazine would selectively block the effects of BN on locomotion, floor activity and rearing.

Method

Ventricular cannulation and behavioral monitoring were carried out as described above for Experiment 1. Rats (n=7) equipped with ventricular cannulae were subjected to the following experimental conditions: 1) saline (IP) + saline (ICV), 2) saline (IP) + BN (ICV), 3) fluphenazine (IP) + saline (ICV), 4) fluphenazine (IP) + BN (ICV). The dose of BN (1.0 ug ICV) was held constant throughout all experimental conditions whereas that of fluphenazine (0.01, 0.025, 0.1, 0.25 mg/kg) was varied randomly until all doses were tested. Following each treatment animals were distributed into their experimental chambers and were given 15 minutes for acclimatization before initiation of data collection. All experimental sessions commenced at 10:15 a.m. every day and were 90 min in duration.

Results

The data for each behavior, when subjected to one-way analysis of variance revealed a significant effect of treatment conditions: locomotion ($F(9,54) = 18.3$, $p < 0.05$), floor activity ($F(9,54) = 26.65$, $p < 0.05$) and rearing frequency ($F(9,54) = 8.47$, $p < 0.05$). Post hoc comparison of means, using least significant differences (L.S.D.) (Kirk, 1982), revealed that administration of

fluphenazine at a dose of 0.1 mg/kg failed to significantly alter the normal behavioral profile. However, at this dose, fluphenazine significantly suppressed the effects of 1.0 ug BN on locomotor, floor, and rearing activity (see Figure 7). At a higher dose (0.25 mg/kg) fluphenazine suppressed normal (saline + saline) as well as BN-induced behaviors. Whereas, at lower doses (0.025, 0.01 mg/kg) it failed to significantly alter either normal or BN-induced behaviors. Thus only at 0.1 mg/kg was there a specific fluphenazine antagonism of BN-induced behavioral changes. As illustrated in Figure 7, administration of BN compared with the control condition (saline + saline) produced significant stimulation of locomotor, floor, and rearing activity.

At every dose of fluphenazine, the fluphenazine + saline group was significantly different from the saline + BN group in behavior. The fluphenazine + saline groups did not differ significantly from the controls (saline + saline) in the behaviors monitored except for floor activity (at 0.25 mg/kg dose). Thus fluphenazine suppressed normal behavior only at doses of 0.25 mg/kg or greater.

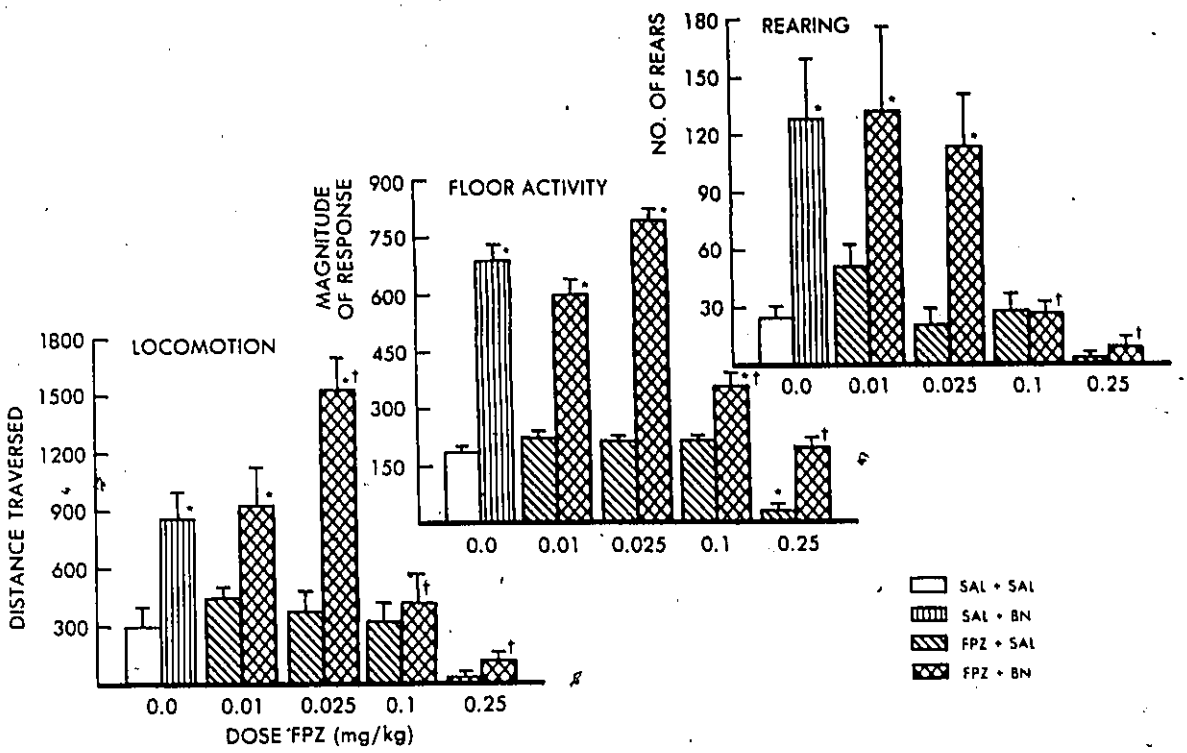

Figure 7. Dose-related effects of fluphenazine on saline- and BN-induced effects on locomotion, floor activity and rearing. The ordinate represents the magnitude of the quantified behavioral response (mean for 90 min); the abscissa represents the dose of fluphenazine administered (mg/kg). The profile of the saline (IP) + saline (ICV) condition is represented by the open bars; the saline (IP) + BN (ICV) condition is represented by the vertically stripped bars; the fluphenazine (IP) + saline (ICV) condition by hatched bar, and the fluphenazine (IP) + BN condition by the cross-hatched bars. Each value represents the mean ± sem of 7 animals. *Significantly different from the controls at $p<0.05$. +Significantly different from the saline-BN condition at $p<0.05$.

Experiment 2.2

Since fluphenazine (a dopamine receptor blocker) selectively blocked the behavioral effects of BN, a second experiment was undertaken to investigate whether the lesioning of the dopamine pathway(s) would have an effect on BN-induced locomotor, floor and rearing activity.

Method

Adult male Sprague-Dawley rats were housed and cannulated as described under Experiment 1, above, and were randomly assigned to sham-lesion (n=5) or 6-OHDA-lesion (n=5) groups. Both groups were pretreated with pargyline (50 mg/kg, i.p.) and desipramine (25 mg/kg, i.p.) in order to pharmacologically restrict the neurotoxic effects to the dopamine neurons (Breese and Traylor, 1971; Hedreen, 1980). Thirty minutes later rats were injected with 6-OHDA (250 ug/10 ul in 0.1% ascorbic acid, ICV) or vehicle (10 ul), over 30 sec (Ungerstedt, 1971b). A 12 day recovery period preceded further experimental manipulation. Behavioral monitoring was conducted as described under Experiment I.

All animals were injected (ICV, over 30 sec) either with saline (5 ul) or BN (0.001, 0.01, 0.1, or 1.0 ug in 5 ul), until all doses were tested. Since animals do not display tolerance to the behavioral effects of repeated

administration of BN (Kulkosky et al, 1982b; Gmerek and Cowan, 1983) and since no significant effect of order of BN administration on locomotion, floor activity or rearing was found (Merali et al, 1983), the ascending dose sequence of BN administration was selected. Following the completion of the BN dose-effect study, all animals were injected with d-amphetamine (0.5 mg/kg; IP) to behaviorally verify the success of the 6-OHDA lesion (Kelly et al, 1975; Kelly and Iversen, 1976; Jeste and Smith, 1980). On the subsequent day all animals were injected with apomorphine (0.25 mg/kg; s.c.) to test whether the rats were capable of motoric activation following a direct stimulation of the dopamine-receptors (Kelly et al, 1975; Fink and Smith, 1980; Zigmond and Stricker, 1980). The behavioral data were collected as described under Experiment 1.

Results

Two way analysis of variance showed a significant interaction of experimental condition (sham-lesion vs 6-OHDA-lesion) and dose of BN, for each behavior: locomotion ($F(4,32) = 4.73$, $p < 0.01$); floor activity ($F(4,32) = 4.7$, $p < 0.01$) and rearing activity ($F(4,32) = 7.55$, $p < 0.001$). The analysis of the simple main effects revealed that with each behavior the interaction was mainly attributable to the differential response of the 6-OHDA-lesioned (nonsignificant effect) vs the sham-lesioned group (significant effect) to

BN. In concordance with the earlier data (Merali et al, 1983), BN was found to stimulate the locomotor, floor and rearing activity of the control (sham-lesioned) animals, in a dose-dependant manner (see Figure 8). However, as illustrated in Figure 8, BN at the doses 0.001, 0.01 and 0.1 ug, did not significantly increase behavior of the 6-OHDA-lesioned animals. Only at the highest dose (1.0 ug BN), did this peptide increase locomotor and floor activity response of the 6-OHDA-lesioned animals. However, the magnitude of this BN-stimulation was significantly smaller in the lesioned animals as compared to the sham-lesioned group.

The direct dopamine agonist apomorphine (0.25 mg/kg) was administered to verify that the dopamine receptors were still functionally intact. Administration of apomorphine, as reported in Table 4, resulted in stimulation of locomotion, floor activity and rearing activity, indicating that the lesioned animals were not only capable of motoric response, but that they were supersensitive to the behavioral effects of apomorphine. It is of interest that Zigmond and Stricker (1980), reported similar supersensitivity to apomorphine only after greater than 80% dopamine loss in the 6-OHDA-lesioned rats.

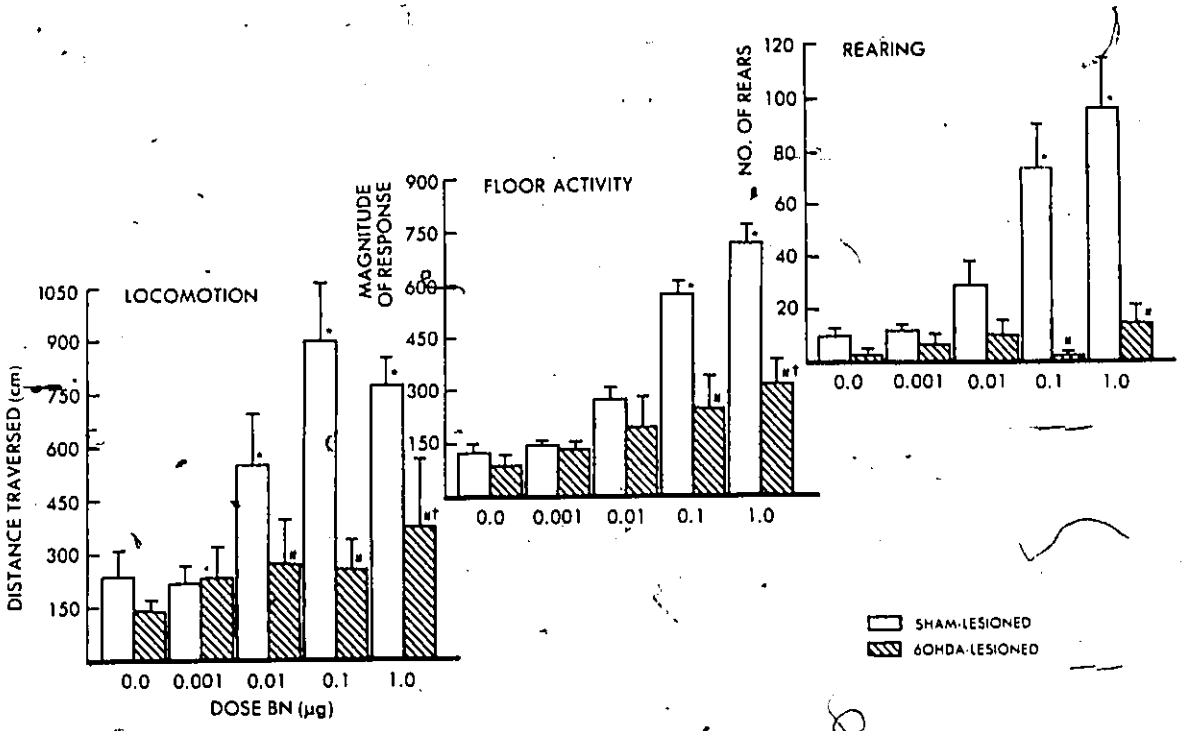

Figure 8. Dose-related effects of BN on locomotion, floor activity and rearing in control and 6-OHDA-lesioned animals. On the ordinate the magnitude of the quantified response (mean for 90 min); on the abscissa dose of BN administered (ug). The open bars represent the control values whereas the hatched bars represent the 6-OHDA-lesioned rats. Each value represents the mean ± sem of 5 animals. *Significantly different from the saline condition (controls, 0.0ug BN) at $p<0.05$. +Significantly different from the 6-OHDA-lesioned saline condition (0.0 ug BN) at $p<0.05$. #Significantly different from the dose-matched control condition, at $p<0.05$.

Table 4. Effect of Dopamine Agonists (Apomorphine and D-amphetamine) on the Behavior of 6-OHDA- and Sham-Lesioned Rats.

Drug	Group	Locomotion (cm traversed)	Floor activity (# beams broken)	Rearing frequency
Apomorphine (0.25 mg/kg)	Sham	1263 ± 223	580 ± 41	39 ± 11
Apomorphine (0.25 mg/kg	6-OHDA	6943 ± 1693*	1457 ± 109*	216 ± 86
D-amphetamine (0.5 mg/kg)	Sham	3872 ± 948	1296 ± 171	275 ± 50
D-amphetamine (0.5 mg/kg)	6OHDA	301 ± 141*	236 ± 58*	9 ± 3*

Each value represents the mean ± sem of the behavior monitored over 90 min., for each group (n=5).* Significant difference between sham- and 6-OHDA-lesioned groups at $p<0.05$.

In order to determine if behaviorally relevant amounts of dopamine release could be stimulated synaptically in the 6-OHDA-lesioned animals, d-amphetamine was administered. As expected, the lesioned animals showed a significantly lower level of behavioral response to d-amphetamine (0.5 mg/kg) than the sham-lesioned rats in all three behaviors (see Table 4). These data are in concordance with those in the literature implicating depletion of mesolimbicocortical dopamine to the decreased locomotor responsiveness to amphetamine (Kelly et al, 1975; Kelly and Iversen, 1976; Jeste and Smith, 1980).

Discussion

The data from these and previously reported experiments (Merali et al, 1983) demonstrate that fluphenazine, a dopamine receptor blocker, can selectively antagonize the behavioral effects of BN, indicating that dopamine pathway(s) may be involved in the mediation of the behavioral effects of BN. Recently, Gmerek and Cowan, (1983) reported that BN-induced grooming in rats was not markedly affected by a behaviorally non-depressant dose of haloperidol (0.5 mg/kg). However, at a higher dose of haloperidol (5 mg/kg), BN-induced grooming was completely blocked. This discrepancy with our data may be attributed to the limited dose range of haloperidol and/or the difference in behavioral monitoring, particularly the time-course

(Isaacson et al, 1983), employed by Gmerek and Cowan, (1983). It is of interest that Schulz et al, (1984) have succeeded in replicating our finding that neuroleptics (haloperidol) can attenuate BN-induced increase in locomotor activity without significantly altering basal activity. The effects of BN on grooming are not significantly affected by morphine, naloxone, neurotensin (Gmerek and Cowan, 1983), atropine or diphenhydramine (Schulz et al, 1984).

The lesioning of the dopamine neurons (by 6-OHDA) was accompanied by a significant reduction in behavioral response to BN, further supporting the contention that BN may be mediating its behavioral effects through the dopaminergic system. Indeed, fully intact dopamine mesolimbicocortical pathways appear to be critical for the increased locomotor effects of amphetamine, and also for novel exploratory behavior (Kelly et al, 1975; Kelly and Iversen, 1976; Fink and Smith, 1980; Jeste and Smith, 1980; Zigmond and Stricker, 1980). In our experiments, BN and amphetamine increased locomotion in a familiar environment in sham-lesioned, but, not in 6-OHDA-lesioned rats. Furthermore, we found that in a familiar environment there was no difference between the locomotor activity of sham-lesioned and 6-OHDA-lesioned animals, under control conditions (saline injection). This observation is in concordance with that of Jeste et al, 1980. It is of interest to note that increased locomotor activity in response to a novel situation, amphetamine administration,

or BN administration all require, in common, fully intact dopamine pathway(s).

The involvement of the noradrenergic pathways in the mediation of locomotor and exploratory behavior has been shown to be of little significance (Kelly et al, 1975; Kelly and Iversen, 1976; Fink and Smith, 1980; Jeste and Smith, 1980). In the lesion procedure utilized in our experiments, the noradrenergic pathways are spared (Breese and Traylor, 1971; Hedreen, 1980; Zigmond and Stricker, 1980). However, it remains to be directly tested whether or not BN modulates part of its behavioral effects through noradrenergic pathways.

Although centrally administered BN has potent behavioral effects, little is known about the neuroanatomical locus (or loci) or the underlying neural correlates of the central effects of BN. The hypothalamus has a high concentration of BN-like immunoreactivity as well as BN binding sites (Moody et al, 1981, Moody and Pert, 1979), and the lateral hypothalamus is believed to mediate the BN-induced reduction in food intake (Stuckey and Gibbs, 1982). In the case of grooming and locomotor effects, other neuroanatomical structures have been implicated. In comparison to ICV administration, microinjection of BN into the periaqueductal grey (PAG) requires much larger doses to induce grooming (Gmerek and Cowan, 1983). Furthermore, although intra-ventral tegmental area (VTA) and ICV BN were equipotent in increasing locomotor activity, administration

of BN into the nucleus accumbens was considerably more effective (Schulz et al, 1984). These results are supported by observations in our laboratory. However it appears that different neuroanatomical structures, rather than a single critical site, may be involved in the mediation of the various components of BN-induced behavioral changes.

Recent autoradiograhic studies indicate that BN receptors are discretely distributed in the rat brain and that parts of the limbic system, particularly the nucleus accumbens, olfactory tubercle, olfactory bulb, medial and anterior amygdaloid nuclei and hippocampus are endowed with high receptor densities (Pert et al, 1980; Wolf et al, 1983). Certain limbic structures (such as the nucleus accumbens) that have been implicated in the mediation of exploratory and locomotor activity in the rat, are also rich in dopaminergic nerve terminals. Since neuroleptic pretreatment as well as 6-OHDA lesion markedly attenuated or blocked the behavioral effects of BN, it remains likely that BN by interacting with its binding sites on or near the terminal fields of mesolimbicocortical dopamine fibres, may be modulating the dopaminergic neurotransmission and consequently the animal's behavior. This contention is further supported by the recent data of Widerlov et al (1984) demonstrating a dose-dependent increase in dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), the principal dopamine metabolites, in several brain areas following intracisternal injection of BN.

In conclusion, BN may be a neurotransmitter or neuromodulator involved in the mediation of behavioral activation, interfacing with other neuronal systems, particularly dopamine systems, in the integration of stress responses (Tache et al, 1979; Brown et al, 1979; Brown and Vale, 1980; Brown, 1981b; Tache and Brown, 1982).

CHAPTER 4

PHASE 3. THE NEUROANATOMICAL CORRELATES of the BEHAVIORAL EFFECTS of BOMBESIN

Introduction

In addition to the stimulation of locomotor, floor and rearing activity studied thus far, BN ICV elicited a conspicuous alteration in the grooming pattern, a syndrome of vastly increased scratching (Katz, 1980; Kulkosky et al, 1982a; Gmerek and Cowan, 1983; Merali et al, 1983; Schulz et al, 1984; Cowan et al, 1985). The BN-induced grooming syndrome was distinct from the grooming pattern induced by the prototypic groom-inducing peptide ACTH. With ACTH the length of individual bouts and not the proportion of time spent per grooming element was enhanced. Another difference was their time-course; BN-induced grooming began immediately, whereas, ACTH-induced grooming did not occur until 10-15 min following ICV administration (Merali et al, 1983; Spruijt and Gispen, 1983; Gmerek and Cowan, 1983; 1984; Isaacson, 1984; Cowan et al, 1985; Gispen and Isaacson, 1981; 1986;). However both peptides require intact dopamine pathway(s) for their behavioral expression (Isaacson, 1984; Merali et al, 1985). Thus, marked pharmacological and behavioral differences exist between these two groom-inducing peptides.

Behavioral effects of BN can be elicited by central (via the ventricular, intracisternal or intrathecal) but not peripheral administration (Kulkosky, 1982a; b; Schulz et al, 1984; Gmerek et al, 1983). Furthermore BN-induced behavioral effects appear to be independent of the pituitary-adrenal axis (Gmerek and Cowan, 1983). However, little is known about the neuroanatomical locus (or loci) or the neurochemical mechanisms subserving these behavioral effects. Recently, several studies have attempted to delineate neuroanatomical loci of some of the biological effects of BN. Bombesin microinjected at the preoptic area produces hypothermia (Pittman et al, 1980; Wunder et al, 1980); whereas at the dorsal hypothalamus, it produces a specific and rapid rise in plasma epinephrine (Brown, 1983). When microinjected into the lateral hypothalamus, BN appears to reduce food intake (Stuckey and Gibbs, 1982; Gibbs, 1985) and at the the paraventricular nucleus produces a marked rise in gastric pH values and a decrease in secretory volume (Gunion et al, 1983). Recent results indicated that administration of BN, either ICV or intra the ventral tegmental area (VTA), equipotently stimulated the locomotor activity. However, BN infused intra NA was considerably more potent in inducing locomotor stimulation (Schulz et al, 1984).

Although BN-induced behavior has been blocked by haloperidol (Merali et al, 1983; Schulz et al, 1984) benzomorphan (Gmerek and Cowan, 1984) and diazepam (Crawley

and Moody, 1983) these drugs are not known to directly block the BN receptors. Recently, a Substance P analogue, spantide, has been demonstrated to be a competitive BN receptor antagonist (Jensen et al, 1984; Yachnis et al, 1984; Folkers et al, 1984). As well as inhibiting binding of BN-like peptide to central receptors, spantide (ICV) reversed the BN-induced hypothermia and grooming (Yachnis et al, 1984).

The major objective of the next set of experiments was to examine the behavioral effects of BN microinjected at five rat brain sites endowed with a high density of BN binding sites, including the hippocampus (CA4), nucleus accumbens (NA), fundus striati (FST), central nucleus of the amygdala (CE), and the anterior olfactory nucleus (AON)(Pert et al, 1980; Wolf et al, 1983; Zarbin et al, 1985). It was hoped that this might identify the loci which were most behaviorally responsive to BN. Up to this time, no direct monitoring of grooming had been carried out. Since BN had been reported to have a profound effect on the grooming repertoire of the rat behavior (Gmerek and Cowan, 1982, 1983; Kulkosky et al, 1982 a,b), it was decided to include the monitoring of grooming elements in all our subsequent experiments.

Although the NA showed the most efficacious response to BN in regard to exploratory behavior stimulation, the grooming pattern at none of the sites tested showed a similar or as pronounced a response as that seen after BN

injected ICV. Since it has been demonstrated that intrathecally administered BN was slightly more potent in simulating grooming than BN ICV (Gmerek and Cowan, 1983), we postulated that there may be a hind brain site responsible for BN-induced grooming. Thus, one further brain site, the nucleus tractus solitarius (NTS), with high density BN receptors as well as BN immunoreactivity was investigated (Zarbin et al, 1985; Moody et al, 1981).

Furthermore, since BN changed the frequency of at least one grooming element, namely scratching, above what appeared during the saline condition, it was decided to monitor grooming according to its individual components. Grooming is a common species characteristic movement pattern with readily definable components (Bolles, 1960; Fentress, 1973). Constituting components are vibration, face washing, body grooming, scratching, paw licking, head shake, body shake and genital grooming (Gispen and Isaacson, 1981). In contrast to BN, as mentioned above, ACTH enhances grooming by prolonging bout duration rather than changing any particular component. Scratching with the hind paws is unpredictable in the grooming sequence (Spruijt and Gispen, 1983; Richmond and Sachs, 1980). However, the other grooming elements appear to arise in a fixed organized pattern of grooming behavior with a cephalocaudal progression of acts within bouts (Fentress, 1977; Spruijt and Gispen, 1983; Richmond and Sachs, 1980; Gispen and Isaacson, 1981; 1986). The grooming bouts exhibited by rats in their home cages are

of relatively short duration, the majority of episodes do not extend beyond one or two 15 sec sampling intervals. Although Gispen and Isaacson and Spruijt have adopted the 15 sec scoring interval, which is appropriate for comparing ACTH-induced grooming to control conditions, we have adopted a procedure similar to Gmerek and Cowan (1983) of sampling the grooming behavior. Eight rats were monitored at a time. Each rat was observed for 5 sec out of every 40 sec for up to 90 min. This procedure is more appropriate for measuring the increased scratching induced by BN, since the BN-induced scratching appears to result in an abnormal sequence where scratching becomes the predominant grooming element and the normal 15 to 30 sec grooming bout is disrupted for up to 90 min. In addition, we chose the grooming elements which would reflect the BN-induced behavior, namely, facial scratching and licking; body scratching and licking, as well as sniffing. The behaviors are defined below.

Initially, the elements of the grooming behavior where tabulated by hand. Shortly thereafter a customized data logger was manufactured by our workshop technicians for computer assisted scoring. Eight different events can be recorded for up to 14 animals. The observer is prompted with an audio tone and a digital display as to the current subject. The sampling interval can be varied from 1 to 120 seconds. The data recorded can be retained indefinitely in memory, and can be transmitted to an IBM PC through a

parallel bus. A software program compiles the data for the desired time intervals, for subsequent statistical analysis.

Objectives Experiments 3.1-7

The overall objective of the next series of experiments was to attempt to identify the central locus (or loci) involved in elicitation of the behavioral effects (locomotor activities and grooming elements) of BN. Hence, experiments were undertaken to examine the behavioral effects of BN microinjected at several rat brain sites endowed with a high density of BN binding sites, including the NTS, CA4, NA, FST, CE and AON (Pert et al, 1980; Wolf et al, 1983; Zarbin et al, 1985). In addition for the sake of comparison the behavioral effects of BN microinjected ICV were examined. Furthermore, the effect of spantide on BN-induced behavior was investigated at the NTS, CA4 and NA.

Animals

Adult male Sprague-Dawley rats (275-300g)(St. Constant, Quebec) were housed individually with free access to food (Master Laboratory Chow) and water. The environment was maintained at 24^{o}C, 60% relative humidity and with 12 hr of light (6 a.m. to 6 p.m.).

Cannulation

Separate groups of rats under sodium pentobarbitol (50mg/kg, IP) anesthesia were stereotaxically implanted, with 24 gauge adjustable stainless-steel guide cannulae (Kinetrods, Ottawa, Canada) aimed at the NTS, A-P -13.3mm, lateral +0.7mm, and depth -7.8mm; CA4, A-P -3.3mm, lateral ± 1.8mm, and depth -3.5mm; NA, A-P +2.2mm, lateral ± 0.8mm, and depth -6.7mm; FST, A-P -2.8mm, lateral ± 4.7mm, and depth -7.2mm; CE, A-P -2.8, lateral ± 4.7mm, and depth -7.9mm; AON A-P +4.2mm, lateral ± 2.2mm and depth -5.5mm or ICV (22 gauge; Plastic Products, Roanoke, VA), A-P -0.8mm, lateral -1.6mm, and depth -4.2mm (Paxinos and Watson, 1982) as illustrated in Figures 9-14.

The guide cannulae were cemented with dental acrylic to 4 jewelers' screws placed in the calvarium. Stainless-steel obturators were in the guide cannulae at all times except during injections. The animals were allowed a minimum of 5 postoperative recovery days prior to commencement of experiments. At the termination of the experiments cannulae placements were verified using cresyl violet dye substitution followed by standard histological procedures.

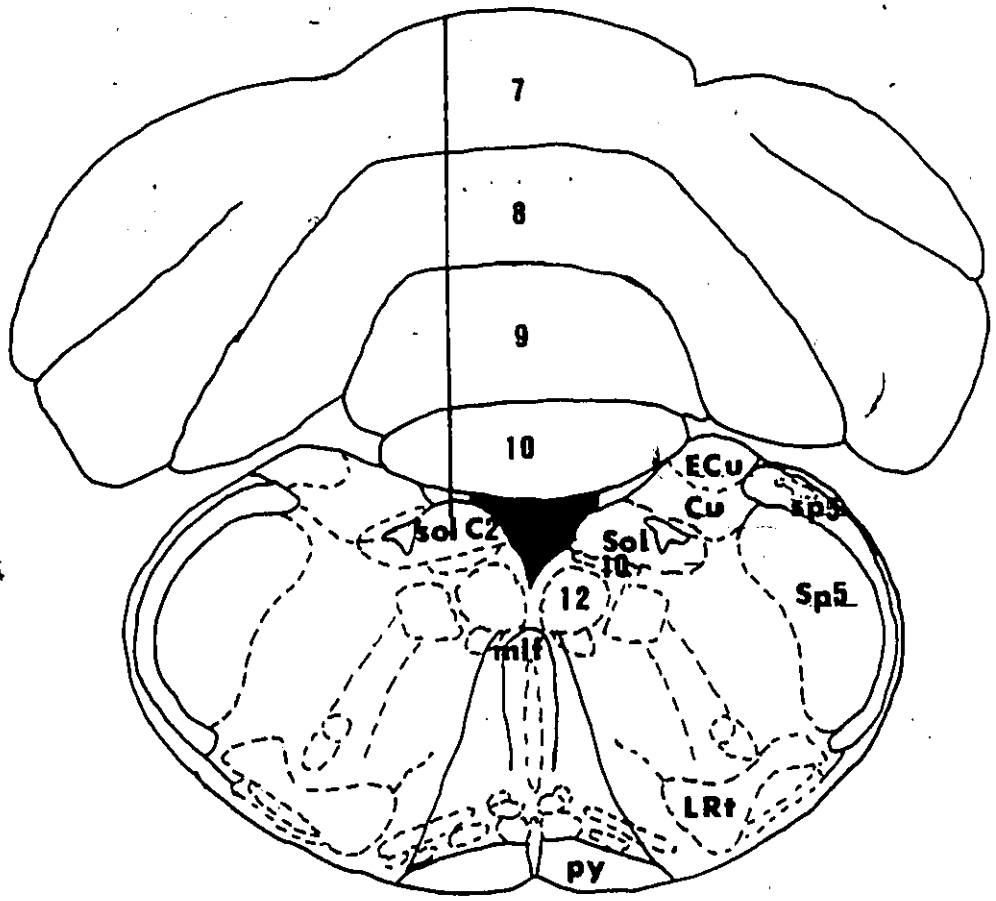

Figure 9: Location of cannula implantation site in the nucleus tractus soliarius of the rat (coronal view: A-P - 13.3, L 0.7, D-V 7.8 mm); (Paxinos and Watson, 1982).

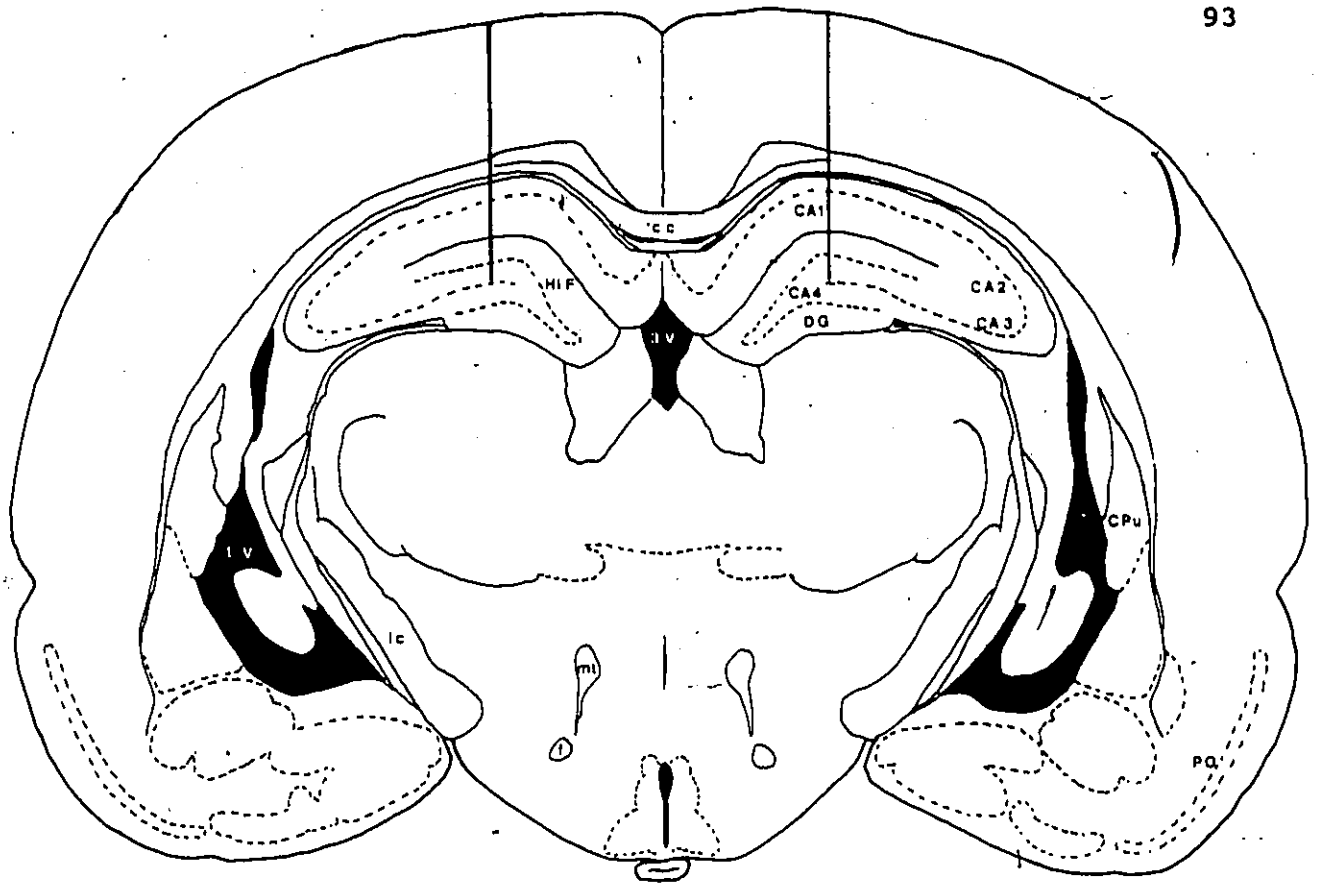

Figure 10: Location of cannula implantation site in the dorsal hippocampus of the rat (coronal view: A-P -3.3, L ± 1.8, D-V 3.5 mm; Paxinos and Watson, 1982).

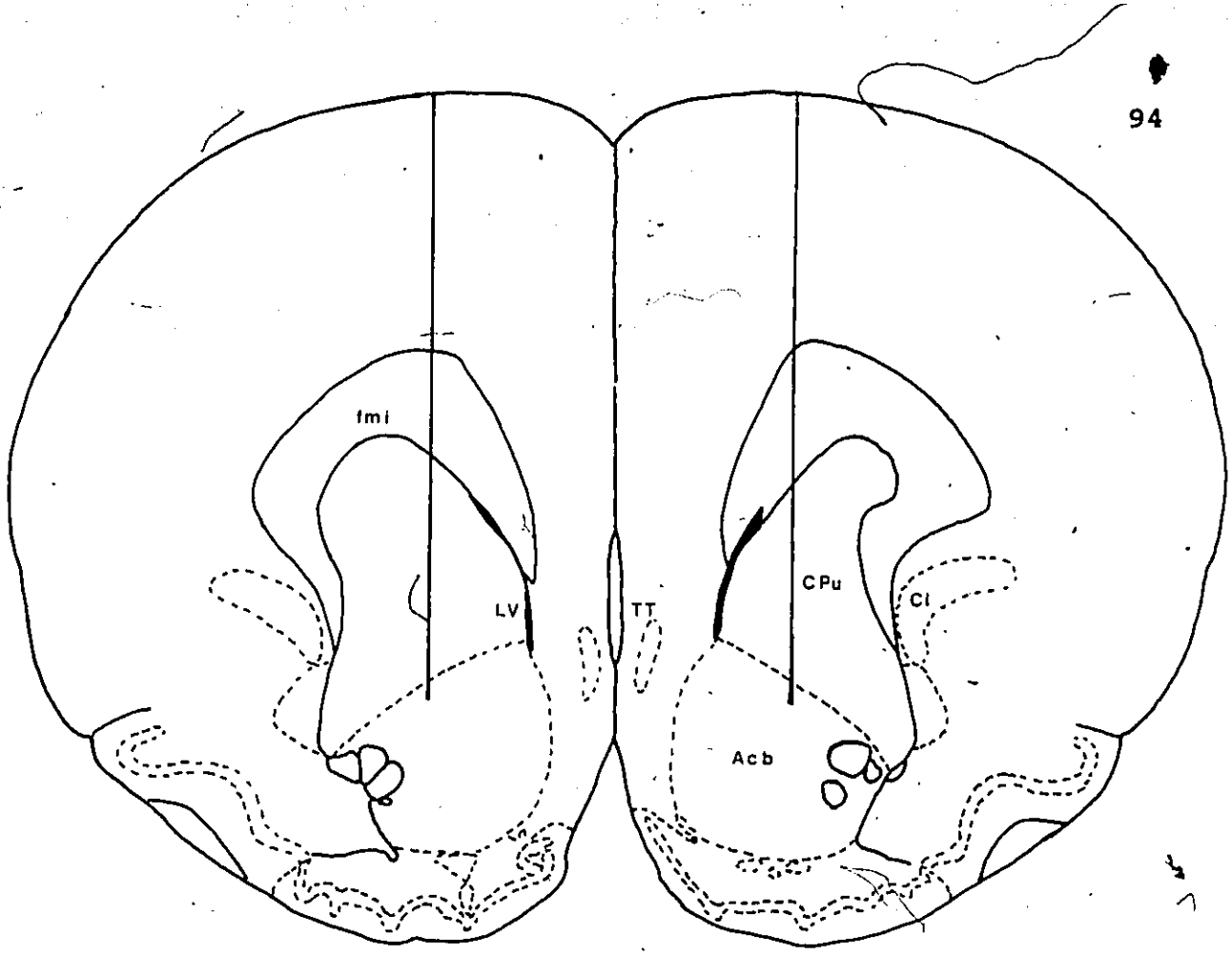

Figure 11: Location of cannula implantation site in the nucleus accumbens of the rat (coronal view: A-P 2.2, L ±. 1.8, D-V 6.7 mm; Paxinos and Watson, 1982).

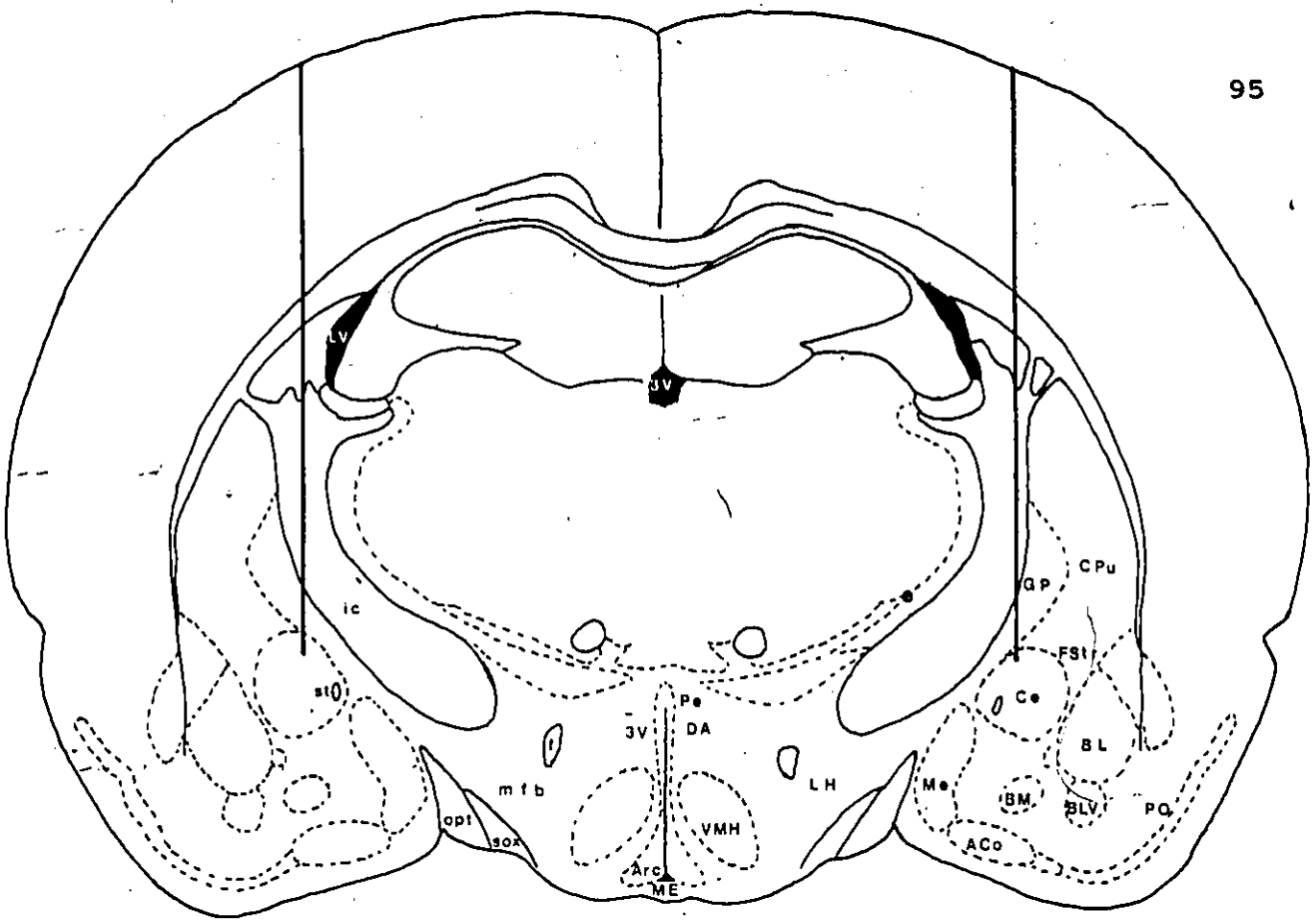

Figure 12: Location of cannula implantation site in the central amygdaloid nucleus of the rat (coronal view: A-P - 2.8, L ± 4.7, D-V 7.9 mm) and fundus striati (A-P-2.8, L ± 4.7, D-V 7.2 mm) (Paxinos and Watson, 1982).

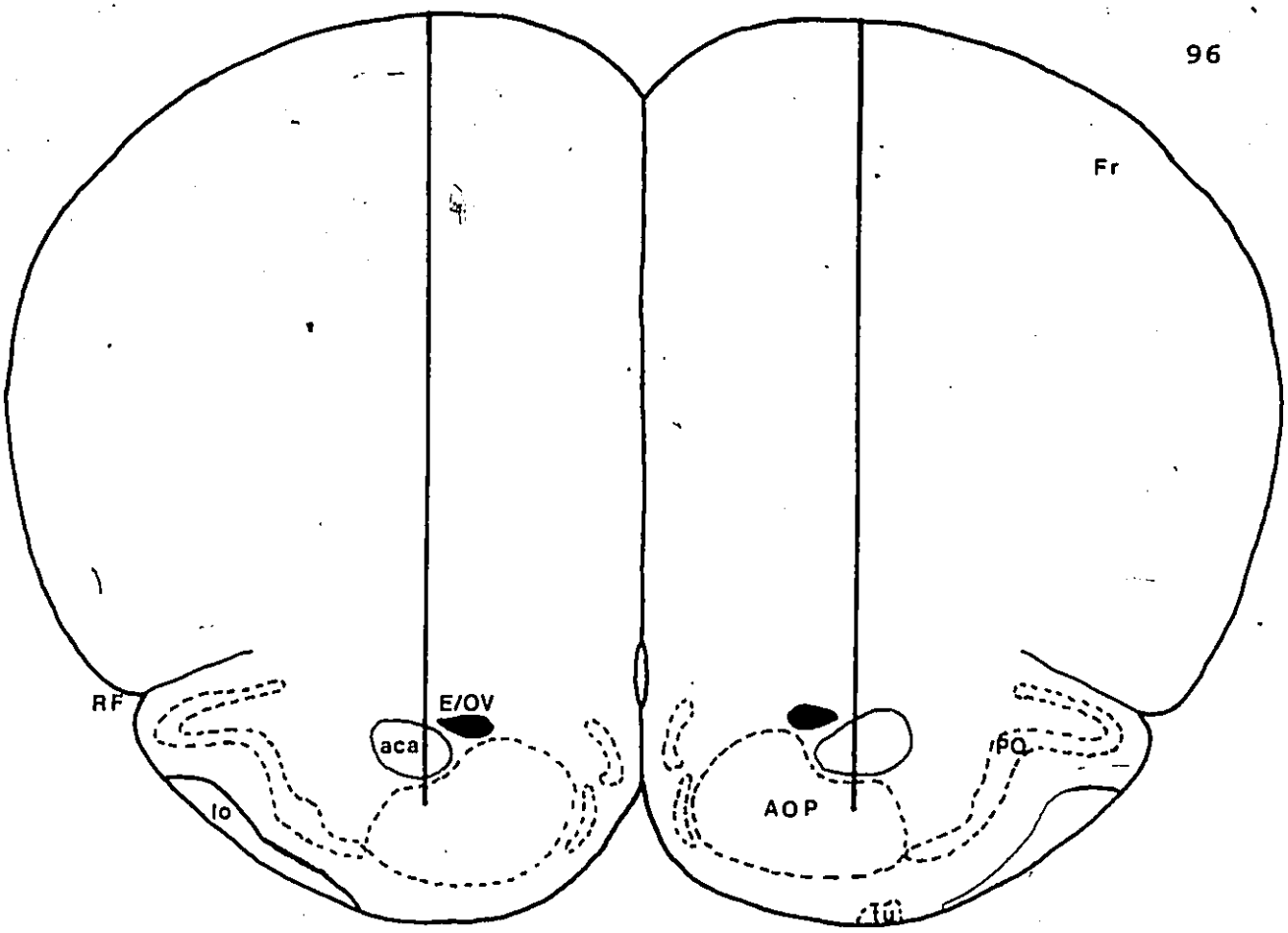

Figure 13: Location of cannula implantation site in the anterior olfactory nucleus of the rat (coronal view: A-P 4.2, L ± 2.2, D-V 5.5 mm; Paxinos and Watson, 1982).

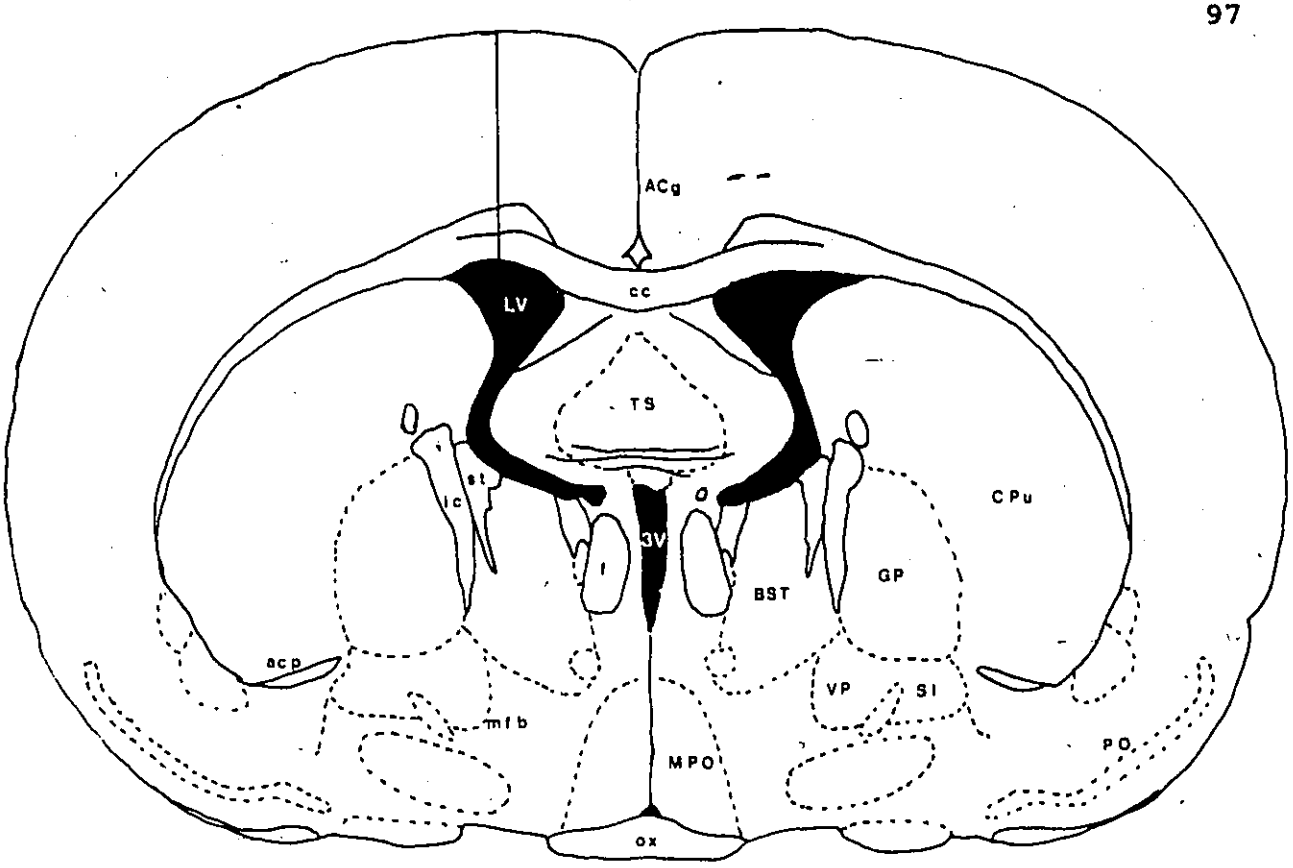

Figure 14: Location of cannula implantation site in the lateral ventricle of the rat (coronal view: A-P -0.8, L 1.6, D-V 4.2 mm; Paxinos and Watson, 1982).

Behavioral Monitoring

Monitoring of the locomotor, floor and rearing activity was conducted by the procedure described previously (Merali et al 1983; 1985; see also Experiment 1, Behavioral Monitoring). Simultaneous monitoring of the grooming activity by human raters was conducted from an adjacent room, through a one-way mirror, using a modification of the procedures described by Gispen and Isaacson (1981) and Gmerek and Cowan (1983). A maximum of 8 rats were monitored at a time. Each rat was observed for one 5 sec interval out of each 40 sec observation period recycled for 60 min. Thus, every 5 sec the observer recorded whether or not a given rat displayed the following grooming elements: facial licking, facial scratching, body licking, body scratching and sniffing as illustrated in Figures 15 and 16.

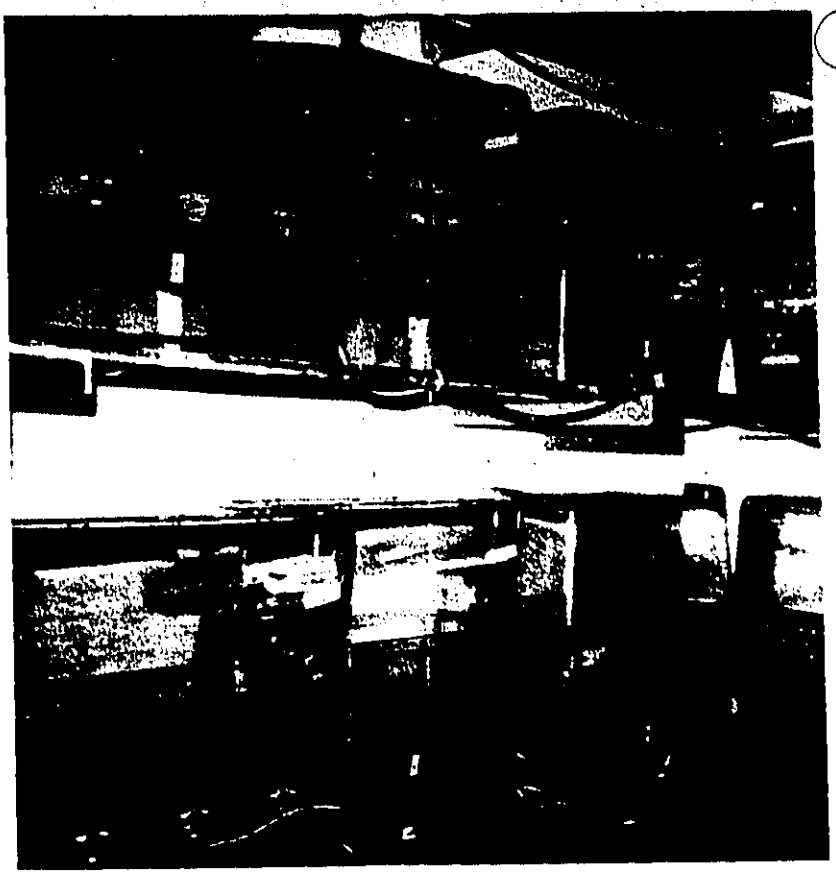

Figure 15: The infrared monitoring cages. The triangular patches on the side of the cages are the infrared transmitters, matched on the opposite side of the cage by the receivers. The rat is housed in the center in a third cage which sits suspended in the bottom cage which holds the infrared monitors. The top box which is inverted and sits on the top of the rat cage keeps the rat in, holds the water bottle and has a wire mesh on the top for free air circulation. All three cage boxes are made of clear polycarbonate. The cages are 4 per shelf, with 12 cages on three shelves. They are in a sound proof enclosed cubicle 8 x 8 ft, with the matching sound proof monitoring cubicle directly opposite, with a connecting one way viewing window 2 x 4 ft.

Figure 16: Sheila Johnston sitting at the one way window, monitoring grooming, with the monitoring box in hand pressing the 8 buttons according to which grooming behaviors occur. Notice the auditory device in her ear cueing each 5 sec interval with a beep. The infrared monitoring cubicle is directly opposite the room with the infrared monitoring cages, which are shelved on the parallel wall opposite to the window.

These behaviors were operationally defined as follows: facial licking, use of the forepaws either placed in the mouth before or after passing in a wiping motion over the face/head region; facial scratching, use of the digits of the hind legs to scratch the head and neck region; body licking, use of the mouth and forepaws in a wiping/gliding motion over the entire body excluding the head region; body scratching, scratching of any part of the body region below the neck with the digits of the hind legs, sniffing: vibration of the nostrils which is often accompanied by either rearing or movement across the floor (Gispen and Isaacson, 1981; Gmerek and Cowan, 1983; Johnston et al, 1986). All doses of BN are expressed in microgram per animal.

Experiment 3.1

This experiment was designed to delineate the dose-effect of BN, microinjected at the NTS, on the behavioral profile of rats. Furthermore the possible antagonism of BN-induced behaviors by the Substance P analogue, spantide was also tested.

Eight animals, equipped with guide cannulae unilaterally implanted at the NTS, were randomly assigned to individual behavioral chambers. After 1 hr acclimatization animals were microinjected with one of the following doses

were administered at the rate of 0.5 ul/ 30 sec using a Harvard infusion pump. In addition the injection cannulae (30 gauge), which protruded past the guide cannulae by 0.05 mm were left in place for 30 sec, after the injection. On the last injection day, 1 ug spantide + 0.5 ug BN was microinjected. After treatment the animals were distributed into their experimental chambers and data collection began. All sessions commenced at 11:00 a.m. and were 60 min in duration except the last injection day (BN + spantide) when data collection lasted for 30 min. The behavioral effects were observed and quantitated as described under the behavioral monitoring section.

Experiment 3.2

In Experiment 3.2, the protocol of Experiment 3.1, was repeated on a separate group of animals (n=8), except that these rats were bilaterally implanted with guide cannulae at the CA4. In addition, the doses per animal were: vehicle, 0.01, 0.1, 1.0 or 2.0 ug BN. On the last injection day, 2 ug spantide + 1.0 ug BN was microinjected.

Experiment 3.3

This experiment was designed to delineate the dose-effect of BN, microinjected at the NA, on the behavioral

profile of rats. Furthermore, the possible antagonism of BN-induced behaviors by spantide, was also tested. The protocol of Experiment 3.2 was followed on seven animals equipped with guide cannulae aimed at the NA.

Experiments 3.4, 3.5, 3.6

In Experiment 3.4, 3.5, and 3.6, Experiment 3.1 protocol was repeated on a separate group of rats except that the guide cannulae were implanted bilaterally at the FST (n=7), CE (n=7), or AON (n=5), respectively. However, the behavioral effects of only a single dose of BN (2.0 ug) was tested.

Experiment 3.7

In Experiment 3.7 rats (n=8) were implanted with guide cannulae aimed at the right lateral ventricle. Animals were randomly assigned to individual experimental chambers. After 1 hr of acclimatization all rats were injected with saline (control) or one of the following doses of BN: 0.01, 0.1 or 1.0 ug. All injections were administered at the rate of 3 ul/ 35 sec using a Harvard infusion pump. In addition, the injection cannulae (which protruded past the guide cannulae by 0.5mm) were left in place for 30 sec, after the injection. Following treatment the animals were distributed

into their behavioral chambers and data collection began as described under the behavioral monitoring section.

Results

Experiment 3.1

Analyses of the data from the NTS revealed a significant effect of dose of BN for floor activity $F(4,28)=8.6$, $p<0.001$; rearing $F(4,28)=6.9$, $p<0.001$; facial scratching $F(4,28)=62.7$, $p<0.001$; facial licking $F(4,28)=27.2$, $p<0.001$; body licking $F(4,28)=4.8$, $p<0.01$; and sniffing $F(4,28)=3.8$, $p<0.05$. Significant differences occurred between control condition and groups administered BN at a dose of 0.05 ug or greater, for floor activity and facial scratching (Least Significant Difference, Kirk, 1982). For both facial and body licking, significant differences occurred at doses of 0.5 ug BN or higher. Rearing and sniffing were significantly altered only at selective doses of BN, namely 0.5 and 0.05 ug, respectively.

Analysis of variance revealed no significant difference between the group administered BN (0.5 ug) alone and the group exposed to BN (0.5 ug) and spantide (1.0 ug) concurrently, on any of the behaviors recorded.

Experiment 3.2

Analysis of the data from the CA4 revealed a significant effect of dose of BN for locomotion $F(4,28)=2.7$, $p<0.05$; floor activity $F(4,28)=9.4$, $p<0.001$; facial scratching $F(4,28)=19.5$, $p<0.001$; and facial licking $F(4,28)=4.38$, $p<0.01$. Comparison of means indicated significant differences between the saline condition and groups administered 1.0 ug BN and higher, for locomotion, floor activity, facial scratching, and facial licking.

No significant difference was found between the 2 ug spantide + 1 ug BN group and the 1 ug BN group, for any of the recorded behaviors.

Experiment 3.3

One way analysis of variance (repeated over dose) revealed a significant effect of dose of BN administered intra NA on locomotion, $F(5,30)=3.7$, $p<0.01$; floor activity, $F(5,30)=2.5$, $p<0.05$; rearing $F(5,30)=4.0$, $p<0.01$; facial scratching $F(5,30)=5.4$, $p<0.001$; facial licking $F(5,30)=4.1$, $p<0.01$; and sniffing $F(5,30)=7.9$, $p<0.001$. No significant effect of BN on either body licking or body scratching was found. Comparison of means between the saline condition and various BN doses revealed that stimulation of facial scratching was evident already at the lowest dose used

(0.001 ug), as was suppression of facial licking. However, the stimulatory effects on other behaviors, namely, locomotion, floor activity, rearing and sniffing were significant only at 1.0 ug or greater. Facial licking appeared to be suppressed by the 1.0 ug, but not the 2.0 ug dose of BN.

Analysis of variance (repeated over dose) also revealed that at the NA there was a significant effect of spantide (2 ug) when coadministered with BN (1.0 ug) as compared with BN (1.0 ug) alone, on locomotion $F(1,6)=47.2$, $p<0.001$; floor activity $F(1,6)=22.3$, $p<0.001$; rearing $F(1,6)=15.9$, $p<0.01$; facial scratching $F(1,6)=12.3$, $p<0.01$; facial licking $F(1,6)=12$, $p<0.01$; and sniffing $F(1,6)=28.5$, $p<0.001$. The stimulatory effects of BN on locomotion, floor activity, rearing, facial scratching and sniffing were significantly attenuated by spantide; whereas suppression of facial licking was reversed by spantide.

Experiment 3.4

One way analysis of variance (repeated over dose) revealed a significant effect of dose of BN (2 ug) administered intra FST on locomotion, $F(1,6)=20.8$, $p<0.001$; floor activity, $F(1,6)=38.1$, $p<0.001$; rearing $F(1,6)=41.1$, $p<0.001$; facial scratching $F(1,6)=14.3$, $p<0.01$; facial licking, $F(1,6)=6.0$, $p<0.05$; body scratching $F(1,6)=7.6$, $p<0.05$, body licking $F(1,6)=12.8$ $p<0.01$; and sniffing

$F(1,6)=46.6$, $p<001$. There was a general stimulation of all grooming elements as well as exploratory activity by BN microinjection at the FST.

Experiment 3.5

One way analysis of variance (repeated over dose) revealed a significant effect of dose of BN (2 ug) administered intra CE on locomotion, $F(1,6)=18.5$, $p<0.01$; floor activity, $F(1,6)=23.1$, $p<0.001$; rearing $F(1,6)=15.8$, $p<0.01$; and sniffing $F(1,6)=8.2$, $p<0.05$. No significant stimulation of any grooming element was evident at the CE.

Experiment 3.6

At the AON, one way analysis of variance revealed no significant effect of BN (2 ug) on any of the behavioral parameters monitored.

Experiment 3.7

Analysis of variance revealed a significant effect of dose of BN administered ICV, on locomotion $F(3,18)=5.2$, $p<0.01$; floor activity $F(3,18)=8.8$, $p<0.001$; rearing $F(3,18)=3.7$, $p<0.05$; facial scratching $F(3,18)=24.2$, $p<0.001$; and facial licking $F(3,18)=20.5$, $p<0.001$. Comparison of means indicated that all doses of BN (1.0,

0.1, or 0.01) were significantly effective in stimulating locomotion, floor activity and rearing over 60 min. However, both for facial scratching and facial licking significant differences occurred only at doses of 0.1 ug BN or greater.

Discussion

Of all the central loci tested, the locomotor stimulatory effect of BN was most pronounced when it was microinjected intra NA, as illustrated in Figure 17. This observation is consistent with the fact that this nucleus is involved in motor responses and that it is also endowed with a very high density of BN binding sites. Although the AON is also endowed with very high density of BN binding sites, local administration of BN there failed to elicit significant behavioral changes. Bombesin microinjected intra CE resulted in a moderate increase in locomotor activities but not grooming activities, whereas, at the FST all activities monitored showed moderate stimulation, as illustrated in Figures 17-19. At the CA4 there was a moderate increase in locomotor activities and some grooming elements (facial scratching and facial licking). The behavioral pattern most conspicuous upon administration of BN at the NTS was the increase in grooming activity, particularly the increase in facial scratching, facial licking and body licking (Figures 18-19).

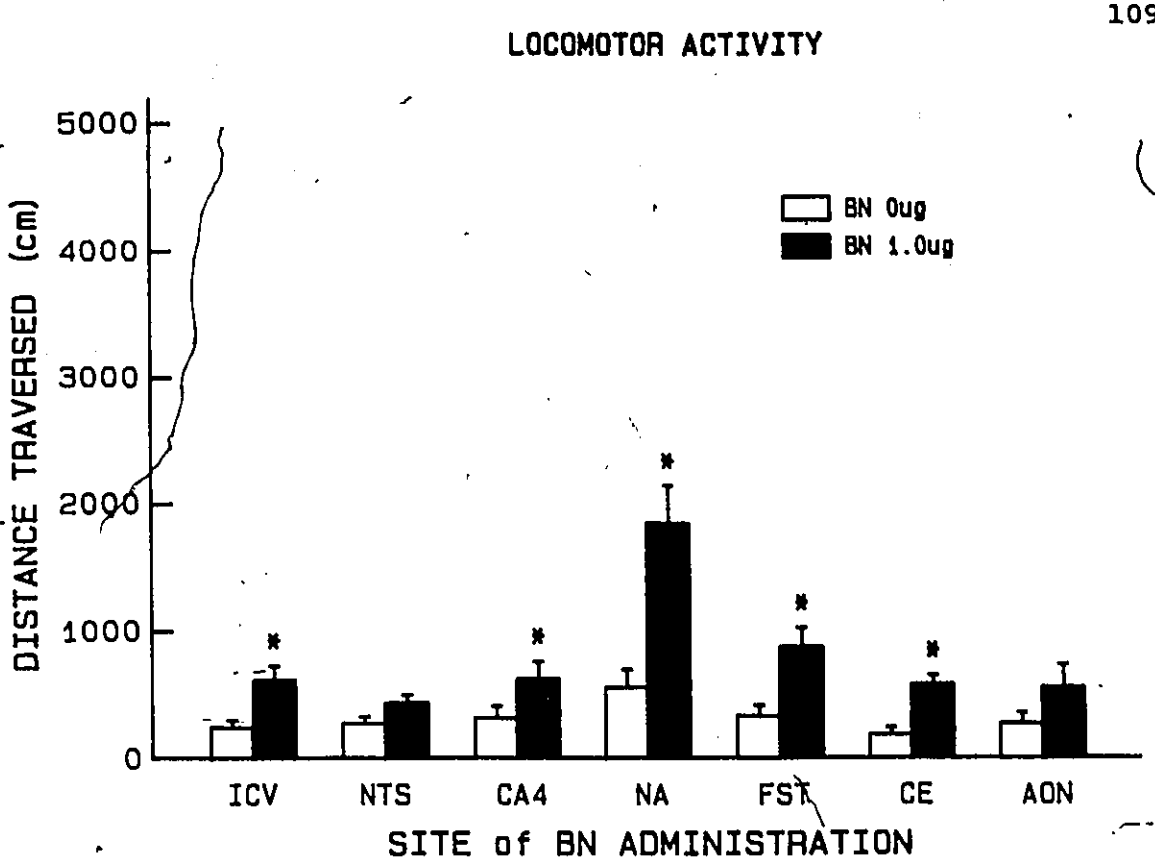

Figure 17: Effects of BN on locomotor activity of rats. On the ordinate: distance traversed (cm) over 60 min; on the abscissa: site of BN administration: ICV (intracerebroventricularly), NTS (nucleus tractus solitarius), CA4 (hippocampus), NA (nucleus accumbens), FST (fundus striati), CE (central nucleus of the amygdala), AON (anterior olfactory nucleus). The profile of vehicle treated rats is represented by the open columns and those of BN-treated groups are represented by the solid columns. Each value represents the mean ± sem of the animals in that group (n = 5 to 8). *Significantly different with respect to the appropriate control value at $p<0.05$. The animals in all groups received 1 ug BN except the FST, CE and AON groups which received 2 ug BN.

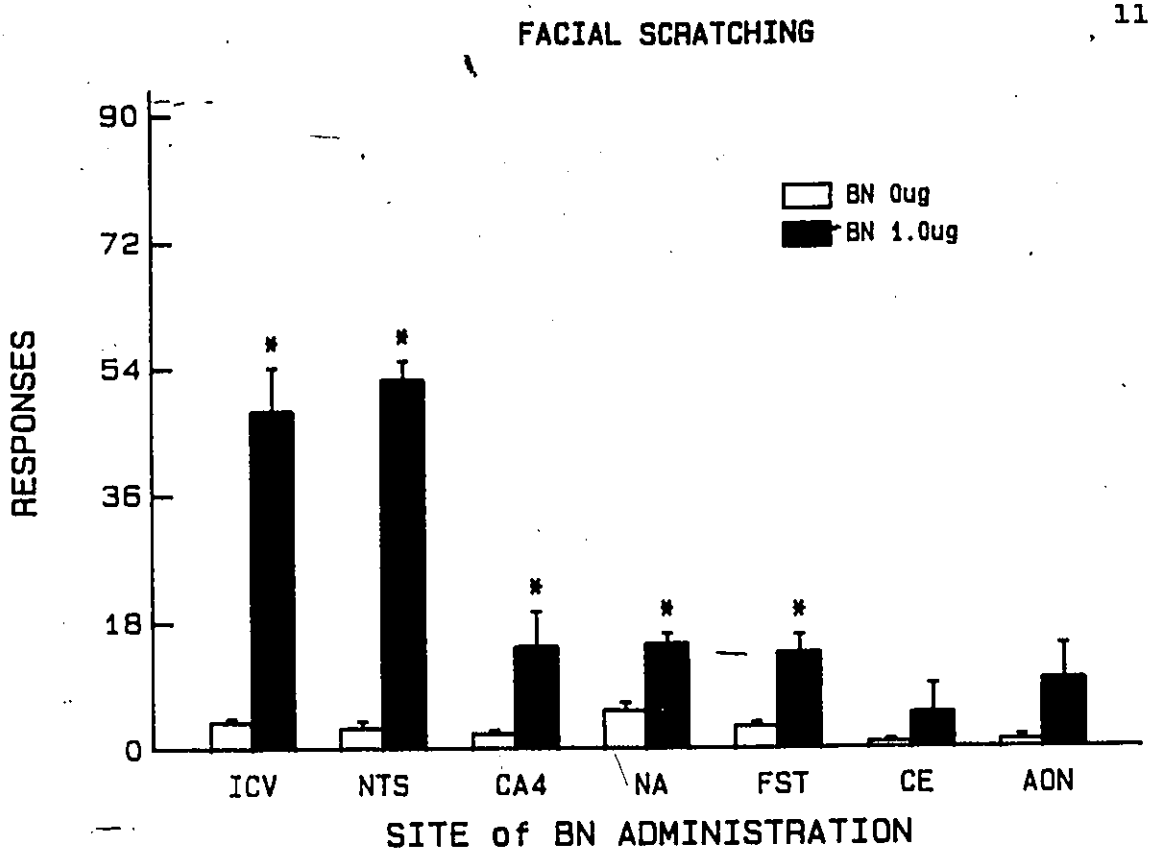

Figure 18: Effects of BN on facial scratching of rats. On the ordinate frequency of responses over 60 min; on the abscissa: site of BN administration: ICV (intracerebroventricular), NTS (nucleus tractus solitarius), CA4 (hippocampus), NA (nucleus accumbens), FST (fundus striati), CE (central nucleus of the amygdala), AON (anterior olfactory nucleus). The profile of vehicle treated rats is represented by the open columns and those of BN-treated groups are represented by the solid columns. Each value represents the mean ± sem of the animals in that group (n = 5 to 8). *Significantly different with respect to the appropriate control value at $p<0.05$. The animals in all groups received 1 ug BN except the FST, CE and AON groups which received 2 ug BN.

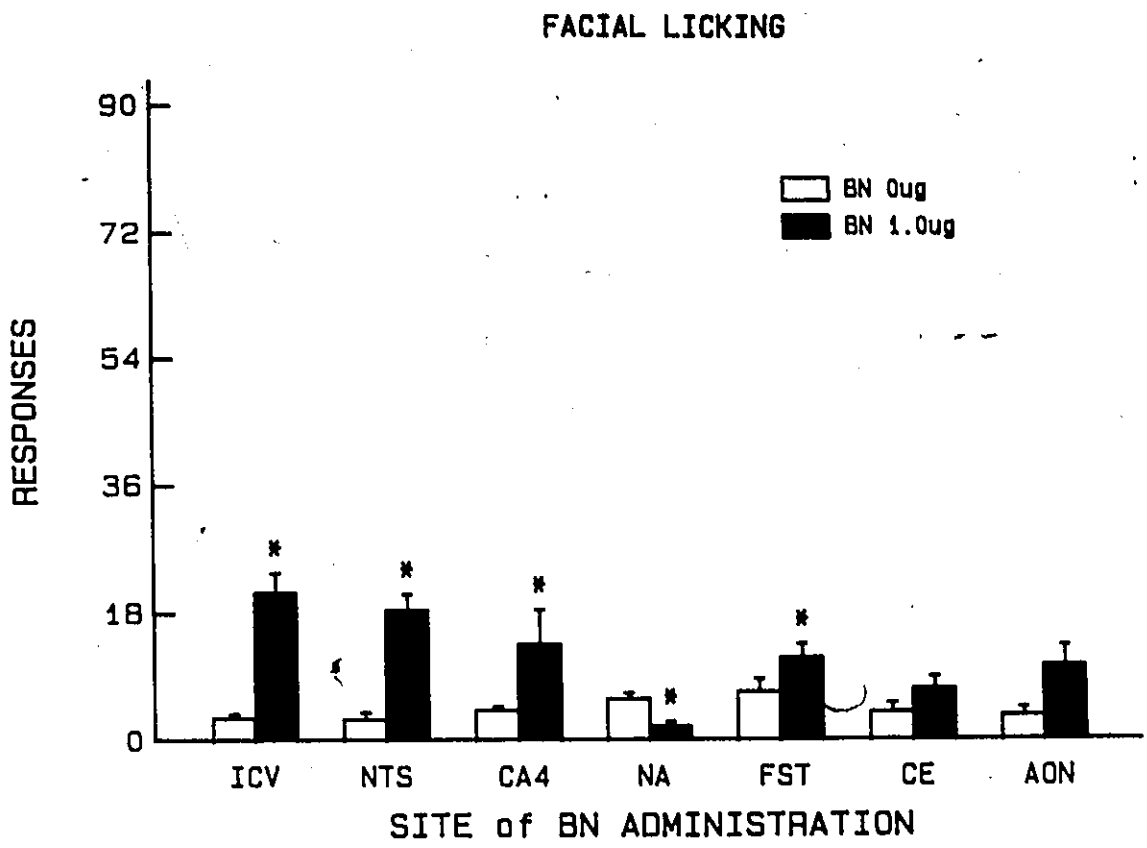

Figure 19: Effects of BN treatment on facial licking. On the ordinate: the frequency of responses over 60 min; on the abscissa: site of BN administration: ICV (intracerebroventricularly), NTS (nucleus tractus solitarius, CA4 (hippocampus), NA (nucleus accumbens), FST (fundus striati), CE (central nucleus of the amygdala), AON (anterior olfactory nucleus). The profile of vehicle treated rats is represented by the open columns and those of BN-treated groups are represented by the solid columns. Each value represents the mean ± sem of the animals in the respective groups (n = 5 to 8). *Significantly different with respect to the appropriate control value (open columns) at $p<0.05$. The animals in all groups received 1 ug BN except the FST, CE and AON groups which received 2 ug BN.

Whereas, at the NTS the grooming profile of BN is quite similar to the grooming profile induced by BN ICV, the locomotor activity is not significantly stimulated at the NTS, in contrast to the locomotor effect of BN administered ICV (Figures 17-19). The effects of BN administered ICV included both components, namely, increased locomotor as well as grooming activity, and may have resulted from the diffusion of BN to various active loci, such as the NA and NTS. Not only does BN alter behavior in a site specific manner, but the antagonism by spantide of BN-induced behaviors appears to vary in a site specific manner from antagonism of all behaviors at the NA to no antagonism of any behavior at the CA4 and NTS.

Gmerek et al, (1983) have shown that intrathecally administered BN was slightly more potent than BN ICV (A_{50} = 0.004 ug vs 0.013 ug respectively) to elicit scratching. Consequently, it is feasible that a brain stem nucleus(i) such as the NTS may be important in triggering the BN-induced scratching response induced by intrathecal BN. Another nucleus that may also be of importance in the above effect is the substantia gelatinosa of the spinal trigeminal nucleus (O'Donohue et al, 1984), since both nuclei appear to be involved in the processing of primary sensory input from the neck and face (Carpenter, 1985) and since both the nuclei contain BN-like immunoreactive peptides (Moody et al, 1981; Panula et al, 1982; Soveny et al, 1984) as well as BN binding sites (Wolf et al, 1983; Zarbin et al, 1985).

Recently, it has been reported that dorsal rhizotomy in the cat resulted in a marked decrease in BN immunoreactivity in the dorsal horn indicating that BN-like peptide(s) may be contained in primary sensory afferents. Furthermore, it was demonstrated that BN injected into the spinal cord caused a biting and scratching response indicative of sensory stimulation and thus may be a neurotransmitter of primary sensory afferents to the spinal cord (O'Donohue et al, 1984). Similarly, it is possible, that BN may cause grooming and scratching by stimulating second-order cranial sensory neurons which may mimic the stimulation of the skin of the face and neck regions (O'Donohue et al, 1984). Furthermore, the wide distribution of BN-like immunoreactivity (Moody et al, 1981a; Panula et al, 1982; Soveny et al, 1984) as well as BN receptors (Pert et al, 1980; Wolf et al, 1983; Zarbin et al, 1985) in limbic and diencephalic nuclei, the cortical layers V and VI, and the dorsal horn of the spinal cord (Moody et al 1981c; O'Donohue et al, 1984), suggest a role for BN in sensory and motor behaviors of a non specific (associative) nature.

Grooming in rodents has been compared to stress-induced displacement behaviors in humans, such as "nail-biting" (Bolles, 1960). Since ICV administration of BN causes alteration of body temperature (Brown et al, 1977a; Tache and Brown, 1982), another cause of grooming could be that rats increase grooming rates for thermoregulatory purposes. Grooming at different sites in the brain may have different

behavioral significance, namely, stress reduction, temperature control, body maintenance, itching or satiety. Certainly the different behavioral profiles at different sites is compatible with this suggestion.

At present two peptides known to induce satiety namely, BN and cholecystokinin (CCK), also induce changes in exploratory behavior (Crawley and Schwaber, 1983; Crawley, 1985; Merali et al, 1983; Kulkosky et al 1982a: b; Gibbs, 1985; Cowan et al, 1985). The CCK-induced satiety syndrome, consisted of reductions in exploratory behaviors in rats and mice, at doses of CCK which inhibited food consumption (Crawley and Schwaber, 1983). Recently it has been reported that radiofrequency lesions of the nucleus tractus solitarius abolished the effects of peripherally administered CCK, on exploratory behaviors (Crawley and Schwaber, 1983). Thus, the NTS may represent a critical relay loop mediating the behavioral actions of CCK (Crawley and Schwaber, 1983). This caudal and medial solitary nucleus receives mainly visceral afferent fibers from the vagus nerve, along with some facial and glossopharyngeal fibers (Carpenter, 1985). The NTS gives rise to ascending projections directly to the hypothalamus, amygdala, NA, and via a single relay, to visceral and taste cortices (Chronister et al, 1981; Crawley and Schwaber, 1983). Our data demonstrated that microinjection of BN at the NTS did not significantly alter the locomotor activity. In sharp contrast, however, BN at the NA caused a profound

stimulation of locomotor activity. It is of interest to note that CCK although ineffective alone, has been reported to potentiate dopamine-induced hyperlocomotion when injected intra NA (Crawley, 1985). Furthermore, at the NTS, BN markedly stimulated grooming, whereas at the NA grooming activity was enhanced only moderately. Since grooming typically follows satiety (Gibbs, 1985), the increased grooming following intra NTS administration, further indicates that BN at the NTS may play a role in the satiety related behavioral sequence.

Of particular interest is the blockade of BN-induced behavior by spantide, a competitive BN receptor antagonist at the NA, but not at the NTS. Yachnis et al (1984), have reported that the IC_{50} value of spantide was 1 uM to inhibit radiolabeled BN binding in the CNS. Furthermore, they report that spantide (2 ug; ICV), although ineffective alone, reversed the BN-induced (1 ug; ICV) hypothermia and grooming. Our results indicated that spantide (2 ug intra NA) blocked all the BN-induced (1 ug intra NA) behavioral changes. The failure of BN to block BN (intra NTS)-induced grooming suggest the possibility of different BN receptor subtypes at the NTS as compared to the NA.

These data indicate that some of the BN-binding sites recently identified autoradiographically (Zarbin et al, 1985), may represent functionally important receptor sites, and that the endogenous BN-like peptide(s) may subserve a physiological role in behaviors associated with exploration,

satiety and care of body surfaces. Finally, the differential antagonism by spantide may implicate different receptor subtypes; some being blocked and others not affected by spantide.

CHAPTER 5

PHASE 4. SPECIFIC NEUROANATOMICAL AND NEUROCHEMICAL CORRELATES OF LOCOMOTION, GROOMING AND SATIETY EFFECTS OF BOMBESIN

Introduction

Of the brain sites tested in the above Experiments (3.1-3.7) the nucleus accumbens, where locomotion was most highly stimulated, and the nucleus tractus solitarius, where grooming was most highly stimulated, were selected for further investigation in the subsequent experiments.

The Nucleus Accumbens

The nucleus accumbens (NA) is an important part of the basal ganglia complex (Pycock and Phillipson, 1984). Together with the olfactory tubercle it has been classified as part of "ventral striatum" (Heimer and Wilson, 1975). This structure can be seen as a differentiated part of the striatum with a similar, general cortico-strio-pallidal organization like the dorsal striatum. The output from accumbens projects heavily to the ventral pallidum (Swanson and Cowan, 1975; Conrad and Pfaff, 1976; Nauta et al, 1978; Chronister et al, 1981). The NA is a structure which provides a route for information travelling from limbic

structures to motor structures and thus is of key interest in understanding how motivation is translated into action (Pycock and Phillipson, 1984). Recent work, suggests that the type of motor behavior subserved by the limbic forebrain dopamine system, terminating in the NA (Kelly and Iversen, 1976), involves investigative aspects of movement, particularly mechanisms underlying the exploration of novel as distinct from familiar environments (Fink and Smith, 1980; Merali et al, 1985). However, the projection from the ventral striatum to the thalamus is not to the motor relay nuclei but to the mediodorsal thalamus and the lateral habenula; furthermore, the mediodorsal nucleus projects to the prefrontal cortex and the lateral habenula to the mesencephalic reticular formation (Bjorklund and Lindvall, 1986). The dorsal striatum, in contrast, projects to the ventral lateral and ventral anterior thalamus, motor relay nuclei that project to motor cortex.

The ventral striatum is also characterized by projections to the medial hypothalamus as well as to the parabrachial and central grey areas of the mesencephalon (Nauta et al, 1978; Bjorklund and Lindvall, 1986). Significant cell populations of the ventromedial accumbens are aligned with the bed nucleus of the stria terminalis (BST) and the central amygdaloid nucleus (CE). These two latter structures are continuous with each other through a rather extensive area underneath the globus pallidus referred to as the sublenticular substantia innominata (SI).

This functional-anatomical system (de Olmos, 1972; Heimer, 1978), is characterized by prominent relations to the lateral hypothalamus, the paraventicular nucleus (Sawchenko and Swanson, 1983), the ascending catecholamine systems, the parabrachial nucleus, and nucleus tractus solitarius, vagal motor nucleus, and the nucleus ambiguus (Fallon and Moore, 1978; Krettek and Price, 1978; Lindvall and Bjorklund, 1978; Schwaber et al, 1982; Heimer Alheid,& Zaborszky, 1985). These projections of the sublenticular substantia innominata and the possibly associated ventromedial nucleus accumbens to hypothalamic, and brainstem nuclei suggest a basal ganglia interface with endocrine, autonomic, and visceral/gustatory sensory and motor nuclei. This suggests the possibility that BN related behaviors of locomotion, grooming and satiety may be modulated by BN receptors at the nucleus accumbens through the various efferent projections of the NA reviewed above.

The Nucleus Tractus Solitarius

The nucleus tractus solitarius is a sensory nucleus located in the hind brain, receiving afferents from cranial neurons V, VII, IX and X, namely the trigeminal, facial, glossopharyngeal and vagus as illustrated in Figure 20 (Patton et al, 1976; Carpenter, 1985; Hamilton and Norgren, 1984). The NTS appears to have two major functional divisions for ingestion - an anterior-lateral oral-gustatory

half, and a posterior-medial visceral afferent half (Norgren, 1983a). Substantial behavioral evidence implicates visceral afferent activity in the regulation of feeding behavior (Norgren, 1983a,b; Crawley et al, 1983; Taylor et al, 1985; Smith et al, 1985).

The NTS in the rat appears to have separate areas for the distribution of gustatory terminations of cranial nerves V, VII, IX and X, which are distributed primarily to the lateral division of the nucleus from its rostral pole to the obex, whereas the distribution of visceral afferents of the cervical vagus are distributed in the caudal medial NST (Hamilton and Norgren, 1984). In spite of this separation, a large number of interneurons exist in the NTS suggesting afferent interactions of the cranial nerves involved in ingestion (Norgren, 1983a)(ie gustatory and visceral). Substantial behavioral evidence implicates visceral afferent activity in the regulation of feeding behavior. One mechanism often suggested for this influence involves visceral afferent activity interacting with oral or gustatory afferent activity within the solitarius as well as in the other nuclei of the ascending gustatory pathway (Norgren, 1983a,b).

Support for a hind brain site, possibly the NTS for expression of both grooming and satiation is derived from the study of the decerebrate rat. The decerebrate rat fails to initiate food-seeking behavior, or any spontaneous behavior except grooming. Although decerebrate rats do not

eat spontaneously, these rats, fed by gavage, display satiation (Norgren and Grill, 1982).

Although grooming and satiety are evident in the decerebrate rat, the integrated sequence of food seeking activity is missing, suggesting the importance of the neuronal connections between the NTS and forebrain structures in motivated behavior (Norgren and Grill, 1982; Norgren, 1983a,b). The structures of the forebrain projecting to the NTS include the paraventricular nucleus of the hypothalamus (Sawchenko and Swanson, 1982), and the ventromedial accumbens, and the sublenticular substantia innominata BST CE complex (Schwaber et al, 1982; Heimer et al, 1985). The former structures have reciprocal projections from the NTS (Richardo and Koh, 1978; Bystrzycka and Nail, 1985). Further efferent projections of NTS to forebrain structures include third order gustatory neurons projecting to the ventroposterior parvocellular nucleus of the thalamus (Norgren and Leonard, 1973; Voshart and Van Der Kooy, 1981). This thalamic gustatory relay nucleus connects in a reciprocal fashion with a discrete area of the insular cortex (Norgren and Wolf, 1975; Saper, 1982). In addition some ascending fibers from the pontine taste area (which receive secondary taste afferents from the rostral portion of the NTS) do not relay to the thalamus but project directly to the gustatory neocortex (Lasiter and Glanzman, 1983; Faull and Mehler, 1985).

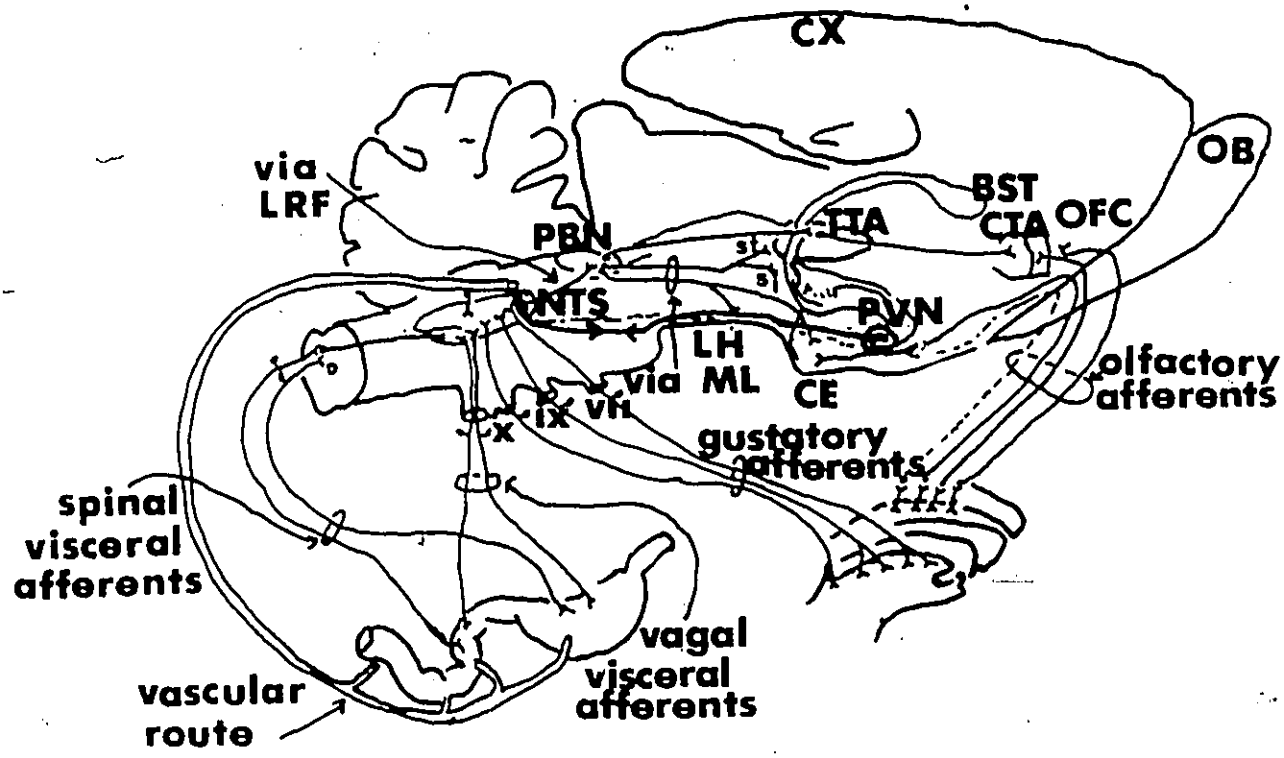

Figure 20: The sensory inputs and outputs of the nucleus tractus solitarius (NTS). This illustration is an adaptation of the illustrations of Keifer, (1985) and Norgren, (1983b). Abbreviations: BST bed nucleus stria terminalis; CE central nucleus of amygdala; CTA cortical taste area; CX cortex; LH lateral hypothalamus; LRF lateral reticular formation; ML medial leminscus; OB olfactory bulb; OFC olfactory cortex; PBN parabrachial nucleus (pons); PVN paraventricular nucleus of the hypothalamus; si substantia innominata; st subthalamic nucleus; TTA thalamic taste area; VII, IX, X, cranial nerves.

Thus, the NTS is reciprocally connected to limbic areas of the brain including the paraventricular nucleus, the CE, the BST, substantia innominata and the NA, and indirectly connected to the thalamus and the neocortex. These anatomical connections suggest the possible involvement of forebrain structures in behaviors such as grooming, initiated by injection of BN into the NTS (Johnston et al, 1986), and possibly satiety as investigated below.

However, other areas of the caudal brainstem, namely, neurons in the trigeminal, facial, hypoglossal, and ambiguus motor nuclei constitute the final common path to ingestive behavior. Both anatomical and electrophysiological evidence indicate that many, perhaps most axons arising from solitary neurons do not ascend, but rather distribute locally into subjacent reticular formation (Norgren, 1983b; Norgren, 1978; Sawchenko, 1983). Assuming that reticular neurons, some of which project to the hindbrain motor nuclei, are involved in coordinating ingestive behavior, then these neuronal systems seem to be a likely a site for interaction of gustatory and visceral afferent information (Norgren, 1983a). Furthermore, the more complex, longer and more variable motor sequences that constitute ingestion are executed normally by the chronically decerebrate rat, indicating that many oral behaviors are sequenced by caudal brainstem mechanisms. Comparative anatomy of the goldfish (Carassius auratus) shows the vagal (gustatory) lobe with sensory and motor components, with an overall viscerotopic

representation of the sensory and motor systems in register (Morita and Finger, 1985). Perhaps in the mammal, there is also a comparative sensory motor interface in the hindbrain, including the NTS and areas in the reticular formation, trigeminal, facial, hypoglossal, and ambiguus motor nuclei.

An additional study suggests that there is a projection from the NTS to preganglionic cell groups of motor nuclei in the dorsal motor nucleus of the vagus and nucleus ambiguus (Sawchenko, 1983). Moreover, unit activity recorded from the dorsal motor nucleus of the vagus from the stomach to the brain indicate that motor neurons project from the dorsal motor nucleus of the vagus to the gastric corpus and antrum (Sharoun et al, 1984). Some of the parasympathetic innervation to the stomach also arises from the nucleus ambiguus. This visceral, sensory-motor innervation was investigated in relation to gastric emptying (Burks et al, 1985). However, this innervation may also be involved in mediating the satiety effects of brian-gut peptides such as BN, which have sensory-motor components, of ingestion and gastrointestinal motility.

Of particular interest are the recent reports that BN, may have a role in sensory processing (O'Donohue et al, 1984; Panula et al, 1983; Fuxe et al, 1983; Bishop et al, 1986). Furthermore, BN is a satiety inducing peptide (Gibbs et al, 1979; Parrott and Baldwin, 1982; Kulkosky et al, 1982 a,b; Gibbs, 1985; Flynn, 1986; Kyrkouli et al, 1986). Recent results indicate that BN induces satiety at lower doses when

administered into the 4th ventricle than IP, or ICV. Thus, BN may act at a hind brain site, possibly the visceral gustatory sensory nucleus, the NTS to induce satiety (Gibbs, 1985; Ladenheim and Ritter, 1985; Flynn, 1986). Bombesin (10 ng) administered into the 4th ventricle produced satiety in 3 hr food deprived rats; in this experimental paradigm grooming did not occur until the dose of BN reached 50 ng (Flynn, 1986).

Thus it appears that there is support for a NTS site operating in relation with forebrain structures, with brain stem motor nuclei and/or with the gut afferents to mediate grooming and/or ingestive behaviors.

Satiety

Studies in which the blood of sated rats reduced the food intake of hungry rats led to the concept of circulating satiety factors. No such hunger inducing factors have been found since the blood of hungry rats would not induce sated rats to eat (Davis et al, 1969). Cholecystokinin octapeptide (CCK-8) was the first peptide that was shown to reduce meal size when administered IP before a meal (Gibbs et al, 1973). Since then many different compounds have been found to reduce meal size. Simply demonstrating that a particular compound reduces meal size is not sufficient to label it a satiety factor. Smith (1982) has outlined four criteria for labelling a substance a satiety factor. First, Booth (1972)

stipulated that a satiety treatment should act in the terminal part of a meal, but not the initial part of a meal. Second, several experiments have described a sequence of behaviors that characterized spontaneous postprandial satiety. Soon after feeding stops, a short period of nonfeeding activities occurs, such as, grooming, sniffing, locomotion, and rearing, which is then followed by rest and/or sleep (Bolles, 1960; Antin et al, 1975; Gibbs et al, 1979, Smith, 1982). When the sequence occurs, it is evidence that satiety is present, but the absence of the sequence is not evidence that satiety is not present. The third criterion, is the ability of a compound to induce satiety in sham feeding rats (Davis and Campbell, 1973; Young et al, 1974; Antin et al, 1975), since sham fed rats, after 17 hour of food deprivation, eat continuously and never display satiety. The fourth event is that these compounds or peptides are present in the gut and are released by food stimuli (Bloom, 1978; Erspamer and Melchiorri, 1976), thus making them candidates for mediating negative feedback information, that is short-term (reduction in meal size) satiety signals.

Bombesin (IP) has been shown to fulfill the four criteria: reduction in eating at the terminal part of a meal, occurrence of the post-prandial sequence of behaviors, induction of satiety in sham feeding rats (Gibbs et al, 1979; Gibbs et al, 1981; Kulkosky et al, 1981, 1982a, b) and in addition the presence of BN in plasma after a meal

(Erspamer and Melchiorri, 1976). However the last result has not been replicated (Bloom, 1978). Bombesin-induced (IP) satiety effects are specific since it has been shown not to effect drinking or any other behavior (Gibbs et al, 1981).

The analysis of satiety has been guided according to Smith (1982) by the further search for the answer to five important questions: 1) Where do food stimuli act to elicit satiety? 2) What neural and/or endocrine mechanisms mediate the effect? 3) Where are the central nervous mechanisms that process the physiological information contained in the neuroendocrine effects of food stimuli? 4) What kind of processing is performed by these central neural mechanisms? and 5) How do the central neural mechanisms produce the changes in behavior that characterize postprandial satiety?

The next step, is the present search for the site of action of BN (IP) on satiety. Thus, presently, effort has been directed at questions 1 and 2. Kulkosky et al (1982a) and Gibbs et al (1981) report BN ICV produced a dose-related suppression of liquid food intake with a threshold dose of 0.1 ug, and the threshold for increased grooming of 0.01 ug. However, BN ICV also inhibited drinking at 0.1 ug. Thus, Gibbs et al (1979, 1981) found BN administered IP was at least 3 times less potent than BN ICV at reducing eating but the effects of BN ICV were unspecific. In addition, ICV BN did not result in the expected post-prandial behavioral sequence of grooming and exploration for approximately 6 min followed by resting. Bombesin ICV resulted in increased

grooming, predominantly scratching, for up to 90 min as well as decreased resting for 90 min. Thus the central administration of BN elicited a totally different behavioral sequence than BN IP.

Attempts to block BN (IP)-induced satiety in rats by the a variety of peripheral and central ablations have failed. The lesions include adrenalectomy (Gibbs et al, 1981), hypophysectomy (Stuckey et al, 1982), subdiaphramatic vagotomy (Smith et al, 1981), celiac ganglionectomy (Gibbs, 1985), spinal cord section at the level of the sixth thoracic vertebra (Stuckey et al, 1982), and lesions of the area postrema and ventromedial hypothalamus (Gibbs, 1985; Geary et al, 1986). However, the neural disconnection (cord section, dorsal rhizotomy and vagotomy) of the gut from the brain blocks BN (IP)-induced reduction of food intake but BN increased the postprandial intermeal interval under these conditions. Thus, the effect of BN (IP) on meal size requires visceral neural elements while the effect on intermeal interval may be humorally mediated. The search for the site of BN-induced satiety continues, it remains controversial whether the site is peripheral or central. The Smith and Gibbs camp appears to favor a peripheral site (Stuckey et al, 1985), whereas, Woods et al (1986) suggest a central site.

Central injection of BN locally into the lateral hypothalamus or the paraventricular nucleus of the hypothalamus has specific effects on satiety. Bombesin does

not induce behavioral activation or effect drinking, however, its effects on satiety are small (20-25%) (Stuckey et al, 1982; Willis et al, 1984). The NTS, in reference to the visceral and gustatory input, as illustrated in Figure 20, seems a very possible site for the mediation of BN induced satiety after either central or peripheral injection of BN. The NTS may be the primary synapse of gut visceral afferent information relative to satiety mediated through bombinergic pathways. In addition, the NTS may be the input centre for information of satiety from the the paraventricular nucleus, the CE and/or other nuclei of the CNS.

Grooming has been implicated as part of the natural behavioral sequence associated with satiety. In the third experiment we microinjected BN at the posterior-medial visceral afferent half of the NTS and as stated above very pronounced grooming resulted. Injection of BN at the anterior NA resulted in pronounced locomotor activity. Thus, the possible association among BN-induced satiety, exploration and grooming was investigated in order to further elucidate the possible physiological role(s) of BN at the NA and NTS.

Neurochemical Bases of Behavioral Effects of BN

Our most active site for grooming, the caudal NTS, has very high density of BN receptors (Zarbin et al, 1985) as

well as very high levels of BLI (Moody et al, 1981a). Furthermore BLI and BN receptors are present in the parabrachial nucleus, the paraventricular nucleus and the amygdala. Limbic forebrain areas such as NA have a very high density of BN receptors but very little BLI. Neuronal cell bodies which synthesize BN-like peptides are present in the stria terminalis and also in the anterior and medial parvocellular part of the paraventricular nucleus (Roth et al, 1982) areas which both receive inputs from and project to the NTS (McKellar and Loewy, 1981). Furthermore, the BN cells of the paraventricular nucleus are ovoid with long varicose processes; it is possible that these cells project to the dorsal vagal nerve (Moody et al, 1987). Bombesin containing perikarya are also present in the NTS. There is possibly a bombinergic pathway between the NTS and the paraventricular nucleus which may be involved in gustatory functions.

Catecholamine assays demonstrate that the highest norepinephrine content in brainstem is present in the NTS. Dopamine neurons provide a very minor component of brain stem innervation (Levitt and Moore, 1979). However, the caudal NTS is an area that projects to the anterior NA (Chronister et al, 1981). The anterior NA, an area where there is a very high density of BN receptors and also a very high density of dopamine terminals, was our most active site for the exploratory behaviors. Interestingly, the behavioral profiles at these two sites are somewhat complimentary with

facial scratching and licking greatly increased at the NTS, and only mildly stimulated at the NA, and body licking insignificant at the NA and significantly stimulated at the NTS, and locomotion, markedly stimulated at the NA and insignificantly stimulated at the NTS. Since a direct anatomical connection between the NA and NTS exists (at least in the rabbit, no such test of HRP retrograde transport from the anterior NA has been done on the rat), there is a possibility that BN microinjected at the NTS may have a modulatory effect on behaviors initiated from the NA. This modulation may occur through either the existing direct pathway whose neurotransmitter(s) is unknown, and/or through modulation of dopamine neurotransmission.

Neuroanatomically, dopamine terminations are evident in several areas along the visceral and gustatory afferent pathway ascending from the NTS, including the lateral parabrachial nucleus, the CE and the lateral hypothalamus and nucleus of the stria terminalis (Pfaffmann et al, 1977; Lindvall and Bjorklund, 1983). Reciprocally, the NA, the parabrachial nucleus, the CE, and bed nucleus of the stria terminalis project to mesencephalic dopamine neurons (Pycock and Phillipson, 1984). These reciprocal connections suggest a possible modulation of gustatory and visceral sensory input and motor output by the dopaminergic mesencephalic neurons as well as, the possible modulation of dopamine function by the visceral and gustatory afferent pathway. In addition, the dopamine system(s) is implicated in the

mediation of peptide induced grooming (Isaacson, 1984). Furthermore, BN caused an increase in dopamine metabolites, homovanillic acid and 3,4 dihydroxyphenylactic acid in the hypothalamus, striatum and olfactory tubercle, areas enriched in BN binding sites (Widerlov et al, 1984). Thus, we investigated the role of dopamine systems in the mediation of the behavioral effects of BN microinjected at the NTS and the NA.

Recently, a substance P analogue, spantide has been shown to antagonize the behavioral effects of BN administered ICV (Yachnis et al, 1984) and at the NA (Johnston et al, 1986) but not at the NTS (Experiment 3.1, above). Spantide has been demonstrated to be a competitive BN receptor antagonist (Jensen et al, 1984; Yachnis et al, 1984; Folkers et al, 1984), consequently, the effects of spantide on BN-induced behaviors were tested. Finally neuromedin B and C, GRP 1-16 and neuropeptide Y were tested, in order to compare the effects of BN to other peptide fragments of the GRP family and to a peptide from a different family (Neuropeptide Y). Comparison of the behavioral effects of BN to GRP 1-16, an inactive fragment of GRP-27, tested for specificity of BN-induced behavioral effects, as did the comparison of effects of BN to neuropeptide Y, a peptide noted to have a different effect on grooming and eating than BN (Gray and Morley, 1986).

Objectives PHASE 4

The overall objectives of the next set of experiments were to further elucidate the relationship(s) between locomotion, grooming and satiety effects of BN, and to determine if differences exist in the mechanisms subserving these effects. Specifically, experiments were proposed to delineate: 1) the time-course and dose-effects of BN administered at the NA, NTS or IP on locomotor activity, grooming and satiety, 2) whether some of these behaviors occur concomitantly 3) whether neurochemical differences exist in the mechanisms subserving these effects with reference to dopaminergic neurotransmission and 4) whether the BN-induced grooming profile differs across two unique paradigms.

Method

Experimental Techniques

In order to more fully understand the physiological significance of BN-induced grooming, different elements of grooming were monitored during feeding to satiety in one paradigm the "satiety" paradigm. In the other paradigm BN-induced grooming was monitored in observation chambers by an experimenter while locomotor activities were monitored by a microprocessor controlled infrared beam grid system.

The latter experimental paradigm, from here on, will be called the "home" paradigm since the observation chambers were identical to the home cage except that the top of the cage was higher to allow for rearing. As in the home cage, food and water were available ad libitum and the rats had not been food deprived prior to experimentation. Furthermore, the animals were acclimatized to the infrared beam monitoring cage each day for 90 min before experimentation. Thus, it was a very familiar environment for the rats, where they spent up to 4 hr a day. Monitoring of grooming elements of 8 rats, each for 5 sec, resulted in 1 observation per rat every 40 sec, for the duration of the experiment. Simultaneous monitoring of grooming and locomotor activities in the home paradigm began about 14-20 minutes after the injection of the first of the 8 rats.

In contrast, in the satiety paradigm eating parameters of the 5 hr food-deprived rats (n = 10-12) were monitored one at a time while, simultaneously, grooming data were collected. Grooming was monitored continuously and scored every 5 sec, for 20 min, starting immediately after the injection. Consequently, the satiety paradigm yielded additional information on the time course of grooming of each rat. It also served to shed more light on the possible physiological or biological significance of BN-induced grooming in the rat.

As well, by comparing the grooming profiles of the two paradigms the experiments addressed the question of whether

the grooming profile was affected by the paradigm conditions in which it occurred. Eight experiments were undertaken as listed below.

PHASE 4 Experimental Organization

Home paradigm.....NTS....Experiments...1 & 2

.....NA....Experiments...3 & 4

Satiety paradigm....NTS....Experiments....5 & 6

.....NA.....Experiments....7 & 8

Animals

All experiments were conducted on adult male Sprague-Dawley rats (275-300 g) (St. Constance, Quebec) housed individually with free access to food (Master Laboratory Chow) and water (except in the satiety paradigm where animals were food deprived between 8 am and 1 pm every day as described below). The environment was maintained at 24°C, 60% relative humidity, and with 12 hr of light (6 a.m. to 6 p.m.). Separate groups of rats under sodium pentobarbitol (50 mg/kg, IP) anesthesia were stereotaxically implanted with 24-gauge stainless-steel guide cannulae (Kinetrods, Ottawa, Canada) aimed unilaterally, at the NTS (A-P -13.8

mm, lateral 0.6 mm, depth 8.8 mm) (Crawley, 1985; Johnston et al, 1986; Paxinos and Watson, 1982) for Experiments 4.1 and 4.5 or bilaterally, at the NA (AP +2.2 mm, lateral ± 1.8 mm, DV 7.2 mm) (Johnston et al, 1986; Paxinos and Watson, 1982) for Experiments 4.3 and 4.7. However, in the four other experiments, (4.2, 4.4, 4.6, and 4.8) in order to save time and money, two groups of rats (n = 8 and n = 12) were simultaneously implanted with bilateral cannulae aimed at the NA and a unilateral cannula at the NTS. Consequently, the same triple implanted rats were used in Experiments 4.2 and 4.4 (n = 8). Similarly the other group was employed for Experiments 4.6 and 4.8 (n = 12). The triple implant surgical procedure took 60 to 90 min, no additional anaesthesia was needed. Furthermore, these rats recovered as quickly as rats with single site implants. The NTS implant site was similar to that in Experiment 3.1 except that the co-ordinates were changed to A-P 13.8 mm, lateral +0.6 mm, and depth 8.8 mm (Paxinos and Watson, 1982). The depth was increased to insure that placement in the NTS would continue as the rats were used for a longer period of time (ie up to approximately 6-8 weeks from surgery) since as the rat grew and the skull thickened. Also, the site was moved 0.5 mm posterior to increase the separation of the site from the the fourth ventricle and at this location the NTS is more ventral (A-P -13.3 to -13.8 mm). The NA site was similar to that in Experiment 3.3 except that the depth was increased by 0.5 mm in this experiment to insure that the injection

remained in the NA and not the ventral caudate-putamen, as the skull thickened.

The guide cannulae were cemented with dental acrylic to four jeweler's screws placed in the calvarium. Stainless-steel obturators were in the guide cannulae at all times except during injections. The animals were allowed a minimum of 5 days postoperative recovery prior to commencement of experiments.

The implant sites chosen avoided crossing the ventricular system (Johnson and Epstein, 1975; Paxinos and Watson, 1982) in order to avoid flow of the injection up the exterior quide cannula tract into the ventricular system.

Injections

At least a full day elapsed between each treatment. All doses are expressed as total dose per animal. All intra brain injections were carried out with the aid of a Harvard infusion pump, at the rate of 0.5 ul/30 sec. In addition, the 30 gauge injection cannula (which protruded past the guide cannula by 0.05 mm) was left in place for an additional 30 sec after the injection. All drugs microinjected at brain sites, were delivered in a volume of 0.5 ul in 0.9% saline vehicle in deionized distilled water (Myers, 1974). Furthermore, the vehicle volume of 0.5 ul was used in accordance with previous experiments (Johnson and Epstein, 1975; Stukey and Gibbs, 1982; Gmerek and Cowan,

1983; Crawley, 1985, Johnston et al, 1986; Spruijt et al, 1986). At the completion of testing, the implants of all animals used in the experiments were verified by injecting cresyl violet, followed by standard histological procedures.

Home Paradigm

Drug injections were carried out at 10 am every day. Behavioral monitoring began immediately after the eighth (last) rat was injected. Grooming elements and locomotor activities were monitored as described previously (Merali et al, 1983; 1985; Johnston et al, 1986 and also in Experiments 3.1-7 above). Briefly locomotor activities were monitored by a custom-designed Z-80 microprocessor-based controller which sampled each of the 9 infrared beams (strategically placed in each of 8 observation chambers) every second and collected data over 60 min. Simultaneous monitoring of grooming elements (facial scratching, facial licking, body scratching and body licking), sniffing and resting by a human rater was conducted from an adjacent room through a one way mirror. Eight rats were monitored serially, each for 5 sec, every 40 sec, for 60 min. Any of the behaviors that occurred for a given rat during the 5 sec was recorded.

Data was collapsed over 60 min and one way analysis of variance (dose-effect) calculated. The Tukey test was used for the comparison of means ($p<0.05$).

The Satiety Paradigm

Ten to twelve animals cannulated at the NTS and/or NA (as described above) were given 5 days of recovery from surgery. Thereafter they were food deprived from 8 am. to 1:00 pm. After 1:00 pm they were trained to obtain a portion of their daily food intake (Wise and Raptis, 1985) as follows: In test boxes 20 meal segments, of ten, 45-mg food pellets each (Dustless Precision Pellets, Bio Serv Inc, Frenchtown, New Jersey), were presented for 58 sec intervals. Meal segments were introduced into a 20 x 27 x 31 cm test box, of clear polycarbonate, by an automatic dispensing apparatus. Pellets were dispensed by an aluminum food delivery platter (31 cm diameter) with seventy-two food cups (1 x 2.5 x 0.8 cm) moulded into it. The platter extended 6 cm into the test box and only one of the cups was exposed, through a mask, at any given time. The platters were indexed one position every 60 sec, and a timer triggered rotation of the platter by a solenoid connected to an electric motor. Solenoid noise was clearly audible but not loud.

Testing began after approximately 15 training days, that is, after the rats eating latency and duration times and number of pellets eaten had stabilized. In each experiment rats were divided randomly into 2 groups of 5-6 rats. Each group received, alternately, a drug injection day followed by a drug-free schedule maintenance day. Thus, for

instance, 10 days were required for completion of 5 drug injections. One group of rats, one rat at a time, was injected and monitored for eating and grooming behaviors, for 20 min each. Simultaneously, the other yoked group was run in an identical feeding paradigm, but no drug was administered and no data was recorded. Between injections, the peptide was kept on ice.. Feeding and grooming behaviors were monitored immediately after the injection, for 20 min.

Latencies to make oral contact with the first pellet of each meal segment and time to consume all 10 pellets in each segment were recorded. Numbers of pellets left uneaten were noted. In addition, the following eight behaviors were recorded: facial scratching, facial licking, body scratching, body licking, sniffing, chewing, rearing and licking (Johnston et al, 1986 and above in Experiments 3.1-7). The satiety room where the experiments took place every day, as well as the setup for collecting the data was displayed in Figures 21 and 22.

The behaviors monitored have been operationally defined above except for chewing, rearing and licking. Chewing was scored when the rat moved his jaws in a motion involving the possible mastication of food. Generally, in this experiment the rat continued to chew after all the food was removed from the food cup. Rearing was scored when the rat stood on his hind legs with his forelegs off the floor in a non-grooming stance and not in contact with the feeding apparatus. Licking was defined as the protrusion of the

tongue out of the mouth and in contact with any part of the testing chamber. This excluded the licking when the rat was feeding or grooming.

The occurrence of each behavior was recorded every 5 sec, over the 20 min of food presentation. Thus, the possible maximum score for any behavior was 240. Behavioral data was collected and compiled on a hyperion computer.

Data was collapsed over 20 min for the Experiments (n = 10-12) and one way analysis of variance carried out. The exception to this occurred for the two way analysis of variance of time-course, and dose-effect of BN intra NTS and IP on latency to eat, pellets left, facial scratching and facial licking. The Tukey test was used for the comparison of means. The level of significance used was $p<0.05$.

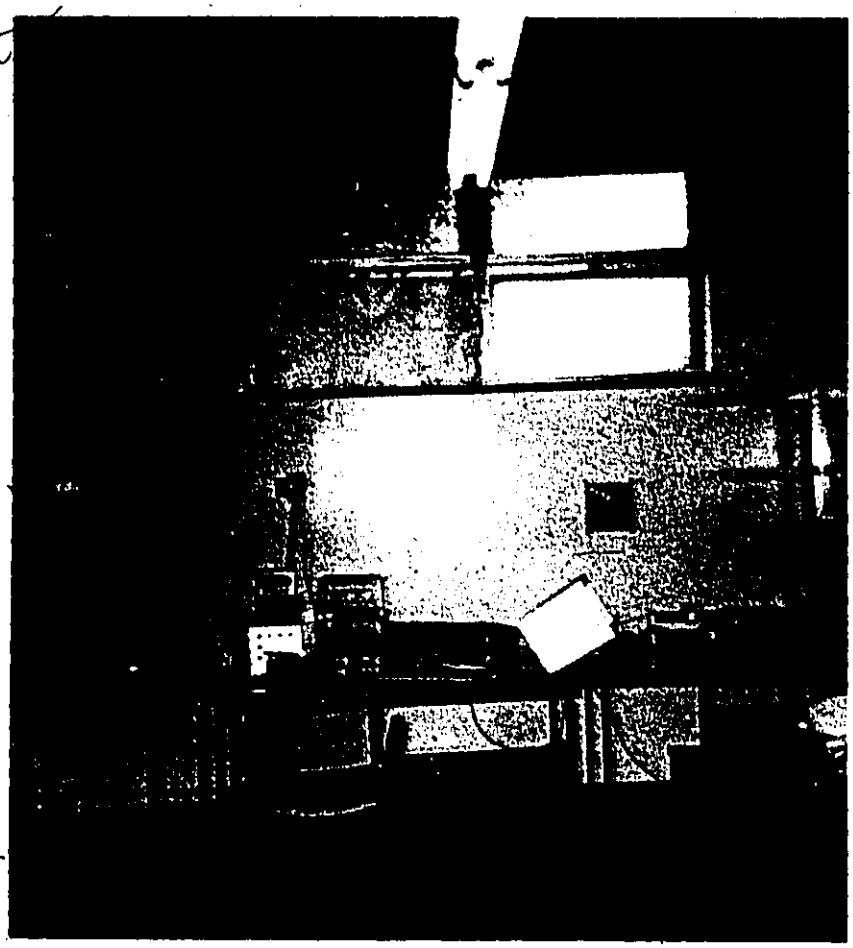

Figure 21: The satiety room: Only the rats in the satiety experiments were housed in this room at all times on shelves on the walls to the left and right. On the right side of the table is a Harvard infusion pump where the rat was held to be infused. On the pump were 50 ul Hamilton syringes; to each was attached a short piece of 50 PE tubing and the injection cannula. The peptide in the tubing was kept on ice between injections. Notice the ice container next to the pump. After the injection the rat was placed immediately into the clear polycarbonate box between the two timers and the Hyperion computer. Monitoring of grooming was done with software programming of 8 keys on the keyboard.

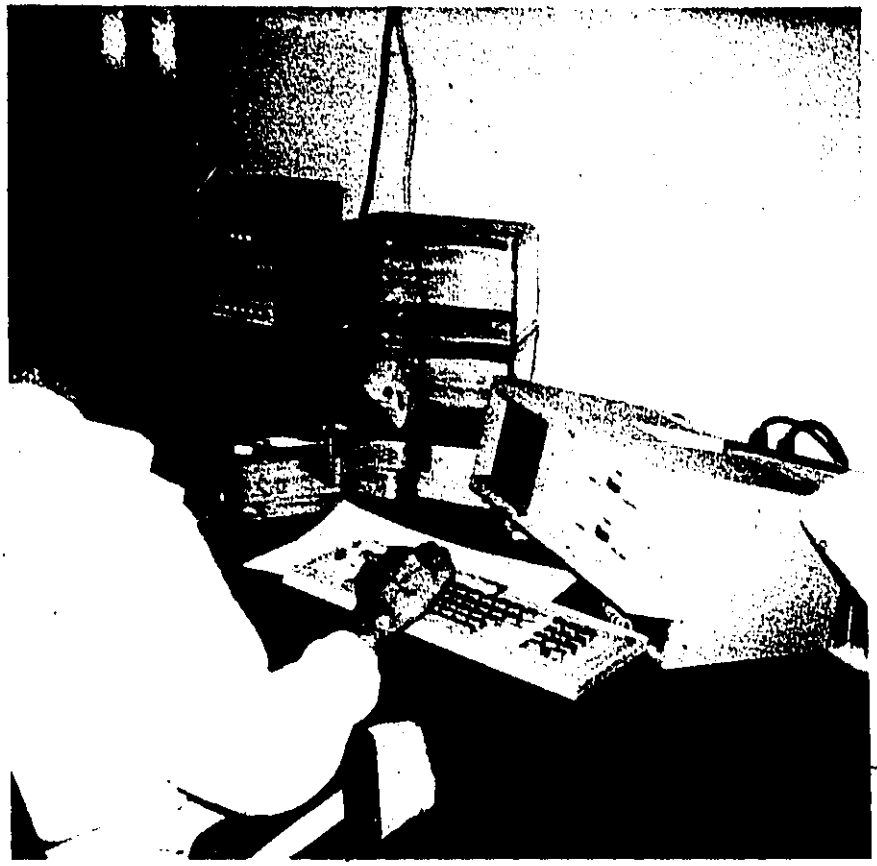

Figure 22: Experimenter at work, monitoring satiety and grooming simultaneously. Right hand on keyboard recording grooming, left hand holding push button for the timer to measure latency to eat and duration of eating.

Figures

Behaviors which showed the most significant variability across drug conditions, sites and paradigms were graphed.

Graphs for the home paradigm (Figures 25-39) included the behaviors: locomotion, facial scratching, facial licking, sniffing and resting. Of the three behaviors monitored by the infrared beam grids (locomotion, floor activity and rearing), locomotion varied the most from the NTS to the NA injection site and thus was illustrated. Body scratching and body licking, and yawning and stretching very often were not significantly changed by these drug treatments and thus were not graphed.

Graphs for the satiety paradigm included the behaviors: latency to eat, pellets left, facial scratching, facial licking, and sniffing (Figures 44-60). Body scratching and body licking, chewing, rearing and licking were not graphed since, for the most part, they did not show significant variation during these experiments. At the NTS BN increased latency to eat to the maximum time of 58 sec often. This extended latency to eat disrupted the duration of eating score since the two measures were in tandem, one following the other over 58 sec, for each food presentation. Thus, latency to eat was graphed instead of duration of eating.

Except for locomotion which had no experimentally imposed maximum score, all graphs followed a 5 decrement scale of the behavioral response on the ordinate, where the

highest score represented the maximum frequency possible (100%). Thus, the ordinate values represented 100, 80, 60, 40, 20 and 0% of the possible total frequency, respectively. In addition, to facilitate comparison of behaviorai data (where possible) the results from drug administration at the NTS and NA as well as the IP injection were illustrated on the same graph. This ability to convert the scores on the graphs to % facilitated the comparison of behavioral values across doses, sites and paradigms. The values of these graphs were derived from the mean $\pm$ sem of oneway analysis of variance of the data (dose-effect).

With the pie charts (Figures 63-64), values for each grooming element were expressed as a per cent of total grooming score. The total grooming score was derived by adding together the means (from one way analysis of variance) of the frequency of each of 4 grooming elements for each drug condition.

The exception to the use of means from oneway analysis of variance involves the 4 graphs (satiety paradigm) from the analysis of time-course, and dose-effect of BN intra NTS and IP on latency to eat, pellets left, facial scratching and facial licking. These graphs were derived from the interaction means of two way analysis of variance. The ordinate scale value for each behavior was the maximum value possible for 1 min for each behavior (ie 58 sec for latency, 10 pellets for pellets left, 12 responses for facial

scratching and facial licking), thus these four behaviors have a comparable scale (divided into 20 units).

Experiments 4.1 and 4.2: The Behavioral Effects of Peptide and/or Drug Administration at the Nucleus Tractus Solitarius and/or Peripherally in the Home Paradigm.

Experiment 4.1

First, Experiment 3.1, designed to elucidate the time- and dose-related behavioral effects of BN administered at the NTS, was replicated. The doses of BN (Peninsula) tested were 0, 0.001, 0.01, 0.1 or 1.0 ug. In addition, rats were injected intra-NTS with d-amphetamine (0, 1, 5, or 10 ug) (Pijnenburg and Van Rossum, 1973). Following the NTS injections these rats were also injected (IP) with the following: fluphenazine (Squibb) (0, 0.05, 0.1, or 0.5 mg/kg) (Merali et al, 1985); BN (0, 1, 2, 4, or 8 ug/kg) (Kulkosky et al, 1982a,b); and d-amphetamine (0, 0.05, 0.1, 0.3, 0.5 mg/kg). A Latin square design for each drug administration was used.

Experiment 4.2

A new set of 8 rats cannulated at the NTS, (triple implanted rats, also used in Experiment 4.4) were microinjected at the NTS with the following drugs:

Neuropeptide Y (Peninsula; porcine) (1 and 0.1 ug); GRP 1-16 (Peninsula) (2ug); saline; BN (0.5 ug); BN (0.5 ug)+ fluphenazine (10 ug); or fluphenazine (10 ug) (Donzanti and Uretsky, 1983), in a random order with a day between each injection. At the end of the experiment, BN (0.5 ug) + spantide (1 ug), were microinjected, followed 1 week later by spantide (1 ug) alone.

Results: Experiments 4.1 and 4.2: The Behavioral Effects of Peptide and/or Drug Administration at the Nucleus Tractus Solitarius and/or Peripherally in the Home Paradigm.

The implants of all animals used in the experiments were illustrated in Figures 23 and 24.

Results Experiment 4.1

One way analysis of variance repeated over subjects, revealed a significant effect ($p<0.05$) of dose of BN when injected at the NTS on floor activity $F(4,28) = 20.77$; rearing $F(4,28) = 3.51$; facial scratching $F(4,28) = 48.25$; facial licking $F(4,28) = 60.68$; body scratching $F(4,28) = 8.19$; body licking $F(4,28) = 6.10$; sniffing $F(4,28) = 3.59$; and resting $F(4,28) = 74.95$. Comparison of means revealed significant decrease in resting over the control condition after 0.01 ug BN or higher (Figure 29). At a dose of 0.01 ug and higher, BN significantly increased floor activity and facial scratching (Figure 26). Facial licking was significantly increased at a dose of 0.1 ug BN or greater (Figure 27). Body scratching was increased at the 0.1 ug dose only. Locomotion (Figure 25), rearing, body licking and sniffing (Figure 28) did not show a significant increase over the saline condition for any of the doses tested. The means $\pm$ sem for locomotion, facial scratching and licking, sniffing and resting were illustrated in Figures 25-29.

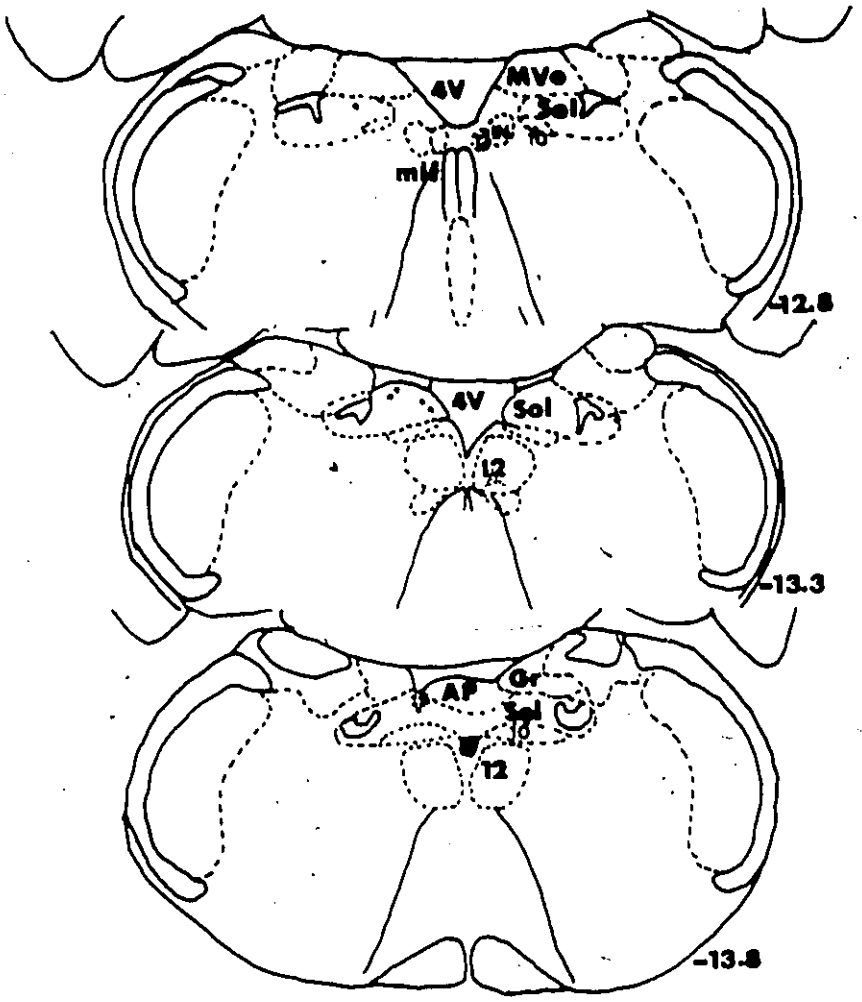

Figure 23: Verification of implantation sites after Experiment 4.1. Location of cannula implantation tip in the caudal nucleus tractus solitarius (NTS) of the rat (n=11) (coronal view: A-P -12.8 to -13.8 mm); (Paxinos and Watson, 1982). Abbreviations: . site of injection; Cu cuneate nucleus; Gr gracile nucleus; In intercalated nucleus of medulla oblongata; 4V fourth ventricle; 12 nucleus of hypoglossal nerve; 10 dorsal motor nucleus of the vagus; mlf medial longitudinal fasciculus; MVe medial vestibular nucleus.

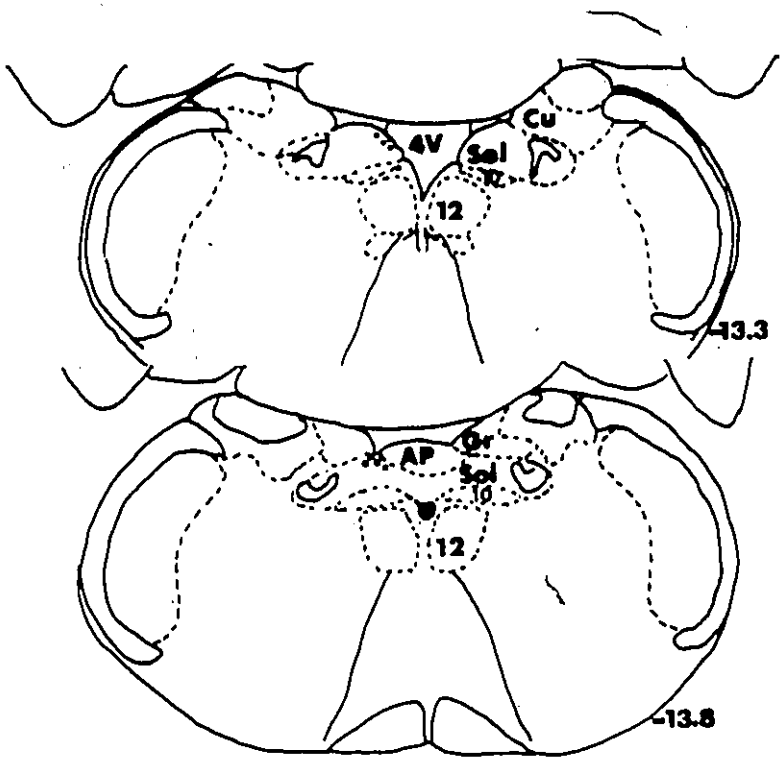

Figure 24: Verification of implantation sites after Experiment 4.2. Location of cannula implantation tip in the caudal nucleus tractus solitarius (NTS) of the rat (n=8) (coronal view: D-V -13.3 to -13.8 mm); (Paxinos and Watson, 1982). Abbreviations: see Figure 23.

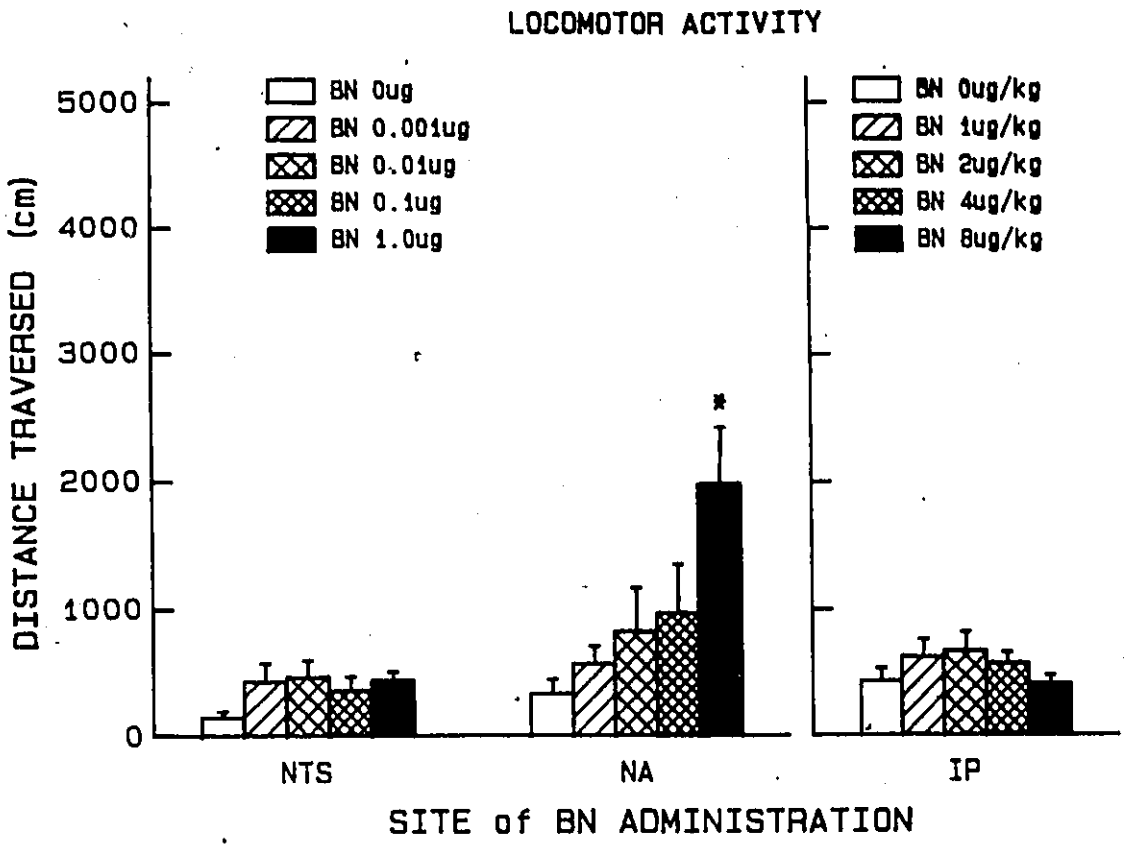

Figure 25: Effects of BN (intra NTS, NA, & IP) on locomotor activity of rats in the home paradigm. On the ordinate: distance traversed (cm) over 60 min; on the abscissa: site of BN administration: NTS (nucleus tractus solitarius), NA (nucleus accumbens), IP (peripheral). The profile of vehicle treated rats is represented by the open columns and those of BN-treated groups are represented by the patterned columns. Each value represents the mean of the animals in that group (n = 8) $\pm$ sem. *Significantly different with respect to the appropriate control value at $p<0.05$.

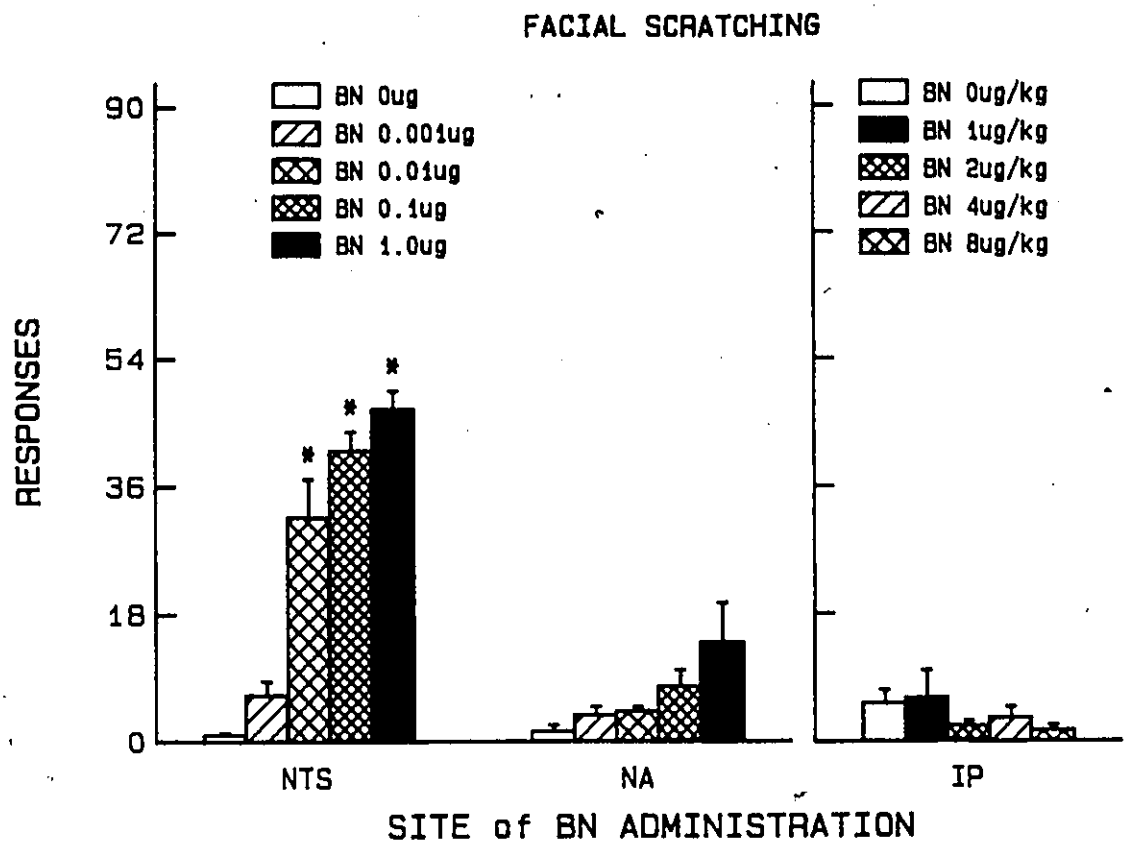

Figure 26. Effects of BN (intra NTS, NA, & IP) on facial scratching of rats in the home paradigm. On the ordinate: the magnitude of the quantified response over 60 min; on the abscissa: site of BN administration: NTS (nucleus tractus solitarius), NA (nucleus accumbens), IP (peripheral). The profile of vehicle treated rats is represented by the open columns and those of BN-treated groups are represented by the patterned columns. Each value represents the mean of the animals in that group (n = 8) ± sem. *Significantly different with respect to the appropriate control value at $p<0.05$.

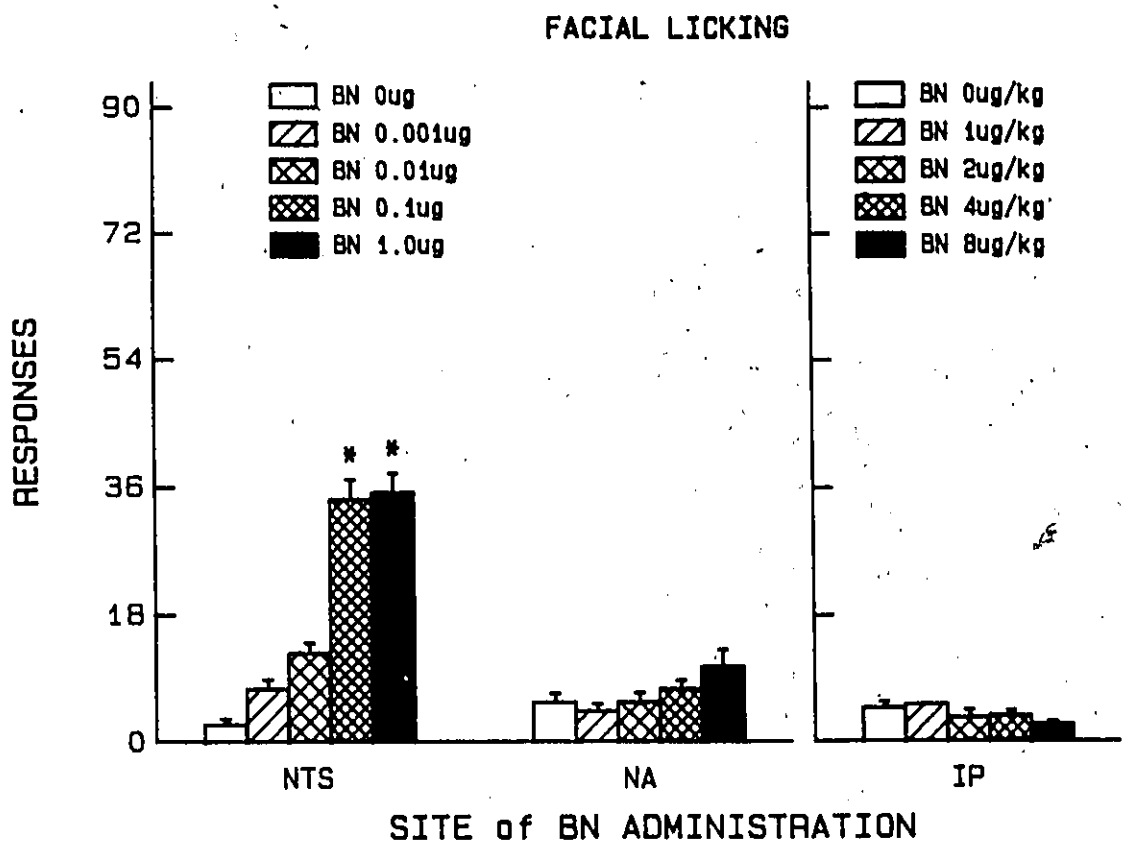

Figure 27: Effects of BN (intra NTS, NA, & IP) on facial licking of rats in the home paradigm. On the ordinate: the magnitude of the quantified response over 60 min; on the abscissa: site of BN administration: NTS (nucleus tractus solitarius), NA (nucleus accumbens), IP (peripheral). The profile of vehicle treated rats is represented by the open columns and those of BN-treated groups are represented by the patterned columns. Each value represents the mean of the animals in that group (n = 8) ± sem. *Significantly different with respect to the appropriate control value at $p<0.05$.

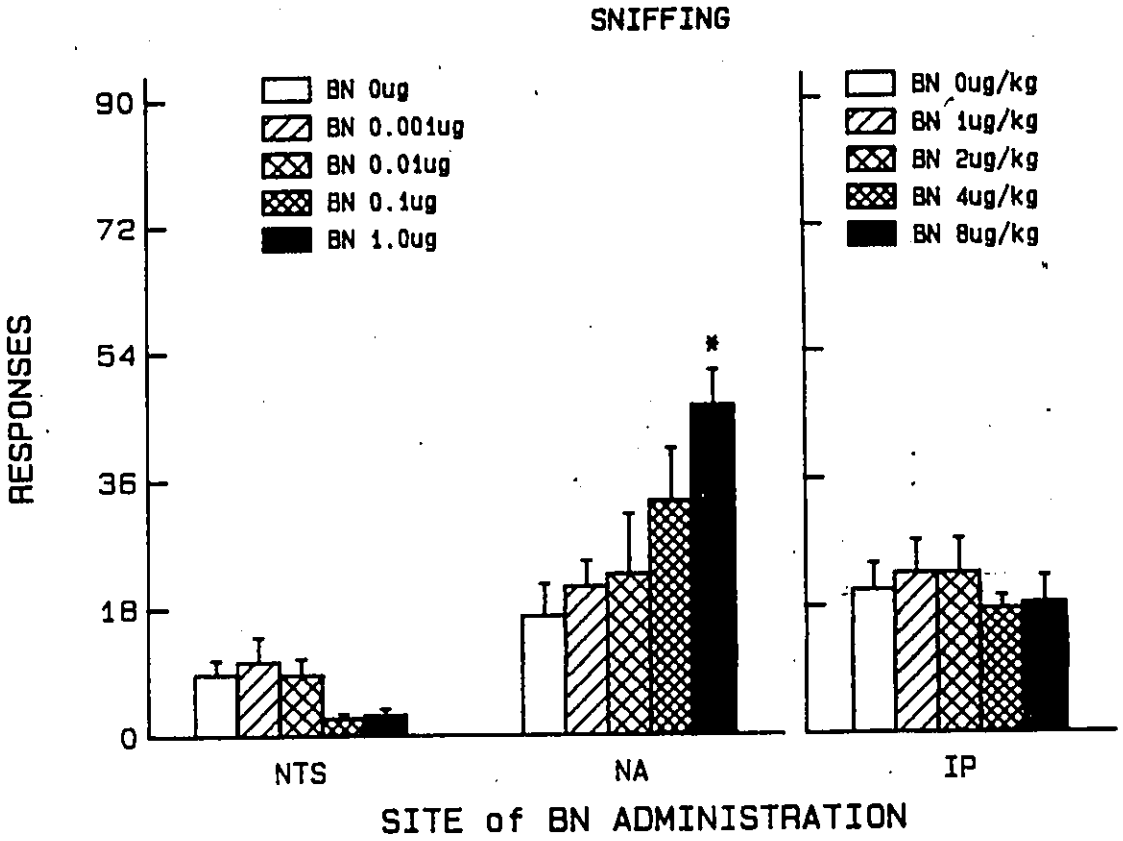

Figure 28: Effects of BN (intra NTS, NA, & IP) on sniffing of rats in the home paradigm. On the ordinate: the magnitude of the quantified response over 60 min; on the abscissa: site of BN administration: NTS (nucleus tractus solitarius), NA (nucleus accumbens), IP (peripheral). The profile of vehicle treated rats is represented by the open columns and those of BN-treated groups are represented by the patterned columns. Each value represents the mean of the animals in that group (n = 8) ± sem. *Significantly different with respect to the appropriate control value at $p<0.05$.

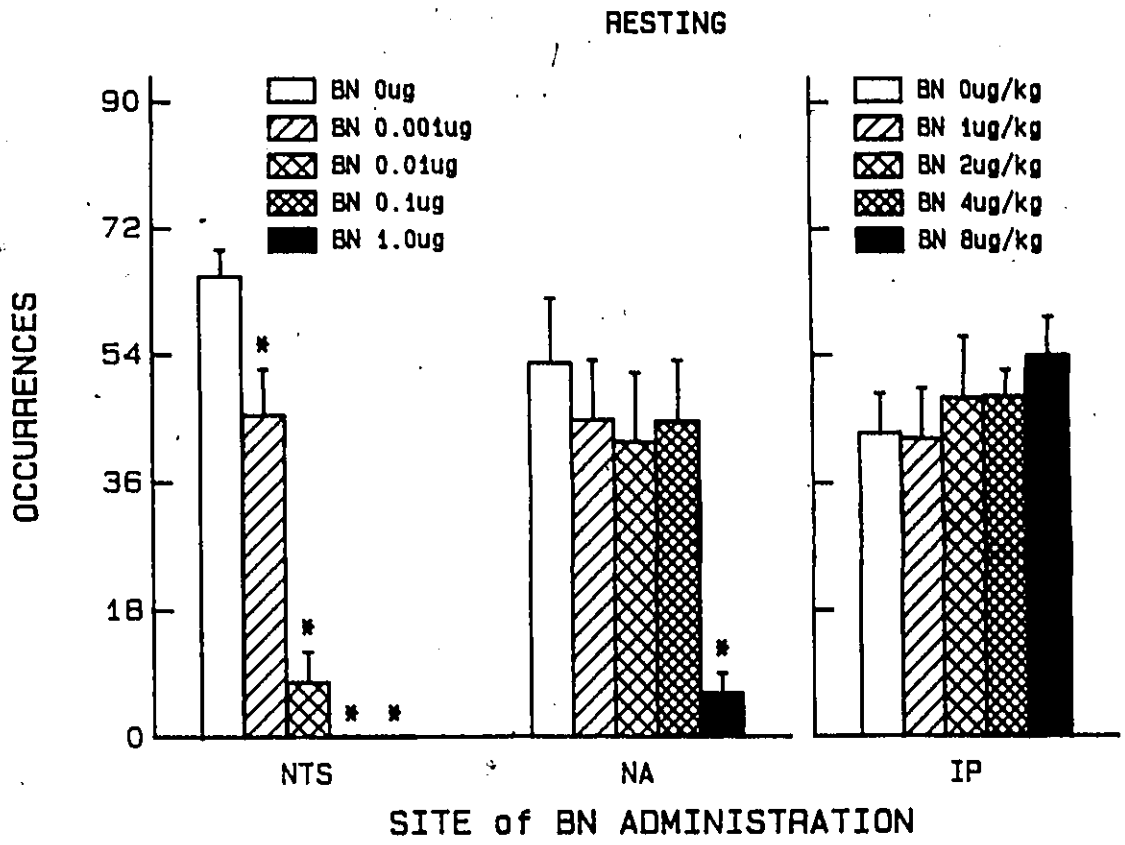

Figure 29: Effects of BN (intra NTS, NA, & IP) on the occurrence of resting of rats, in the home paradigm. On the ordinate: the magnitude of the quantified response over 60 min; on the abscissa: site of BN administration: NTS (nucleus tractus solitarius), NA (nucleus accumbens), IP (peripheral). The profile of vehicle treated rats is represented by the open columns and those of BN-treated groups are represented by the patterned columns. Each value represents the mean of the animals in that group (n = 8) ± sem. *Significantly different with respect to the appropriate control value at $p<0.05$.

Results of Experiment 4.1 continued

Neither d-amphetamine (1, 5, or 10 ug) nor fluphenazine (1, 5, or 10 ug) had a significant effect on behaviors monitored in the home paradigm, when microinjected at the NTS.

Although fluphenazine (0.05, 0.1 0.5 mg/kg) injected peripherally had no significant effects on exploratory and grooming behaviors, d-amphetamine, injected peripherally (0.05, 0.1, 0.3, 0.5 mg/kg) did change some of these behaviors. One way analysis of variance revealed a significant effect of d-amphetamine on locomotion $F(3,21) = 16.58$; floor activity $F(3,21) = 30.01$; rearing $F(3,21) = 28.01$; facial licking $F(3,21) = 3.18$; sniffing $F(3,21) = 29.01$; and resting $F(3,21) = 12.27$. Comparison of means, revealed a significant increase over the control condition, in floor activity, rearing, sniffing and resting with d-amphetamine, at a dose of 0.3 mg/kg or greater; locomotion was significantly increased at a dose of 0.5 mg/kg d-amphetamine. Although facial licking showed an overall significant decrease after d-amphetamine, comparison of means revealed no significant difference between means. The means of the dose effect of d-amphetamine for locomotion, facial scratching and licking, sniffing and resting were illustrated in Figures 30-34, respectively.

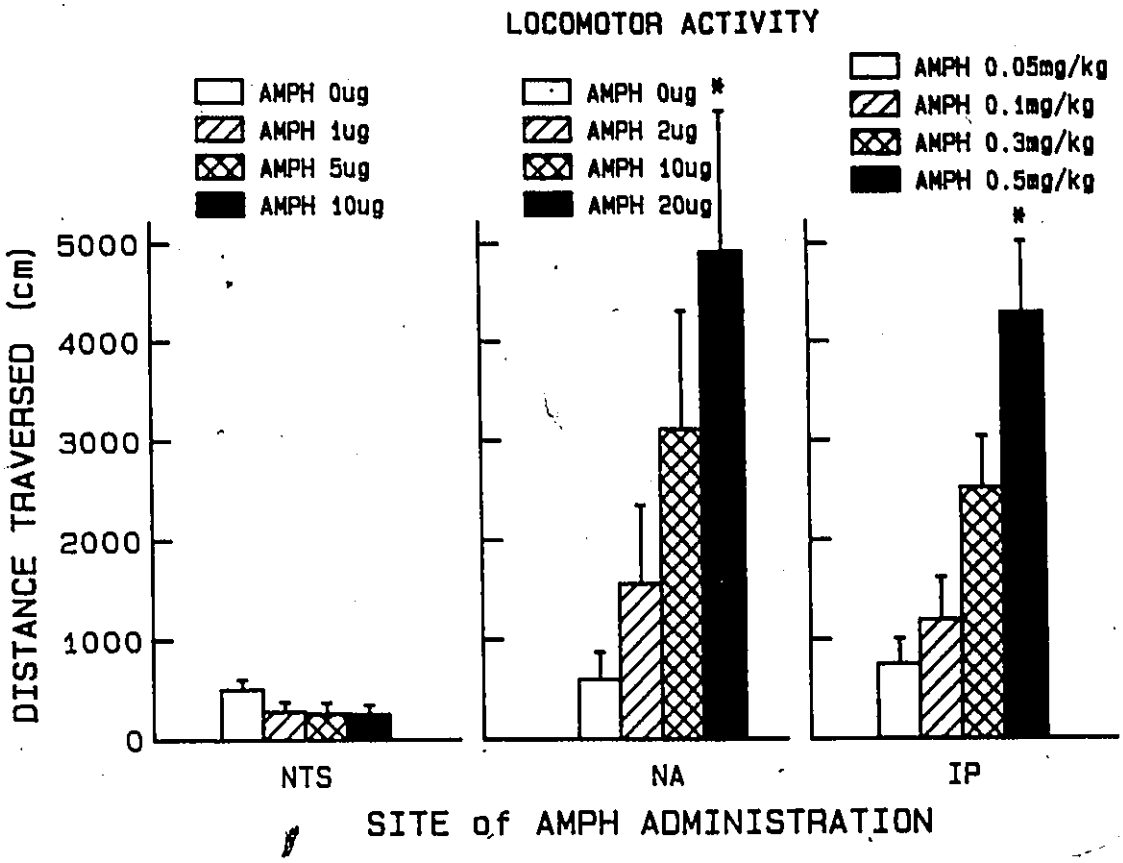

Figure 30: Effects of d-amphetamine (intra NTS, NA, & IP) on locomotor activity of rats in the home paradigm. On the ordinate: distance traversed (cm) over 60 min; on the abscissa: site of d-amphetamine administration: NTS (nucleus tractus solitarius), NA (nucleus accumbens), IP (peripheral). The profile of vehicle treated rats is represented by the open columns and those of d-amphetamine treated groups are represented by the patterned columns. Each value represents the mean of the animals in that group (n = 8) ± sem. *Significantly different with respect to the appropriate control value at $p<0.05$.

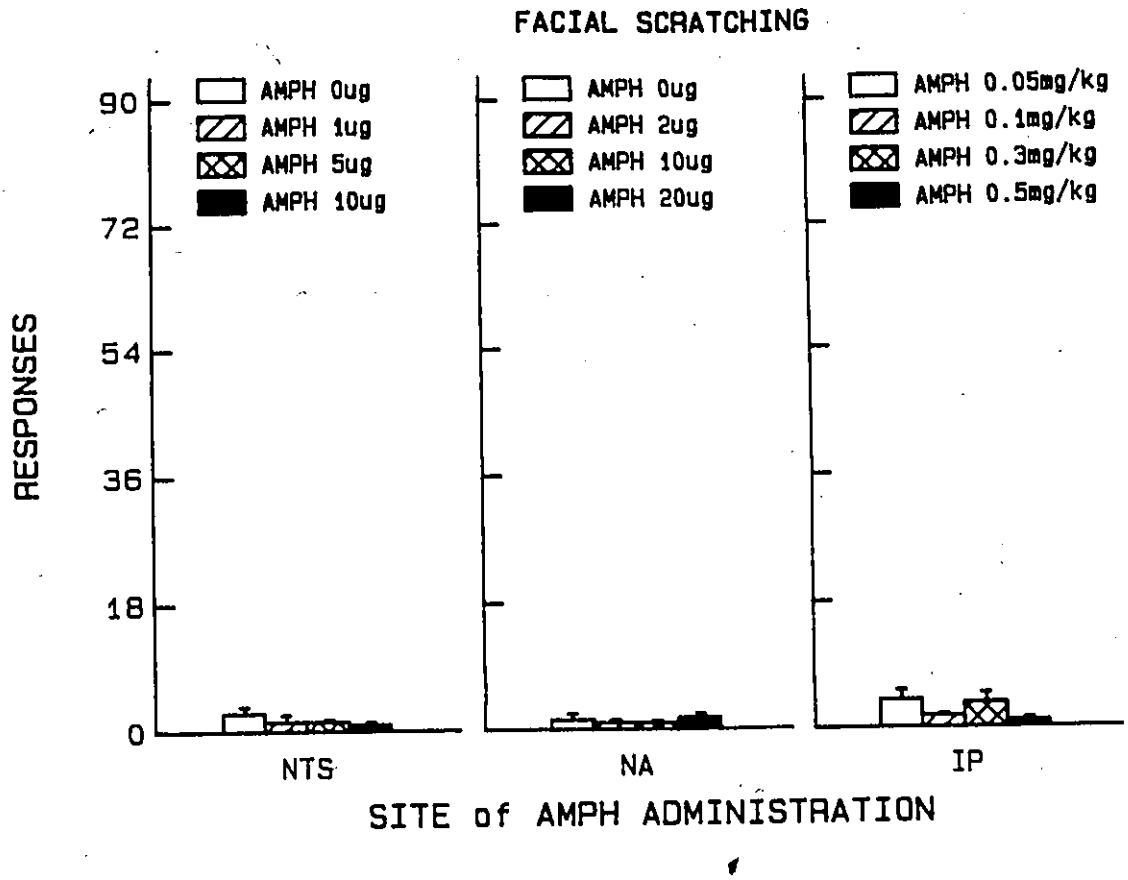

Figure 31: Effects of d-amphetamine (intra NTS, NA, & IP) on the frequency of facial scratching of rats in the home paradigm. On the ordinate: the magnitude of the quantified response over 60 min; on the abscissa: site of d-amphetamine administration: NTS (nucleus tractus solitarius), NA (nucleus accumbens), IP (peripheral). The profile of vehicle treated rats is represented by the open columns and those of d-amphetamine-treated groups are represented by the patterned columns. Each value represents the mean of the animals in that group ($n = 8$) $\pm$ sem. *Significantly different with respect to the appropriate control value at $p<0.05$.

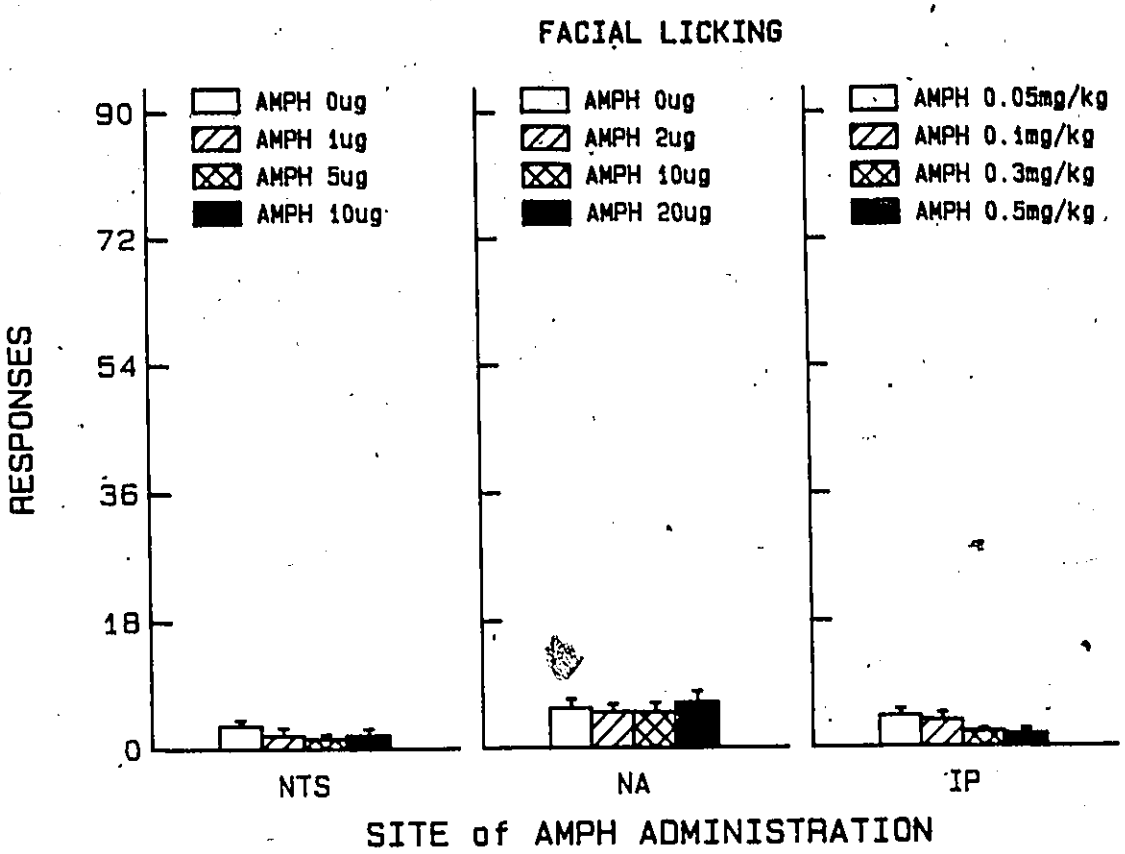

Figure 32: Effects of d-amphetamine (intra NTS, NA, & IP) on the frequency of facial licking of rats in the home paradigm. On the ordinate: the magnitude of the quantified response over 60 min; on the abscissa: site of d-amphetamine administration: NTS (nucleus tractus solitarius), NA (nucleus accumbens), IP (peripheral). The profile of vehicle treated rats is represented by the open columns and those of d-amphetamine-treated groups are represented by the patterned columns. Each value represents the mean of the animals in that group (n = 8) ± sem. *Significantly different with respect to the appropriate control value at $p<0.05$.

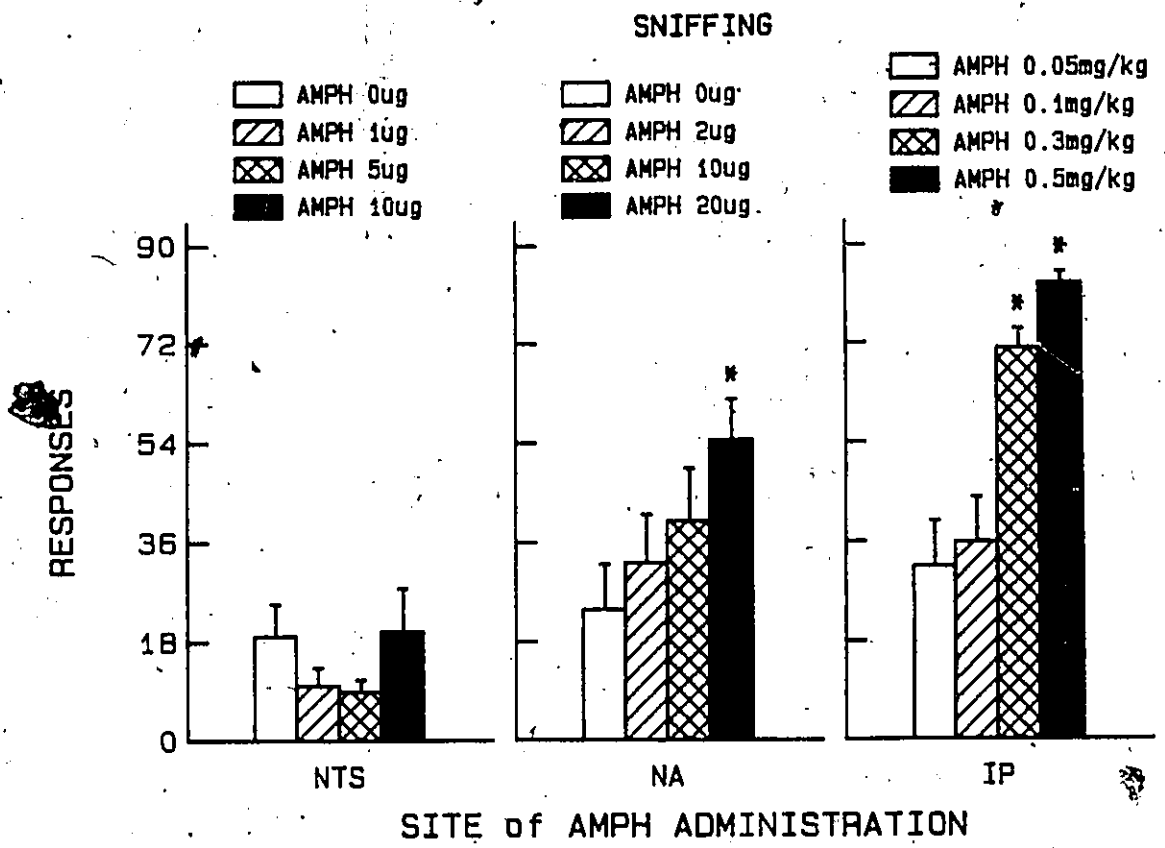

Figure 33: Effects of d-amphetamine (intra NTS, NA, & IP) on the frequency of sniffing of rats in the home paradigm. On the ordinate: the magnitude of the quantified response over 60 min; on the abscissa: site of d-amphetamine administration: NTS (nucleus tractus solitarius), NA (nucleus accumbens), IP (peripheral). The profile of vehicle treated rats is represented by the open columns and those of d-amphetamine-treated groups are represented by the patterned columns. Each value represents the mean of the animals in that group (n = 8) ± sem. *Significantly different with respect to the appropriate control value at $p<0.05$.

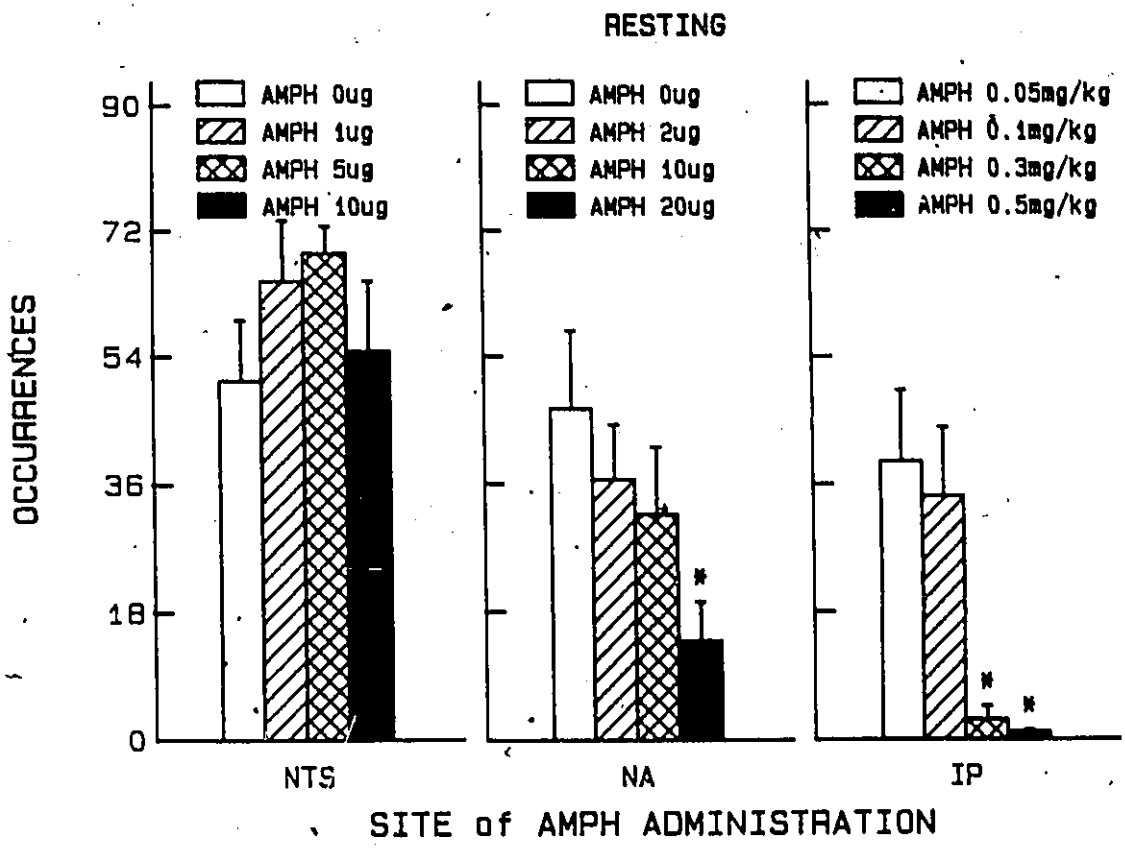

Figure 34. Effects of d-amphetamine (intra NTS, NA, & IP) on the occurrence of resting of rats in the home paradigm. On the ordinate: the magnitude of the quantified response over 60 min; on the abscissa: site of d-amphetamine administration: NTS (nucleus tractus solitarius), NA (nucleus accumbens), IP (peripheral). The profile of vehicle treated rats is represented by the open columns and those of d-amphetamine-treated groups are represented by the patterned columns. Each value represents the mean of the animals in that group (n = 8) ± sem. *Significantly different with respect to the appropriate control value at $p<0.05$.

Results Experiment 4.2

One way analysis of variance repeated over subjects, revealed a significant effect of drug treatment (NPY, 1, 0.1 ug; GRP 1-16, 2 ug; saline; or BN, 0.5 ug) when injected at the NTS, on locomotion $F(3,21) = 4.61$; floor activity $F(3,21) = 4.96$; rearing $F(3,21) = 9.44$; facial scratching $F(3,21) = 73.23$; facial licking $F(3,21) = 75.63$; body scratching $F(3,21) = 2.64$; body licking $F(3,21) = 2.87$; and resting $F(3,21) = 14.11$. Comparison of means revealed a significant increase in floor activity, rearing, facial scratching, facial licking and sniffing for the BN condition (0.5 ug) over the control. Neither NPY (1 ug) nor GRP 1-16 (2 ug) had a significant effect on any of the behaviors monitored in the home paradigm.

One way analysis of variance repeated over subjects revealed a significant effect of drug (saline; BN (0.5 ug); fluphenazine (10 ug) or BN (0.5 ug) + fluphenazine (10 ug) when injected at the NTS, for locomotion $F(3,21) = 4.15$; floor activity $F(3,21) = 9.76$; rearing $F(3,21) = 12.15$; facial scratching $F(3,21) = 57.48$; facial licking $F(3,21) = 36.98$; body licking $F(3,21) = 3.08$; sniffing $F(3,21) = 4.16$; and resting $F(3,21) = 45.85$. Comparison of means revealed that there was no significant difference between the BN condition and the BN + fluphenazine condition for any behavior tested. Furthermore the fluphenazine (10 ug) condition did not result in a significant difference over

the saline condition for any behavior tested. Significant effects in stimulation of behavior by BN and BN + fluphenazine as compared to the saline condition, were evident for floor activity, rearing, facial scratching, facial licking, and resting. The means for the dose-effect of the BN and fluphenazine administered alone and together, for locomotion, facial scratching and licking, sniffing and resting were illustrated in Figures 35-39, respectively.

One way analysis of variance repeated over subjects revealed a significant effect of treatment (saline; BN (0.5 ug); BN (0.5 ug) + spantide (1 ug); spantide(1 ug)) when injected at the NTS on locomotion $F(3,21) = 14.03$; floor activity $F(3,21) = 21.88$; rearing $F(3,21) = 16.87$; facial scratching $F(3,21) = 82.67$; facial licking $F(3,21) = 102.12$; body licking $F(3,21) = 6.81$; and resting $F(3,21) = 121.16$. Comparison of means revealed a significant difference between BN alone and BN + spantide for locomotion and floor activity only. Bombesin + spantide for locomotion and floor activity was not significantly different than the saline condition, however BN alone for locomotion and floor activity was significantly increased over the saline condition. In contrast, BN and BN + spantide were both significantly increased over the saline condition for facial scratching, facial licking and decreased for resting. Spantide alone when injected at the NTS was not different from the saline condition for any behavior monitored in the home paradigm.

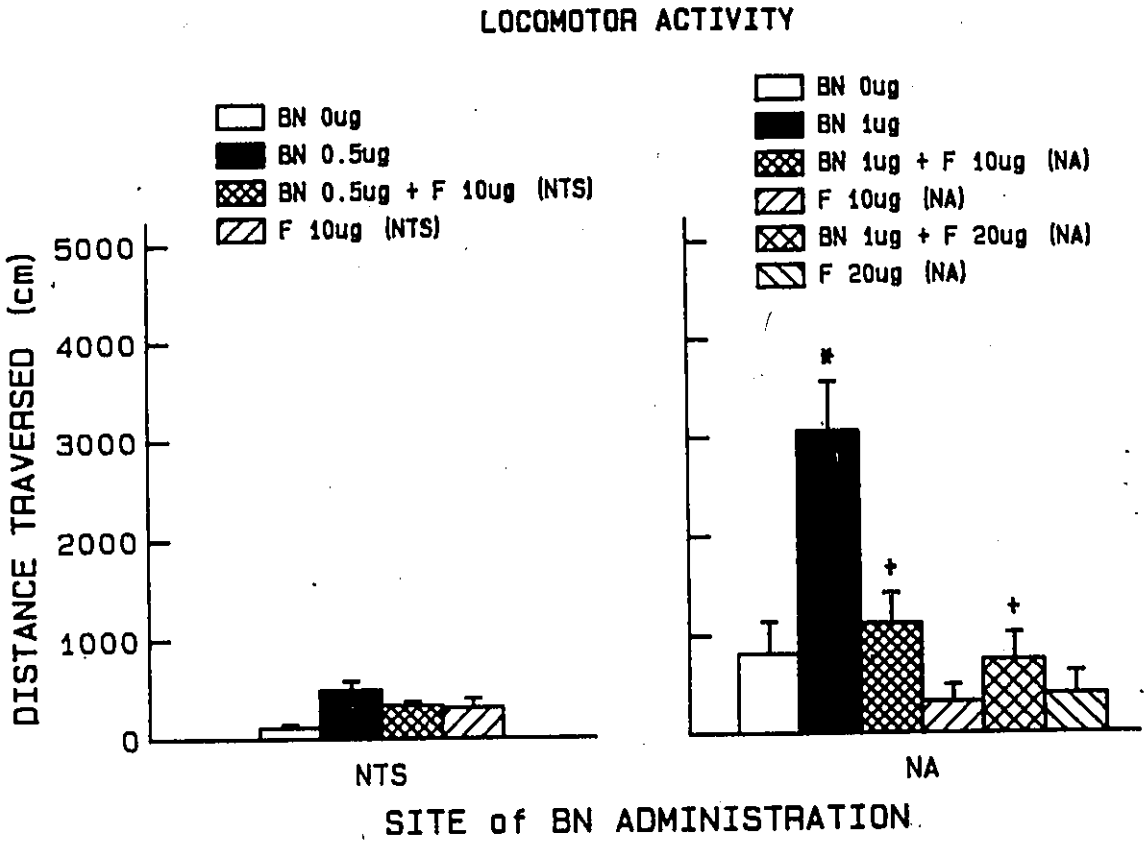

Figure 35: Effects of BN, fluphenazine and BN + fluphenazine on locomotor activity of rats in the home paradigm. On the ordinate: distance traversed (cm) over 60 min; on the abscissa: site of BN administration: NTS (nucleus tractus solitarius), NA (nucleus accumbens). The profile of vehicle treated rats is represented by the open columns and those of BN-alone treated groups are represented by the solid columns, and the fluphenazine alone, and fluphenazine + BN with the patterned columns. Each value represents the mean of the animals in that group (n = 8) ± sem. *Significantly different with respect to the appropriate control value at $p<0.05$. +Significantly different with respect to BN group at $p<0.05$.

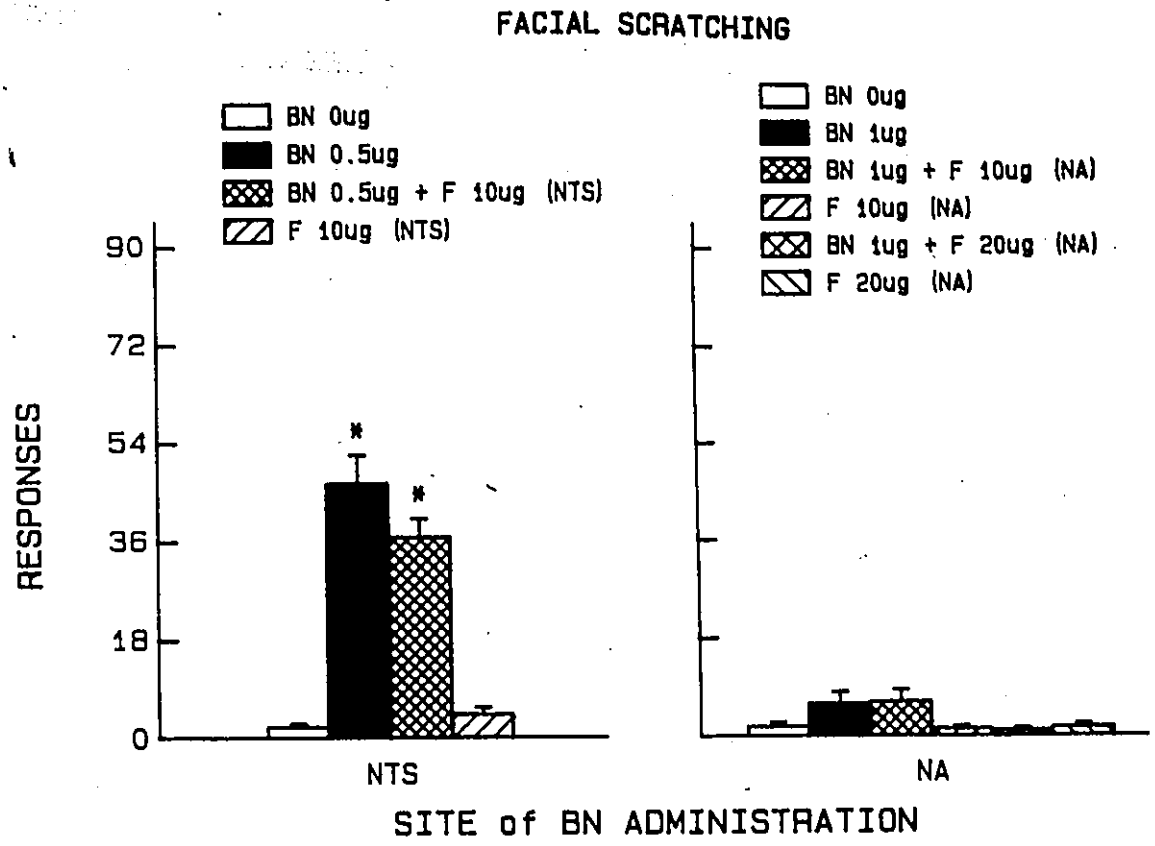

Figure 36: Effects of BN, fluphenazine and BN + fluphenazine on the frequency of facial scratching of rats in the home paradigm. On the ordinate: the frequency of response over 60 min; on the abscissa: site of BN administration: NTS (nucleus tractus solitarius), NA (nucleus accumbens). The profile of vehicle treated rats is represented by the open columns and those of BN-alone treated groups are represented by the solid columns, and the fluphenazine alone, and fluphenazine + BN with the patterned columns. Each value represents the mean of the animals in that group (n = 8) ± sem. *Significantly different with respect to the appropriate control value at $p<0.05$. +Significantly different with respect to BN group at $p<0.05$.

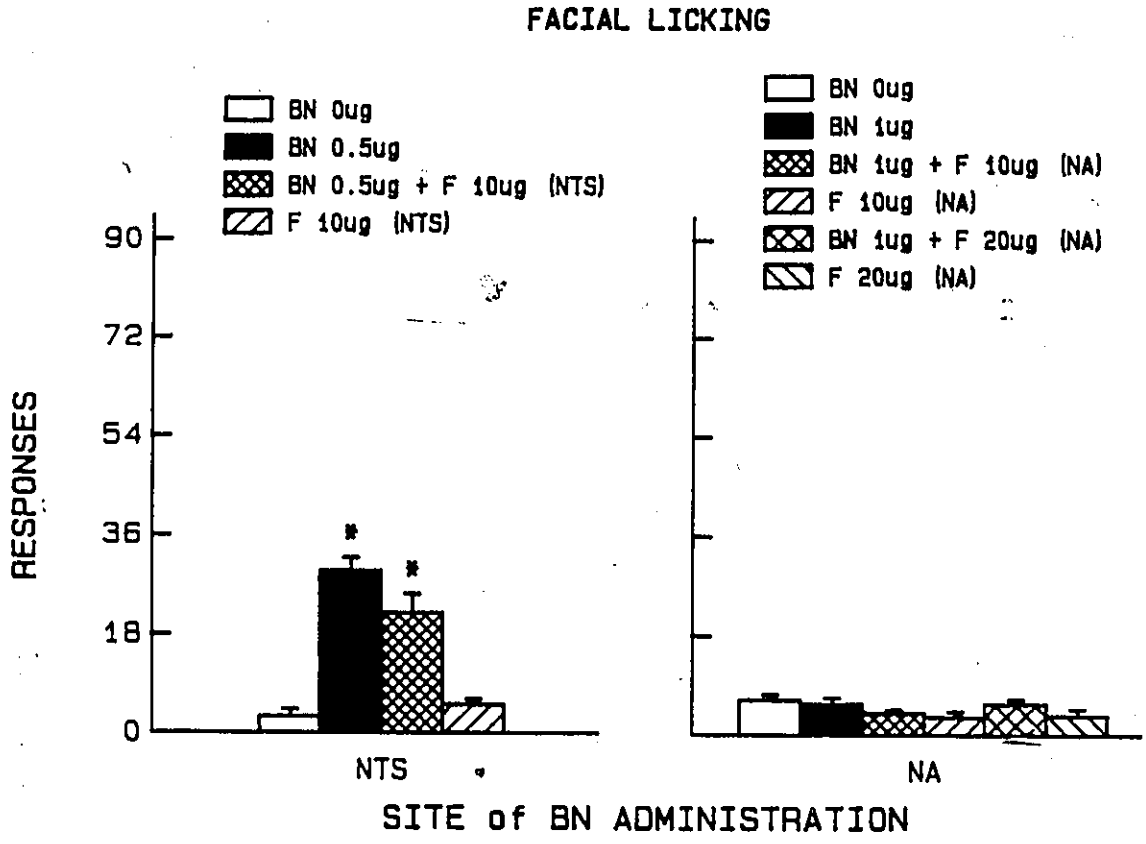

Figure 37: Effects of BN, fluphenazine and BN + fluphenazine on the frequency of facial licking of rats in the home paradigm. On the ordinate frequency of response over 60 min; on the abscissa: site of BN administration: NTS (nucleus tractus solitarius), NA (nucleus accumbens). The profile of vehicle treated rats is represented by the open columns and those of BN-alone treated groups are represented by the solid columns, and the fluphenazine alone, and fluphenazine + BN with the patterned columns. Each value represents the mean of the animals in that group (n = 8) $\pm$ sem. *Significantly different with respect to the appropriate control value at $p<0.05$. +Significantly different with respect to BN group at $p<0.05$.

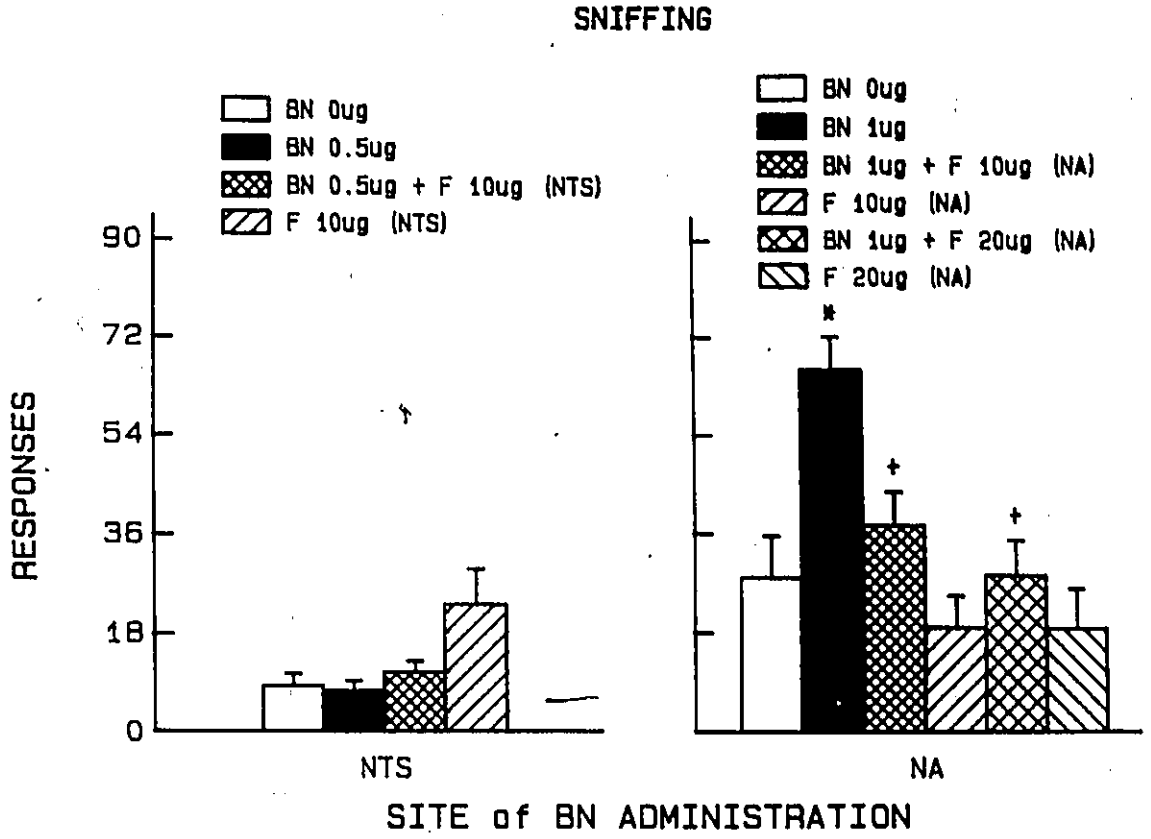

Figure 38: Effects of BN, fluphenazine and BN + fluphenazine on the frequency of sniffing of rats in the home paradigm. On the ordinate: the frequency of response over 60 min; on the abscissa: site of BN administration: NTS (nucleus tractus solitarius), NA (nucleus accumbens). The profile of vehicle treated rats is represented by the open columns and those of BN-alone treated groups are represented by the solid columns, and the fluphenazine alone, and fluphenazine + BN with the patterned columns. Each value represents the mean of the animals in that group (n = 8) ± sem. *Significantly different with respect to the appropriate control value at $p<0.05$. +Significantly different with respect to BN group at $p<0.05$.

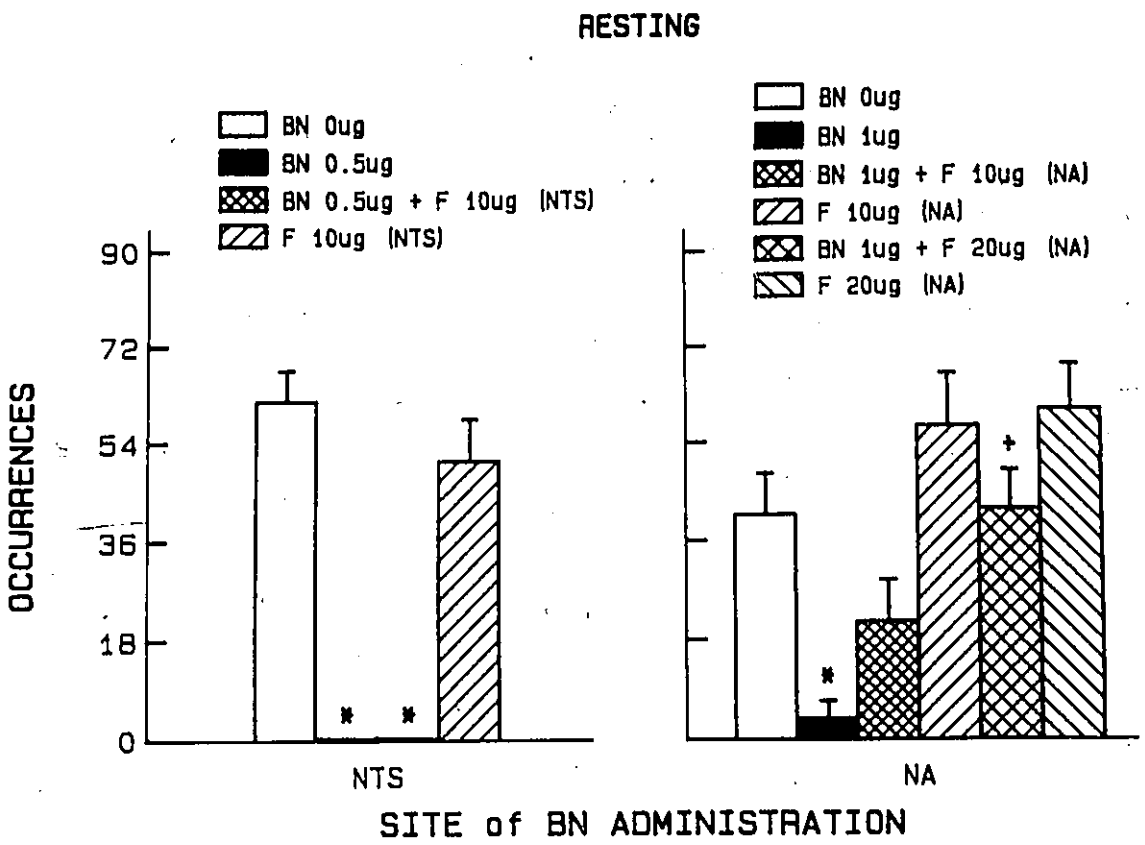

Figure 39: Effects of BN, fluphenazine and BN + fluphenazine on the occurrence of resting of rats in the home paradigm. On the ordinate: the frequency of response over 60 min; on the abscissa: site of BN administration: NTS (nucleus tractus solitarius), NA (nucleus accumbens). The profile of vehicle treated rats is represented by the open columns and those of BN-alone treated groups are represented by the solid columns, and the fluphenazine alone, and fluphenazine + BN with the patterned columns. Each value represents the mean of the animals in that group (n = 8) ± sem. *Significantly different with respect to the appropriate control value at $p<0.05$. +Significantly different with respect to BN group at $p<0.05$.

Experiments 4.3 and 4.4: The Behavioral Effects of Peptides and/or Drug Administration at the Nucleus Accumbens and/or Peripherally in the Home Paradigm.

Experiment 4.3

First, Experiment 3.3, designed to elucidate the time- and dose-related effects of BN-induced behavior when BN is administered at the NA, was replicated. Sixteen rats were bilaterally cannulated, two groups, n=8 per group. Each group was tested on alternate days. For group I, the doses of BN tested were 0, 0.001, 0.01, 0.1 or 1.0 ug. In addition, group I rats were injected, intra-NA with d-amphetamine (0, 2, 10, or 20 ug) (Pijnenburg and Van Rossum, 1973). Group II was injected with fluphenazine (0, 2, 10, or 20 ug); followed by, BN (1 ug), BN (1 ug) + fluphenazine (2 ug), BN (1 ug)+ fluphenazine (10 ug) or BN (1 ug) + fluphenazine (20 ug). Following this, 8 rats randomly chosen from group I and II were injected with haloperidol (0, 0.05, 0.1 or 0.5 mg/kg, IP). A Latin square design for drug administration was used, except for the BN + fluphenazine drugs, which were administered in a random order.

Experiment 4.4

Another set of 8 rats, (the triple implants from Experiment 4.2) were microinjected with the following drugs at the NA: neuropeptide Y (1 and 0.1 ug); GRP 1-16 (2 ug), saline, or BN (0.5 ug), in a random order. At the end of the experiment, BN (0.5 ug) + spantide (2 ug) was microinjected, followed 3 days later by spantide (2 ug) alone.

Results: Experiments 4.3 and 4.4: The Behavioral Effects of Peptides and/or Drug Administration at the Nucleus Accumbens and/or Peripherally in the Home Paradigm.

The implants of all animals used in the experiments were illustrated in Figures 40 and 41.

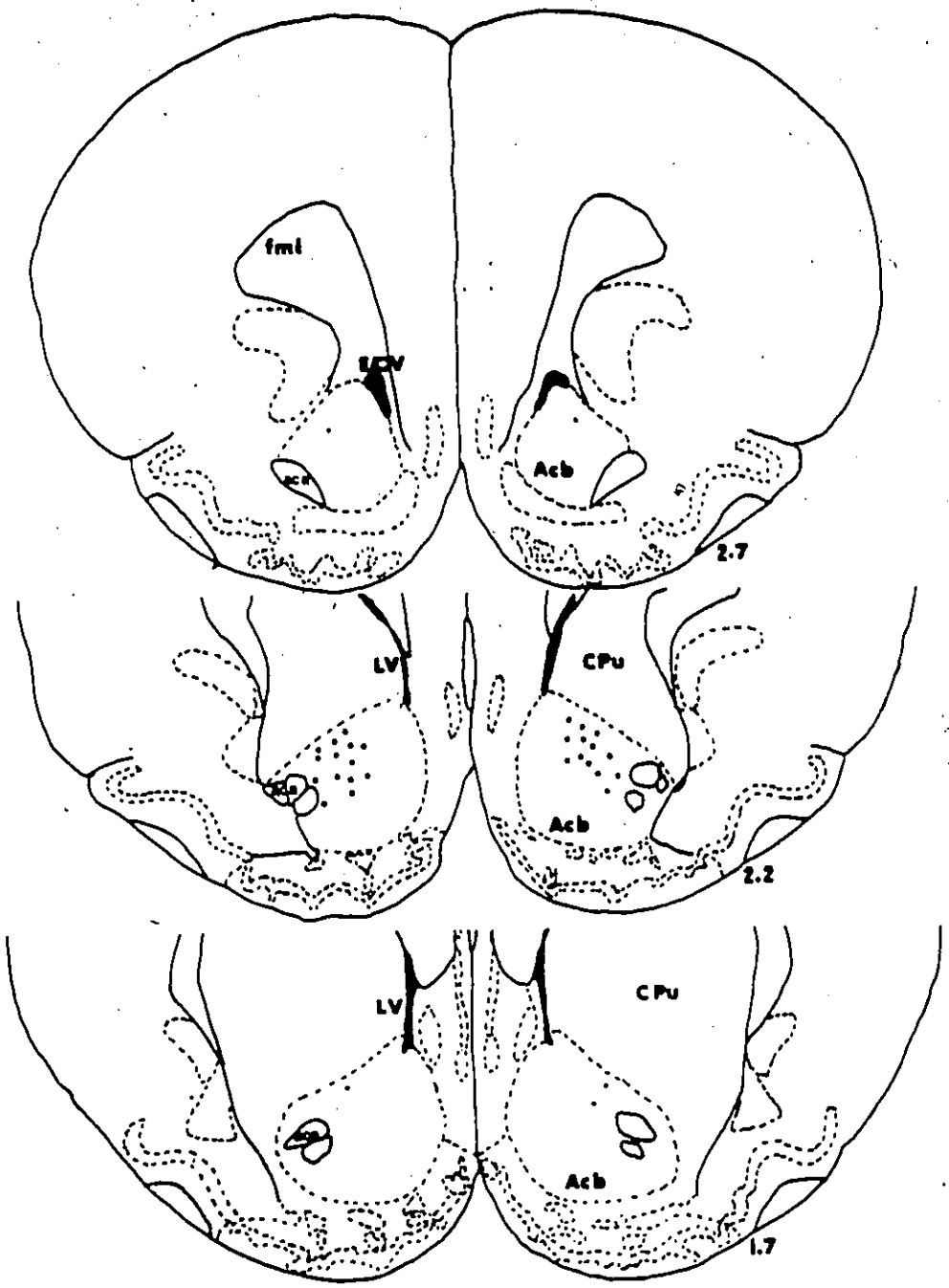

Figure 40: Verification of implantation sites after Experiment 4.3. Location of cannula implantation tip in the nucleus accumbens (NA) of the rat (n = 16) (coronal view: A-P 2.7 to 1.7 mm); (Paxinos and Watson, 1982). Abbreviations: . site of injection; aca anterior commissure; Acb nucleus accumbens; CPu caudate putamen; fmi forceps minor of the corpus callosum; LV lateral ventricle.

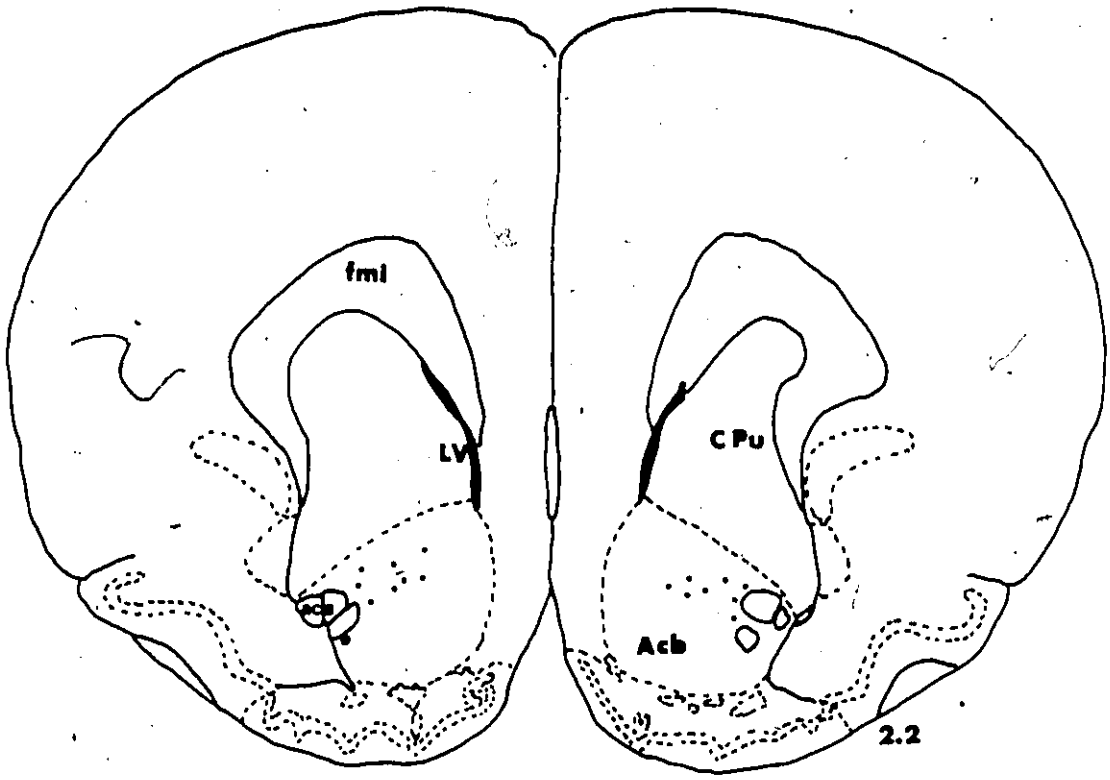

Figure 41: Verification of implantation sites after Experiment 4.4. Location of cannula implantation tip in the nucleus accumbens (NA) of the rat (n = 8) (coronal view: A-P 2.2 mm; Paxinos and Watson, 1982). Abbreviations: see Figure 40.

Results Experiment 4.3

One way analysis of variance repeated over subjects revealed a significant effect of dose of BN (0, 0.001, 0.01, 0.1, 1.0 ug) when administered at the NA, on locomotion $F(4,27) = 4.94$; floor activity $F(4,27) = 8.00$; rearing $F(4,27) = 6.38$; facial scratching $F(4,28) = 3.72$; facial licking $F(4,28) = 2.81$; sniffing $F(4,27) = 4.87$; and resting $F(4,27) = 6.75$. Comparison of means revealed a significant increase in locomotion, floor activity, rearing, and sniffing behaviors at a dose of 1 ug of BN over the saline condition; resting was significantly decreased at a dose of 1 ug BN over the saline condition. Doses lower than 1 ug BN did not significantly alter home paradigm behaviors. Results for locomotion, facial scratching, facial licking, sniffing and resting were illustrated in Figures 25-29, respectively.

One way analysis of variance repeated over subjects revealed a significant effect of dose of d-amphetamine (0, 2, 10, 20 ug) when administered at the NA, on locomotion $F(4,28) = 9.63$; floor activity $F(4,28) = 14.37$; rearing $F(4,28) = 11.72$; sniffing $F(4,28) = 6.47$; and resting $F(4,28) = 5.60$. Comparison of means revealed a significant increase in locomotion, floor activity, rearing and sniffing at a dose of 20 ug d-amphetamine only, with a simultaneous decrease in resting. Results for locomotion, facial scratching, facial licking, sniffing and resting were illustrated in Figures 30-34, respectively.

Fluphenazine (0, 2, 10, or 20 ug) microinjected at the NA did not have a significant effect on the home paradigm behaviors. However, one-way analysis of variance repeated over subjects revealed a significant effect of treatment (saline; BN 1 ug; BN 1 ug + fluphenazine 20 ug; BN 1 ug + fluphenazine 10 ug), when administered at the NA on locomotion $F(3,21) = 16.31$; floor activity $F(3,21) = 15.58$; rearing $F(3,21) = 6.84$; facial scratching $F(3,21) = 5.35$; body scratching $F(3,21) = 3.91$; sniffing $F(3,21) = 12.65$; and resting $F(3,21) = 10.67$. When comparing the means of the BN, and BN + fluphenazine conditions to the saline condition, only the BN conditions were significantly different. Bombesin significantly increased locomotion, floor activity, rearing, and sniffing and significantly decreased resting over the saline condition. Comparison of means revealed a significant decrease from the BN condition to the BN + fluphenazine condition (both the 20 and 10 ug fluphenazine + 1 ug BN conditions) for locomotion, floor activity, rearing and sniffing. However, there was a corresponding significant decrease in resting over the BN condition for only the BN 1 ug + fluphenazine 20 ug condition. Results for locomotion, facial scratching, facial licking, sniffing and resting were illustrated in Figures 35-39, respectively.

Results Experiment 4.4

One way analysis of variance repeated over subjects, revealed a significant effect of drug (NPY, 1, 0.1 ug; GRP 1-16, 2 ug; saline; or BN, 0.5 ug) when injected at the NA on locomotion $F(3,21) = 15.71$; floor activity $F(3,21) = 24.43$; rearing $F(3,21) = 23.71$; facial scratching $F(3,21) = 8.05$; body licking $F(3,21) = 3.51$; sniffing $F(3,21) = 37.80$; and resting $F(3,21) = 25.13$. Comparison of means revealed a significant increase in locomotion, floor activity, rearing, and sniffing for the BN condition (0.5 ug) over the control, as well as a corresponding decrease in resting for the BN condition. Neuropeptide Y (1 ug) had no significant effect on any of the home paradigm behaviors monitored. However, GRP 1-16 (2 ug) increased sniffing and decreased resting over the saline condition. Surprisingly, there was no difference between BN 1ug and GRP 1-16 2 ug for sniffing and resting behaviors.

One way analysis of variance repeated over subjects revealed a significant effect of drug (saline; 0.5 ug BN; 0.5ug BN + 2 ug spantide; 2 ug spantide) when injected at the NA on locomotion, $F(3,21) = 14.20$; floor activity $F(3,21) = 28.93$; rearing $F(3,21) = 23.95$; sniffing $F(3,21) = 51.58$ and resting $F(3,21) = 29.55$. Comparison of means revealed a significant difference between BN alone and BN + spantide for sniffing only. The effects of BN + spantide and BN alone, significantly increased locomotion, floor

activity, rearing, and sniffing over the saline condition; and decreased resting. Spantide alone, when injected at the NA, was not different from the saline condition for any behavior tested in the home paradigm.

Experiments 4.5 and 4.6: The Behavioral Effects of Peptides and\or Drugs Administration at Nucleus Tractus Solitarius and\or Peripherally in the Satiety Paradigm.

Experiment 4.5

Ten animals cannulated at the NTS (as described above ie single implants) received BN (0, 0.0001, 0.001, 0.01, or 0.1 ug). Following this, the rats received BN (0, 1, 2, 4, or 8 ug/kg; IP). In a third part of the experiment, rats were injected with fluphenazine (0, 0.05, 0.1 or 0.5 mg/kg; IP), haloperidol (0, 0.05, 0.1, or 0.5 mg/kg; IP), or d-amphetamine (0, 0.1, 0.3 or 0.5 mg/kg; IP). A Latin square design for all drug administration was used.

Experiment 4.6

Twelve animals cannulated at the NTS (and the NA, triple implants, also used in Experiment 4.8) received intra NTS, neuropeptide Y (1 ug); GRP 1-16 (2 ug); fluphenazine (10 ug); fluphenazine (10 ug) + BN (0.5 ug), BN (0.5 ug), or saline (n=12), in a randomized sequence. In addition, the

rats were divided into two groups (n=6) and each group was randomly chosen to receive fluphenazine (0.5 mg/kg, IP) + BN (0.5 ug intra NTS), 10 min later; fluphenazine (10 ug, intra NA) + BN (0.5 ug, intra NTS); neuromedin C (Peninsula) (0.35 ug), or neuromedin B (Peninsula) (0.35 ug). Finally, at the end of the experiment one group was randomly chosen to receive spantide, (1 ug, intra NTS), followed seven days later, by spantide (1 ug) + BN (0.5 ug), coadministered at the NTS.

Results: Experiments 4.5 and 4.6: The Behavioral Effects of Peptides and\or Drugs Administration at Nucleus Tractus Solitarius and\or Peripherally in the Satiety Paradigm.

The implants of all animals used in the experiments were illustrated in Figures 42 and 43.

Results Experiment 4.5

One way analysis of variance repeated over dose revealed a significant effect of BN intra NTS, on latency to eat $F(4,36) = 17.88$; duration of eating $F(4,36) = 9.23$; pellets left $F(4,36) = 17.77$; facial scratching $F(4,36) = 12.73$; facial licking $F(4,36) = 24.05$; body licking $F(4,36) = 12.25$; sniffing $F(4,36) = 7.73$; and chewing, $F(4,36) = 16.68$. Comparison of means demonstrated a significant increase over control conditions for latency to eat, and

number of pellets left, at a dose of 0.01 ug and higher. Duration of eating, sniffing and chewing were significantly decreased over the control condition at a dose of 0.1 ug BN, only. On the other hand facial scratching and licking, body licking, were significantly increased at the 0.1 ug dose. Results for latency to eat, pellets left, facial scratching, facial licking, and sniffing were illustrated in Figures 44-48, respectively.

Two way analysis of variance repeated over time and dose (for time-course dose-effect, Figures 49-52) demonstrated a significant interaction of time and dose of BN (intra NTS) on latency to eat $F(12,108) = 2.74$; pellets left $F= (12,108) = 2.95$; and only a main effect on dose of BN for facial scratching $F(12,108) = 12.73$; and facial licking $F(12,108) = 24.05$. Comparison of interaction means demonstrated a significant increase over control conditions for latency to eat and pellets left for BN (0.0001 ug and greater) at 20 min after injection. At 15 and 10 min after injection of BN there was a significant difference on BN at dose of 0.01 and 0.1 ug; at 5 min after injection of BN only the 0.1 ug dose demonstrated a significant increase over control conditions for latency to eat and pellets left as illustrated in Figures 49 and 50. Comparison of interaction means demonstrated a significant increase over control conditions for facial scratching at a dose of 0.01 ug BN at 10, 15 and 20 min and facial licking, at 15 min only. At a dose of 0.1 ug BN both facial scratching and licking showed

a significant increase at all time periods as illustrated in Figures 51 and 52

One way analysis of variance repeated over dose demonstrated a significant effect of BN, (IP), on latency to eat $F(4,36) = 21.03$; duration of eating $F(4,36) = 4.54$; pellets left $F(4,36) = 21.34$; chewing $F(4,36) = 11.95$; and licking $F(4,36) = 3.13$. Comparison of means revealed a significant increase over the saline condition of latency to eat, and pellets left at a dose of 2 ug BN or higher. Chewing was significantly decreased at a dose of 4 ug BN or higher. Grooming scores in the satiety paradigm were not significantly effected by peripherally administered BN. Results for latency to eat, pellets left, facial scratching, facial licking, and sniffing were illustrated in Figures 44-48, respectively.

Two way analysis of variance repeated over time and dose (for time-course dose-effect, Figures 49-52) demonstrated a significant interaction of time and dose of BN (IP) on latency to eat $F(12,108) = 3.54$; pellets left $F(12,108) = 4.34$. Neither facial scratching nor facial licking were significant on time, dose or interaction. Comparison of interaction means demonstrated a significant increase over control conditions for latency to eat and pellets left for BN (1.0 ug and greater) at 20 min after injection. At 15 min after injection of BN there was a significant difference at a dose of 2.0 ug and greater; At 10 min after injection of BN there was a significant

difference at a dose of 4.0 ug and greater as illustrated in Figures 49 and 50.

One way analysis of variance repeated over dose of fluphenazine (0, 0.05, 0.1, 0.5 mg/kg; IP) demonstrated no significant effects of any dose of fluphenazine in comparison to the saline condition for any behavior measured in the satiety paradigm.

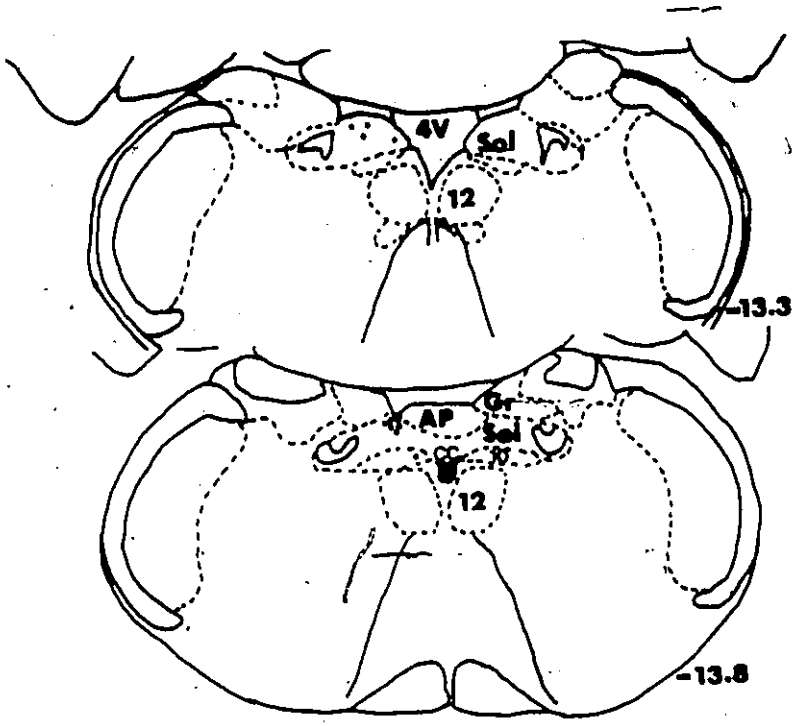

Figure 42: Verification of implantation sites after Experiment 4.5. Location of cannula implantation tip in the caudal nucleus tractus solitarius (NTS) of the rat (n = 10) (coronal view: A-P -13.3 to -13.8 mm; Paxinos and Watson, 1982). Abbreviations: . site of injection; AP area postrema; Cu cuneate nucleus; Gr gracile nucleus; In intercalated nucleus; 4V fourth ventricle; 12 nucleus of hypoglossal nerve; 10 dorsal motor nucleus of the vagus; mlf medial longitudinal fasciculus; MVe medial vestibular nucleus.

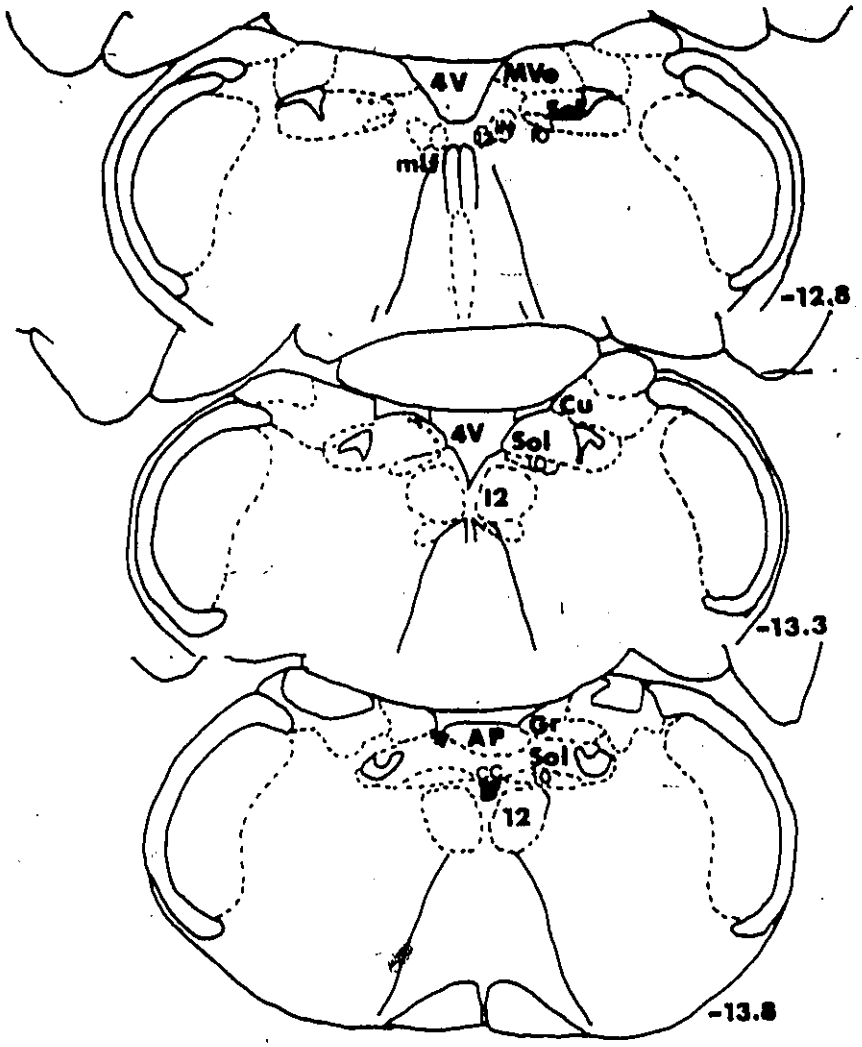

Figure 43: Verification of implantation sites after Experiment 4.6. Location of cannula implantation tip in the caudal nucleus tractus solitarius (NTS) of the rat (n = 12) coronal view: (A-P -12.8 to -13.8 mm; Paxinos and Watson, 1982). Abbreviations: see Figure 42.

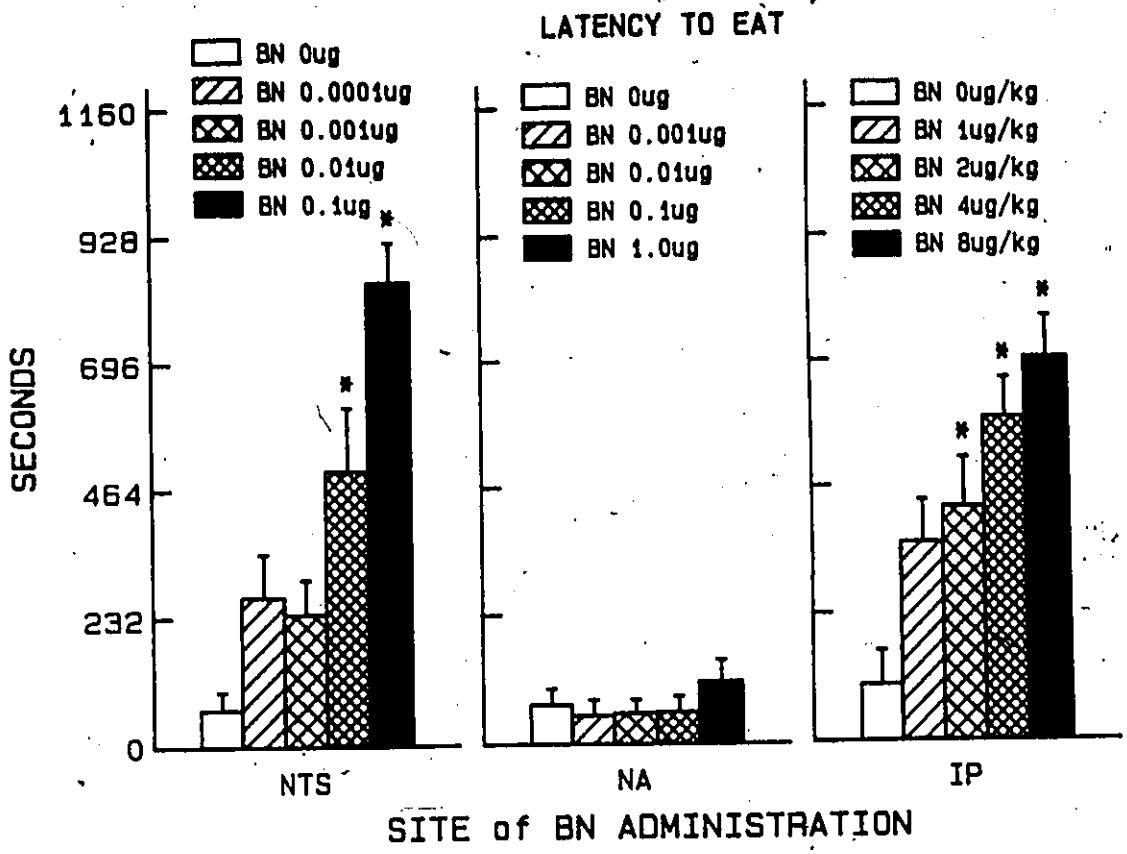

Figure 44: Effects of BN on the latency to eat in the satiety paradigm. On the ordinate seconds of latency to eat, over 20 min; on the abscissa: site of BN administration: NTS (nucleus tractus solitarius, NA (nucleus accumbens), IP (peripheral). The profile of vehicle treated rats is represented by the open columns and those of BN treated groups are represented by the patterned columns. Each value represents the mean ± sem of the animals in the respective groups (n = 10). *Significantly different with respect to the appropriate control value (open columns) at $p<0.05$.

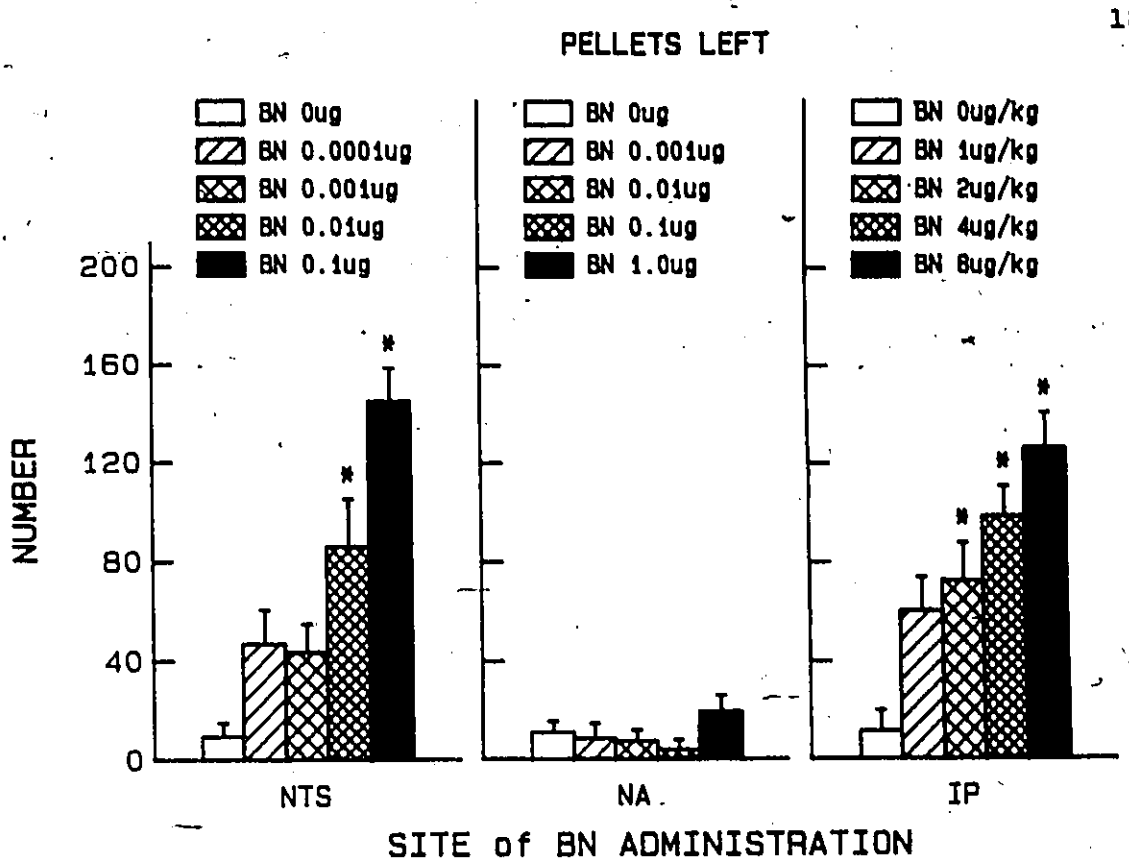

Figure 45: Effects of BN on the number of pellets left uneaten, in the satiety paradigm. On the ordinate number of pellets left, over 20 min; on the abscissa: site of BN administration: NTS (nucleus tractus solitarius, NA (nucleus accumbens), IP (peripheral). The profile of vehicle treated rats is represented by the open columns and those of BN-treated groups are represented by the patterned columns. Each value represents the mean ± sem of the animals in the respective groups (n = 10). *Significantly different with respect to the appropriate control value (open columns) at $p<0.05$.

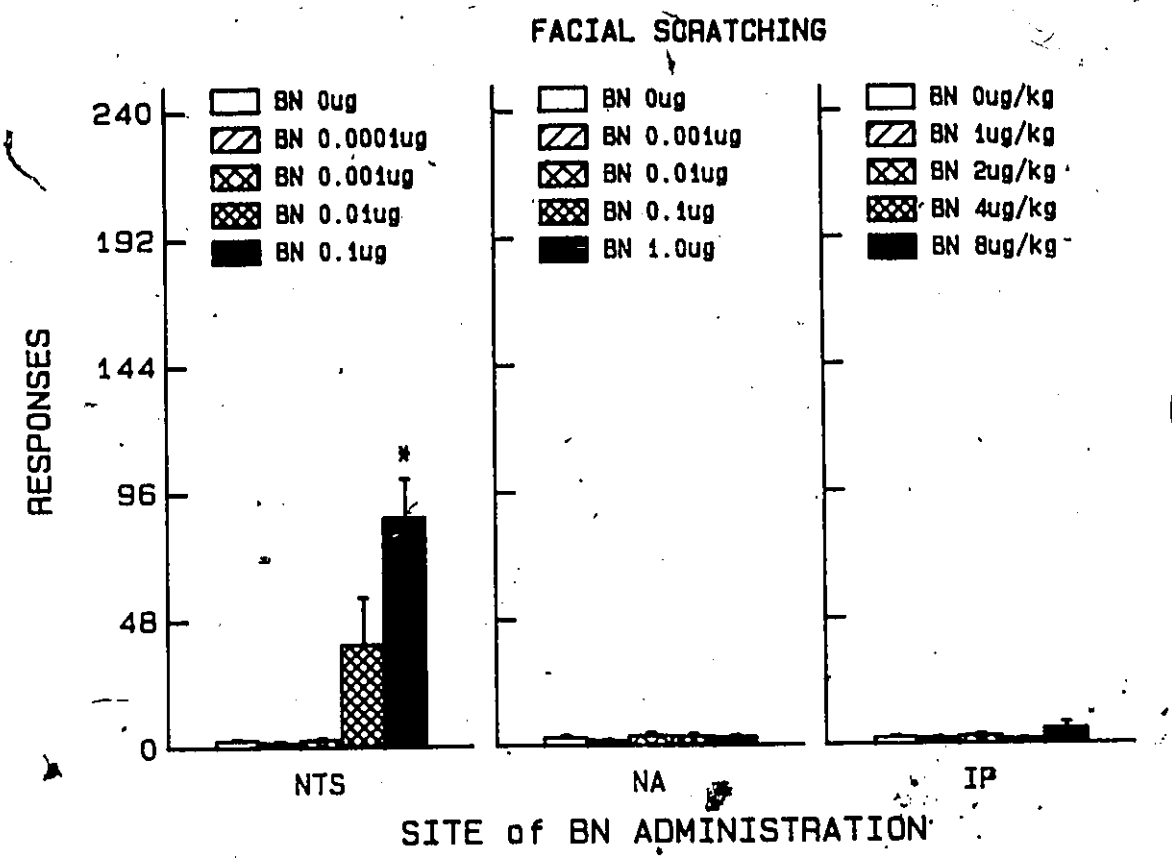

Figure 46: Effects of BN on the frequency of facial scratching, in the satiety paradigm. On the ordinate the frequency of responses, over 20 min; on the abscissa: site of BN administration: NTS (nucleus tractus solitarius, NA (nucleus accumbens), IP (peripheral). The profile of vehicle treated rats is represented by the open columns and those of BN-treated groups are represented by the patterned columns. Each value represents the mean ± sem of the animals in the respective groups (n = 10). *Significantly different with respect to the appropriate control value (open columns) at $p<0.05$.

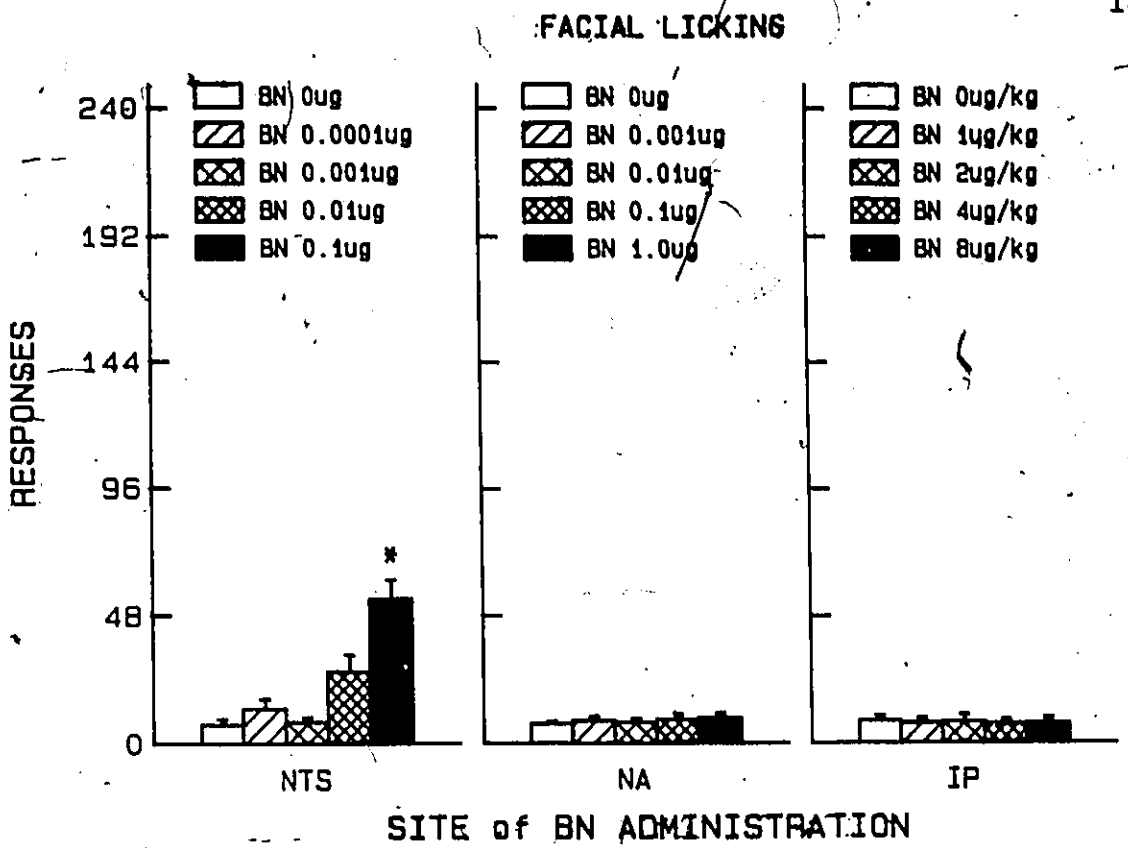

Figure 47: Effects of BN on the frequency of facial licking, in the satiety paradigm. On the ordinate: the frequency of responses, over 20 min; on the abscissa: site of BN administration: NTS (nucleus tractus solitarius, NA (nucleus accumbens), IP (peripheral). The profile of vehicle treated rats is represented by the open columns and those of BN-treated groups are represented by the patterned columns. Each value represents the mean ± sem of the animals in the respective groups (n = 10). *Significantly different with respect to the appropriate control value (open columns) at $p<0.05$.

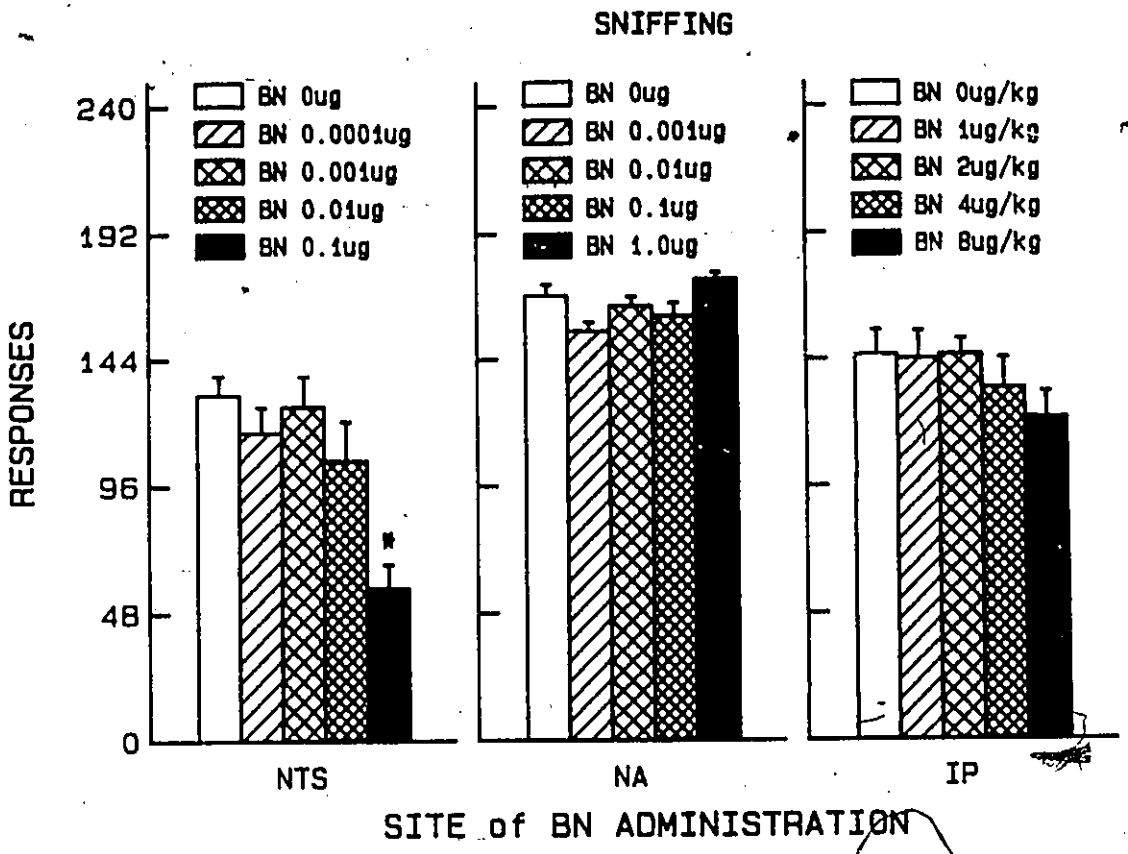

Figure 48: Effects of BN on the frequency of sniffing, in the satiety paradigm. On the ordinate: the frequency of responses over 20 min; on the abscissa: site of BN administration: NTS (nucleus tractus solitarius, NA (nucleus accumbens), IP (peripheral). The profile of vehicle treated rats is represented by the open columns and those of BN treated groups are represented by the patterned columns. Each value represents the mean ± sem of the animals in the respective groups (n = 10). *Significantly different with respect to the appropriate control value (open columns) at $p<0.05$.

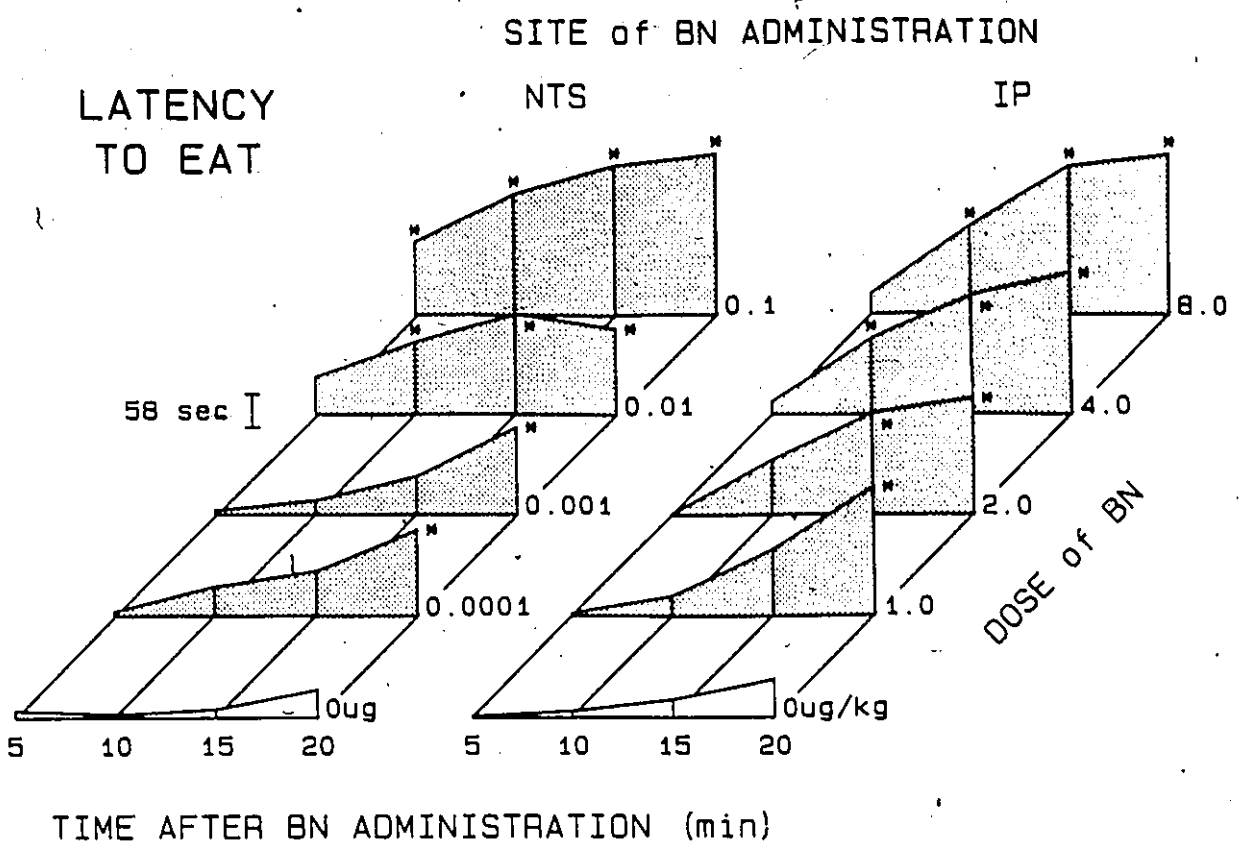

Figure 49: Comparison of time- and dose-related effects of BN on latency to eat of rats, in the satiety paradigm, after BN administered either at the NTS or IP. Tridimensional figure: On the ordinate: the magnitude of the quantified response (mean for 5 min.); on the horizontal abscissa: time after BN administration (min); on the oblique abscissa: dose of BN administered (ug). The profile of vehicle-treated controls is represented by the unshaded area and those of BN-treated groups are represented by the subsequent shaded areas. For each session, n = 10. *Significantly different with respect to the appropriate control value at $p<0.05$.

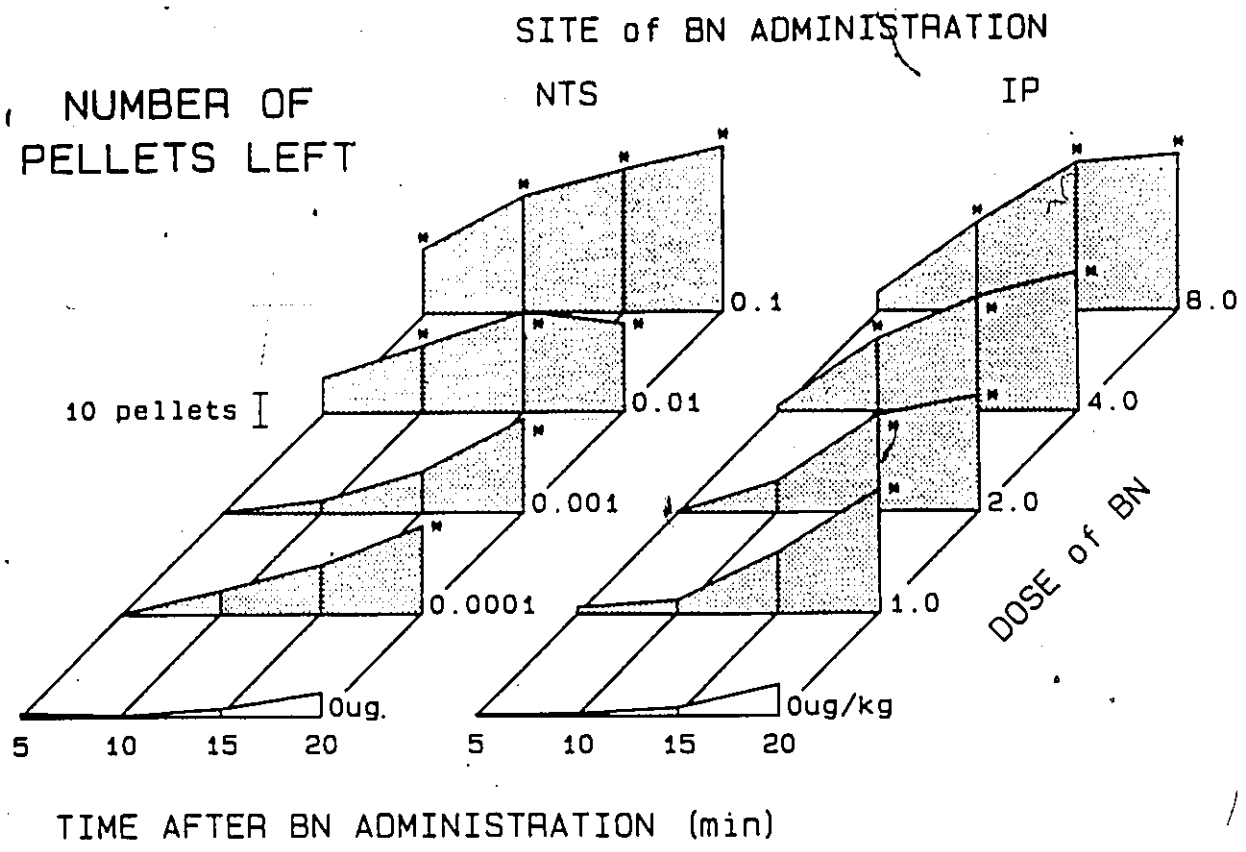

Figure 50: Comparison of time- and dose-related effects of BN on number of pellets left uneaten of rats, in the satiety paradigm, after BN administered either at the NTS or IP. Tridimensional figure: On the ordinate: the magnitude of the quantified response (mean for 5 min.); on the horizontal abscissa: time after BN administration (min); on the oblique abscissa: dose of BN administered (ug). The profile of vehicle-treated controls is represented by the unshaded area and those of BN-treated groups are represented by the subsequent shaded areas. For each session, n = 10. *Significantly different with respect to the appropriate control value at $p<0.05$.

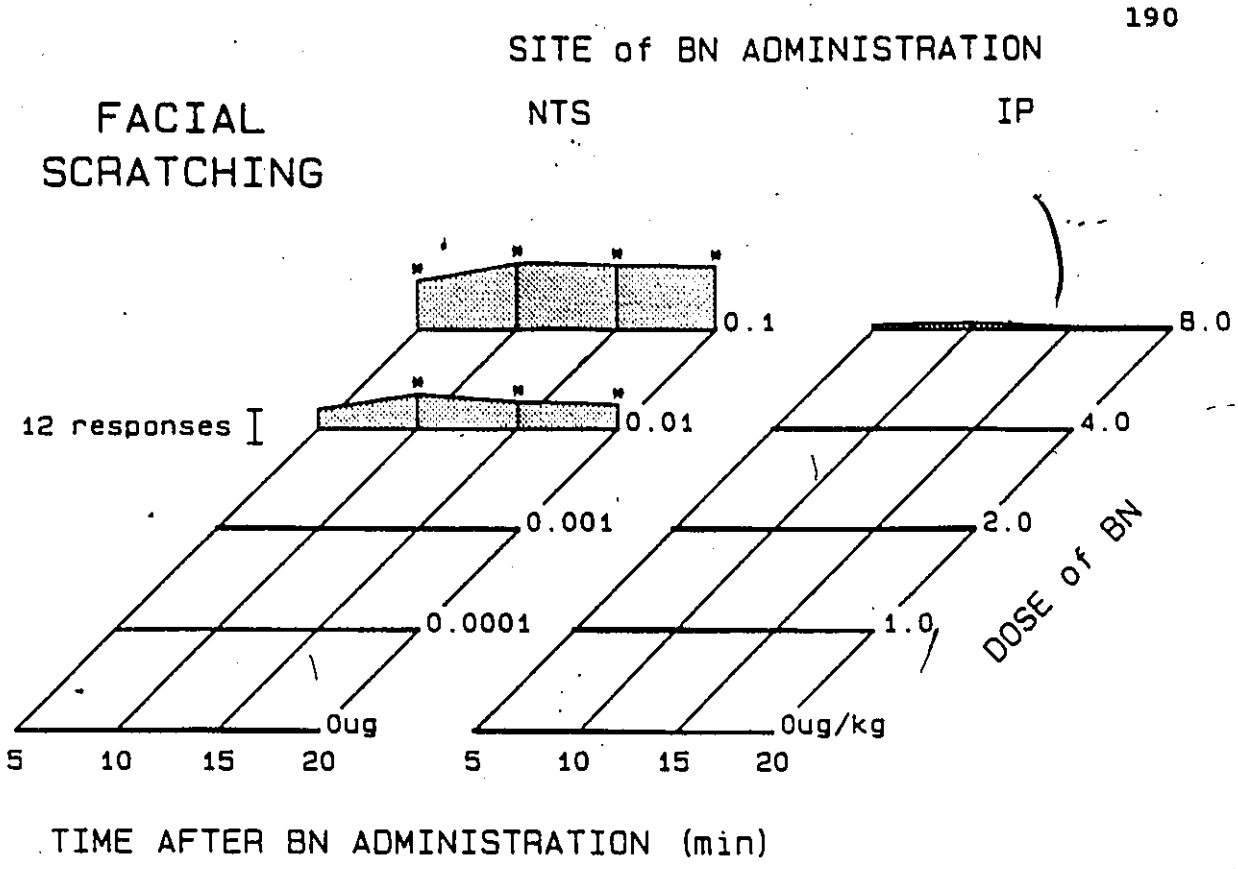

Figure 51: Comparison of time- and dose-related effects of BN on the facial scratching of rats, in the satiety paradigm, after BN administered either at the NTS or IP. Tridimensional figure: On the ordinate: the magnitude of the quantified response (mean for 5 min.); on the horizontal abscissa: time after BN administration (min); on the oblique abscissa: dose of BN administered (ug). The profile of vehicle-treated controls is represented by the unshaded area and those of BN-treated groups are represented by the subsequent shaded areas. For each session, n = 10. *Significantly different with respect to the appropriate control value at $p<0.05$.

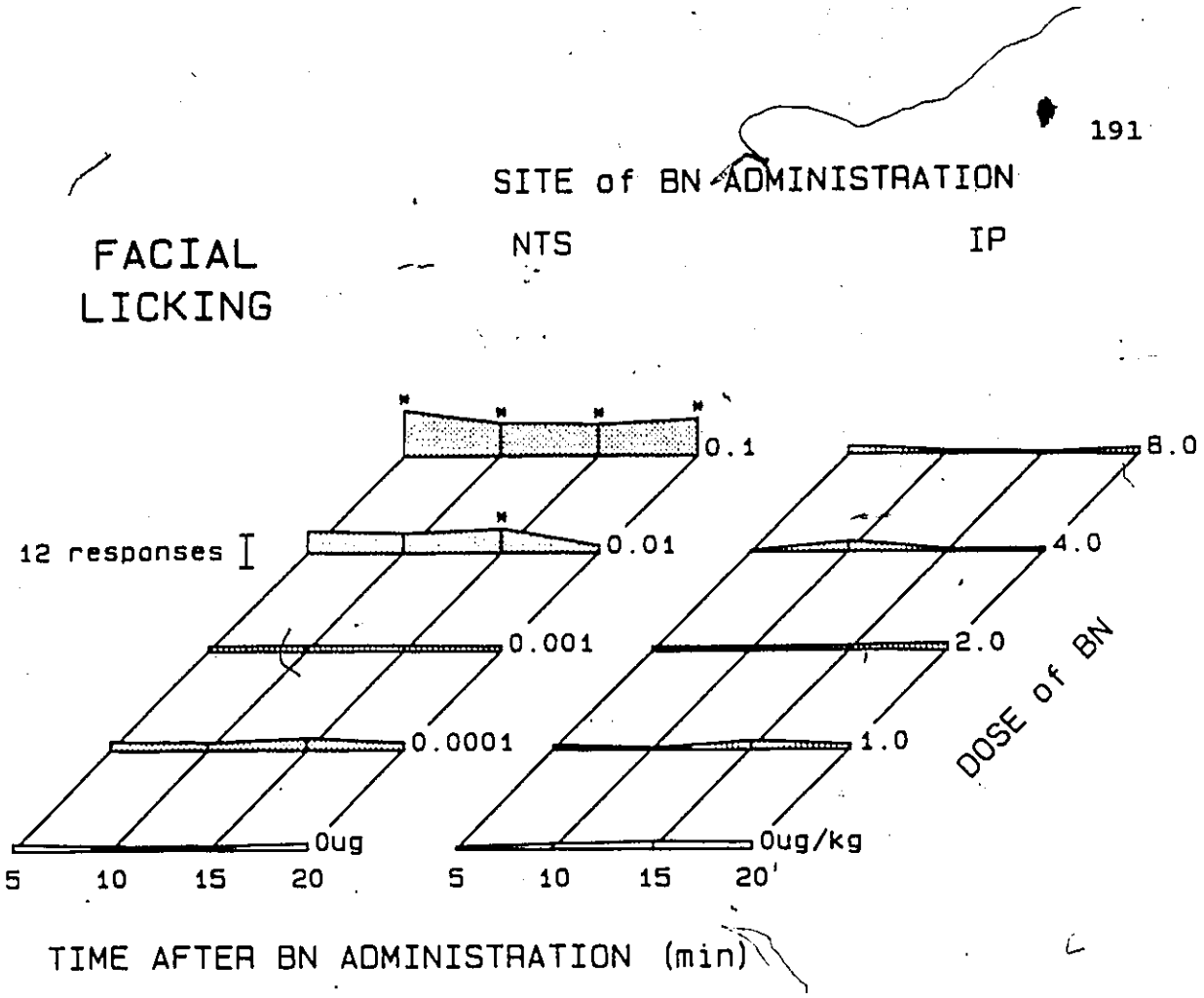

Figure 52: Comparison of time- and dose-related effects of BN on the facial licking of rats, in the satiety paradigm, after BN administered either at the NTS or IP.

Tridimensional figure: On the ordinate: the magnitude of the quantified response (mean for 5 min.); on the horizontal abscissa: time after BN administration (min); on the oblique abscissa: dose of BN administered (ug). The profile of vehicle-treated controls is represented by the unshaded area and those of BN-treated groups are represented by the subsequent shaded areas. For each session, n = 10.

*Significantly different with respect to the appropriate control value at $p<0.05$.

Similar analysis of variance repeated over dose of d-amphetamine (0.05, 0.1, 0.3, 0.5 mg/kg; IP) revealed a significant increase in latency to eat $F(3,24) = 6.86$; pellets left $F(3,24) = 8.35$; sniffing $F(3,24) = 8.59$; and rearing $F(3,24) = 7.72$; and a significant decrease in chewing $F(3,24) = 7.02$. Comparison of means demonstrated a significant difference between the saline condition and 0.5 mg/kg d-amphetamine for latency to eat, pellets left, sniffing and rearing only.

Analysis of variance demonstrated a significant effect of dose of haloperidol (0, 0.05, 0.1, 0.5 mg/kg, IP) on latency to eat $F(3,27) = 54.13$; duration of eating $F(3,27) = 11.07$; pellets left $F(3,27) = 37.98$; facial scratching $F(3,27) = 4.15$; facial licking $F(3,27) = 9.18$; sniffing $F(3,27) = 10.24$; chewing $F(3,27) = 32.01$; and licking, $F(3,27) = 8.72$. Comparison of means revealed a significant increase over the saline condition for the haloperidol at a dose of 0.5 mg/kg for latency to eat and pellets left; similarly, there was also a significant decrease in duration of eating, facial scratching, facial licking, sniffing, chewing and licking. The means for the dose effect of d-amphetamine, fluphenazine and haloperidol (IP), for latency to eat and pellets left uneaten, facial scratching and licking, and sniffing, were illustrated in Figures 53-57.

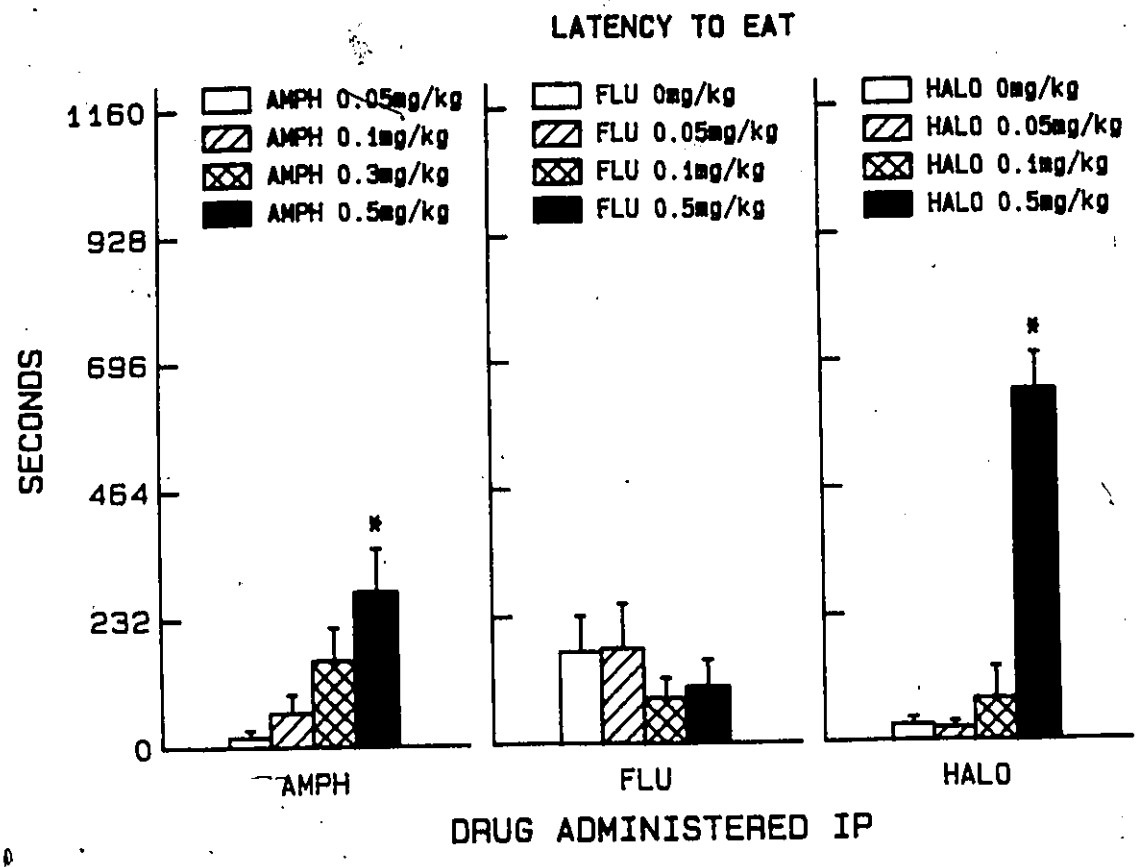

Figure 53: Effects of d-amphetamine, fluphenazine, or haloperidol (IP) on the latency to eat, in the satiety paradigm. On the ordinate seconds of latency to eat, over 20 min; on the abscissa: drug administered IP (peripherally): AMPH (d-amphetamine), FLU (fluphenazine), HALO (haloperidol). The profile of vehicle-treated controls are represented by the open columns and those of drug-treated groups are represented by the patterned columns. Each value represents the mean ± sem of the animals in the respective groups (n = 12). *Significantly different with respect to the appropriate control value (open columns) at $p<0.05$.

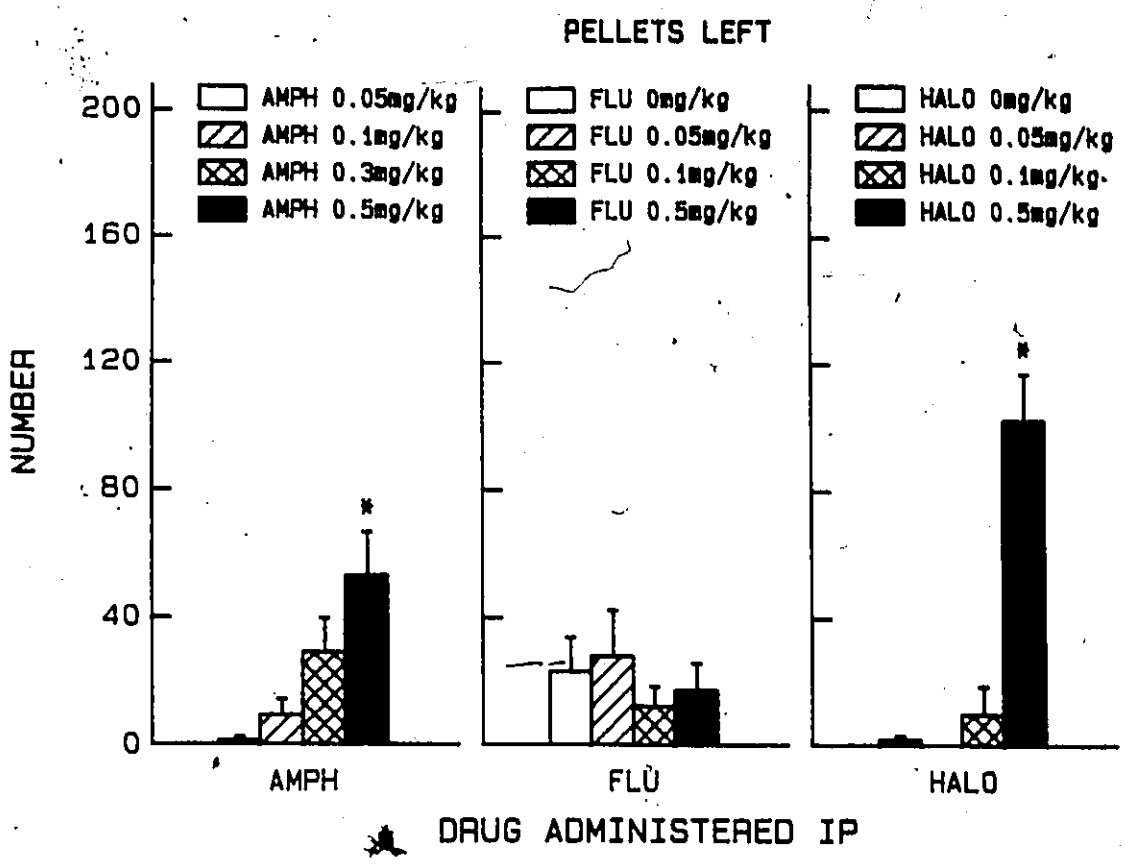

Figure 54: Effects of d-amphetamine, fluphenazine, or haloperidol (IP) on the number of pellets left uneaten, in the satiety paradigm. On the ordinate number of pellets left, over 20 min; on the abscissa: drug administered IP (peripherally): AMPH (d-amphetamine), FLU (fluphenazine), HALO (haloperidol). The profile of vehicle-treated controls are represented by the open columns and those of drug-treated groups are represented by the patterned columns. Each value represents the mean ± sem of the animals in the respective groups (n = 12). *Significantly different with respect to the appropriate control value (open columns) at $p<0.05$.

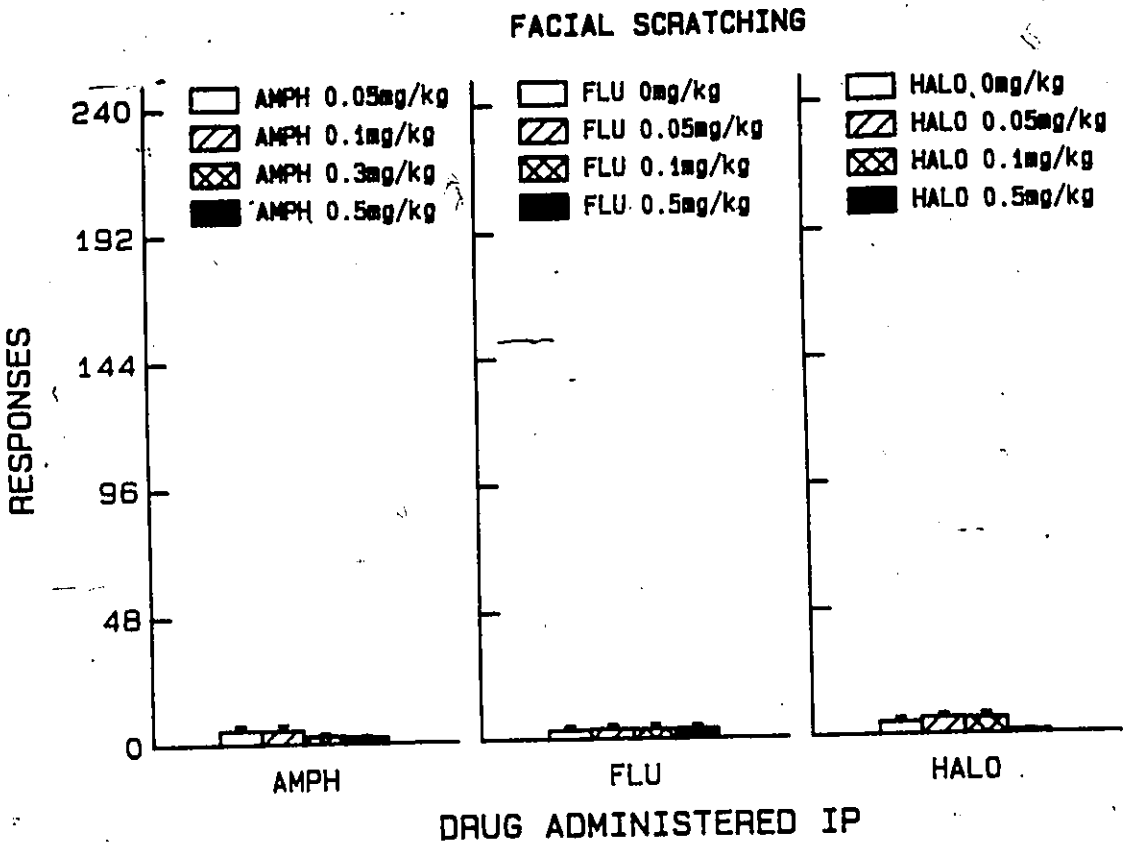

Figure 55: Effects of d-amphetamine, fluphenazine, or haloperidol (IP) on the frequency of facial scratching, in the satiety paradigm. On the ordinate the number of responses, over 20 min; on the abscissa: drug administered IP (peripherally): AMPH (d-amphetamine), FLU (fluphenazine), HALO (haloperidol). The profile of vehicle-treated controls are represented by the open columns and those of drug-treated groups are represented by the patterned columns. Each value represents the mean ± sem of the animals in the respective groups (n = 12). *Significantly different with respect to the appropriate control value (open columns) at $p<0.05$.

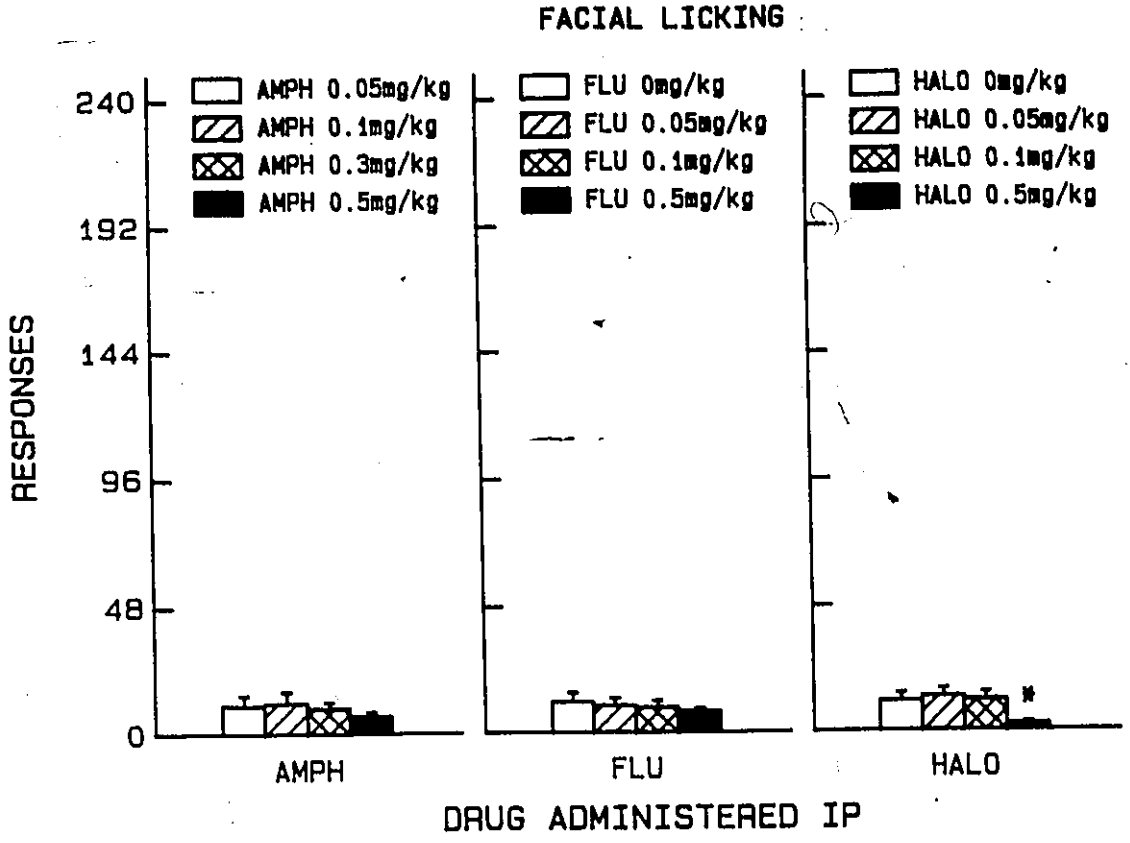

Figure 56: Effects of d-amphetamine, fluphenazine, or haloperidol (IP) on the frequency of facial licking, in the satiety paradigm. On the ordinate the number of responses, over 20 min; on the abscissa: drug administered IP (peripherally): AMPH (d-amphetamine), FLU (fluphenazine), HALO (haloperidol). The profile of vehicle-treated controls are represented by the open columns and those of drug-treated groups are represented by the patterned columns. Each value represents the mean ± sem of the animals in the respective groups (n = 12), *Significantly different with respect to the appropriate control value (open columns) at $p<0.05$.

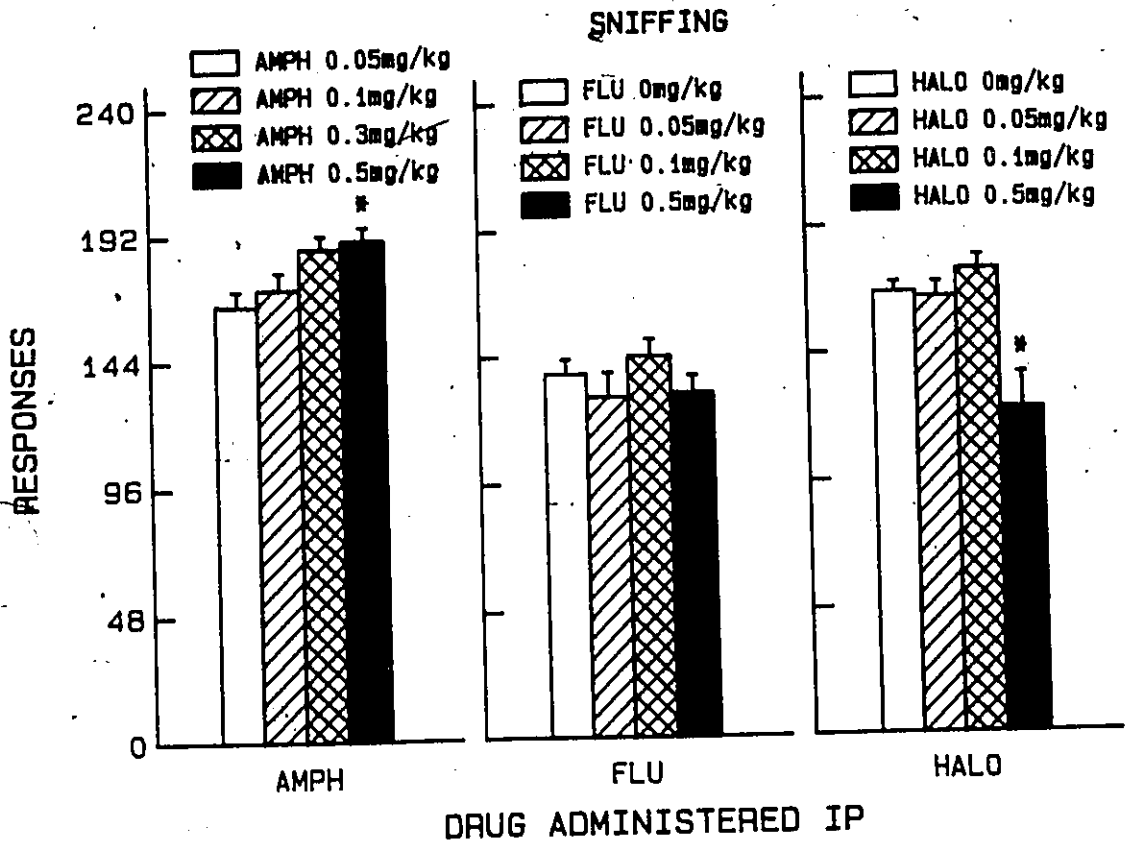

Figure 57: Effects of d-amphetamine, fluphenazine, or haloperidol (IP) on the frequency of sniffing, in the satiety paradigm. On the ordinate the number of responses, over 20 min; on the abscissa: drug administered IP (peripherally): AMPH (d-amphetamine), FLU (fluphenazine), HALO (haloperidol). The profile of vehicle-treated controls are represented by the open columns and those of drug-treated groups are represented by the patterned columns. Each value represents the mean ± sem of the animals in the respective groups (n = 12). *Significantly different with respect to the appropriate control value (open columns) at $p<0.05$.

Results Experiment 4.6

One way analysis of variance repeated over peptides (neuropeptide Y, 1 ug; GRP 1-16, 2 ug; BN 0.5 ug or vehicle), revealed a significant effect of peptide for latency to eat, $F(3,33) = 54.2$; duration of eating, $F(3,33) = 21.79$; pellets left, $F(3,33) = 66.71$; facial scratching $F(3,33) = 116.97$; facial licking, $F(3,33) = 137.54$; body scratching $F(3,33) = 18.16$; body licking $F(3,33) = 13.34$; sniffing $F(3,33) = 58.64$; chewing $F(3,33) = 102.58$; and rearing $F(3,33) = 9.38$. Comparison of means demonstrated a significant effect of BN (0.5 ug) as compared to the saline condition for all the behaviors that were mentioned just above. Neither neuropeptide Y nor GRP 1-16 had a significant effect on any behavior measured.

One way analysis of variance repeated over drug and/or peptide treatment (vehicle, BN 0.5 ug, fluphenazine 10 ug + BN 0.5 ug or fluphenazine 10 ug) microinjected intra NTS revealed a significant effect on latency to eat, $F(3,33) = 114.64$; duration of eating, $F(3,33) = 25.85$; pellets left, $F(3,33) = 157.47$; facial scratching $F(3,33) = 58.83$; facial licking, $F(3,33) = 86.39$; body scratching, 13.83; body licking, $F(3,33) = 7.14$, sniffing, $F(3,33) = 88.99$, chewing, $F(3,33) = 172.70$; and rearing, $F(3,33) = 16.33$. Comparison of means demonstrated a significant difference between the control condition and BN for all the behaviors listed just above. As well fluphenazine + BN also demonstrated

significant differences in the same behaviors with the exception of body scratching and licking, which were not significantly changed. Fluphenazine alone did not alter any behavior significantly as compared to the vehicle. When comparing the BN condition to the fluphenazine + BN condition, there were no significant differences using the Tukey test, however using the Least Significant Differences test (Kirk, 1982), there was a significant decrease after fluphenazine + BN, of facial scratching, and body scratching and licking, however, the satiety scores remained unaltered. Moreover, whether fluphenazine was injected intra NTS, NA (10 ug) or peripherally the effects on BN (intra NTS)-induced behaviors were similar. The means for the dose-effect of the BN and fluphenazine administered alone and together, for facial scratching and licking, sniffing, latency to eat and pellets left uneaten were illustrated in Figures 58 and 59.

Overall fluphenazine injected intra NTS, NA or IP, had no effect on BN (intra NTS) -induced satiety however it appeared to partially suppress BN (intra NTS) -induced grooming.

One way analysis of variance repeated over treatment (vehicle, BN 0.5 ug, neuromedin C 0.35 ug) intra NTS, revealed as expressed above a significant effect of BN on the satiety and grooming scores. Comparison of means demonstrated a significant decrease for chewing only, after neuromedin C as compared to the vehicle condition (Tukey).

However the LSD test revealed a significant increase in latency to eat and pellets left, and a decrease in sniffing as compared to the vehicle condition. Thus neuromedin C (0.35-ug) appeared to have a slight effect on satiety but not grooming at the NTS.

One way analysis of variance repeated over treatment (vehicle, BN 0.5 ug, neuromedin B 0.35 ug) intra NTS, revealed as expressed above, a significant effect of BN on the satiety and grooming scores. Comparison of means demonstrated a significant decrease in duration of eating and an increase in facial scratching (Tukey) only, for neuromedin B in relation to the vehicle condition. There was no significant effect of neuromedin B on the other satiety behaviors (either Tukey or LSD) or the other grooming behaviors.

One way analysis of variance repeated over treatment (vehicle, BN 0.5 ug, BN 0.5 ug + spantide 1 ug, or spantide 1 ug) intra NTS, revealed a significant effect on latency to eat, $F(3,15) = 16.03$; pellets left, $F(3,15) = 18.62$; facial scratching, $F(3,15) = 64.05$; facial licking, $F(3,15) = 33.96$; body scratching $F(3,15) = 6.46$; body licking $F(3,15) = 13.68$; sniffing $F(3,15) = 12.24$; chewing, $F(3,15) = 16.31$; and licking $F(3,15) = 4.05$. Comparison of means revealed, as reported above, significant effects of BN on the satiety and grooming scores in relation to the saline condition. Spantide + BN induced significantly greater latency to eat, and pellets left, and significantly less chewing, than the

saline condition. In comparison to the BN condition, spantide + BN, induced significantly less facial scratching and licking, and body scratching and licking than BN alone; however, the satiety scores were not significantly different. Spantide alone, when compared to the vehicle condition, significantly increased latency to eat, and pellets left, (Figure 60) but did not significantly effect grooming or the other behaviors.

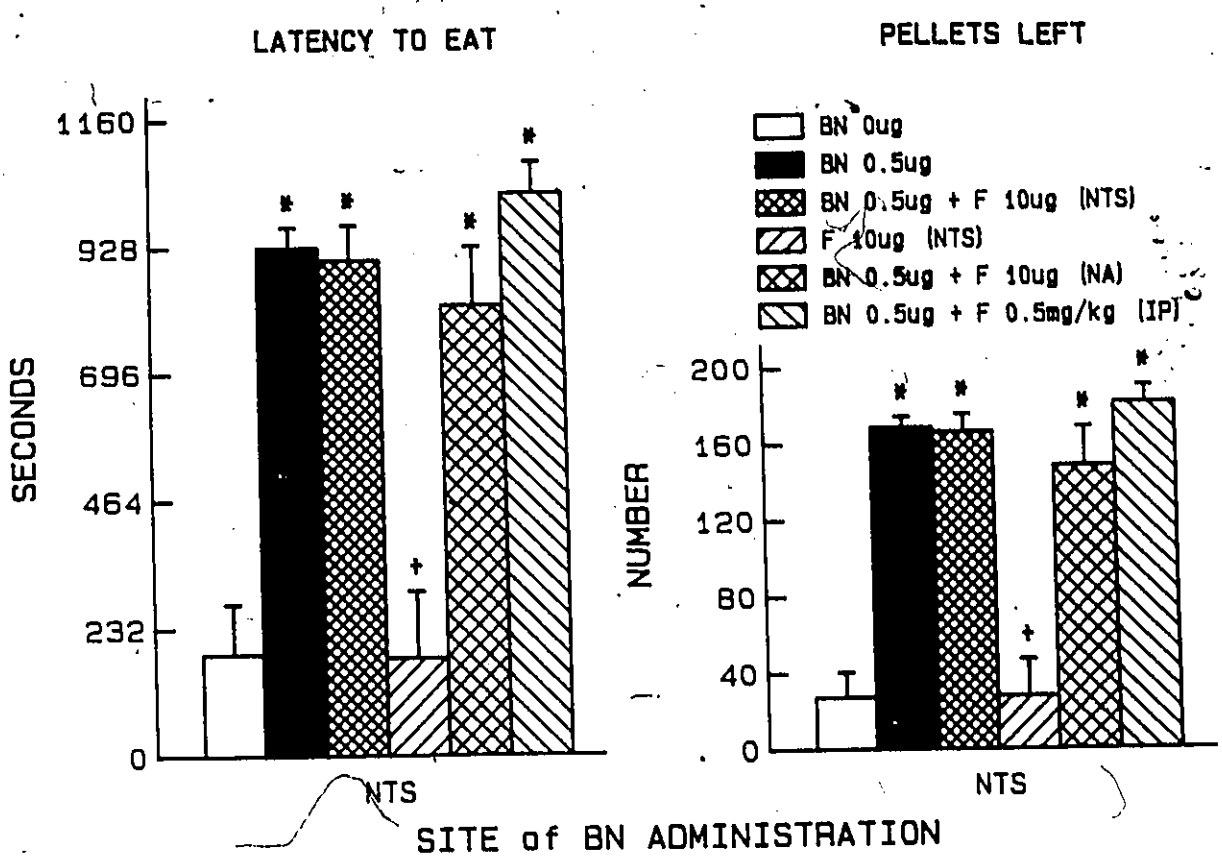

Figure 58: Effects of BN, fluphenazine (F), and BN + fluphenazine (BN + F), on the latency to eat or on the number of pellets left uneaten. On the ordinate: the magnitude of the quantified response over 20 min; on the abscissa: site of BN administration: NTS (nucleus tractus solitarius). The profile of vehicle-treated controls are represented by the open columns, those of BN-alone treated groups by the solid columns, and the fluphenazine alone and fluphenazine + BN by the patterned columns. Each value represents the mean ± sem of the animals in the respective groups. *Significantly different with respect to the vehicle control at $p<0.05$. +Significantly different from the BN control condition at $p<0.05$.

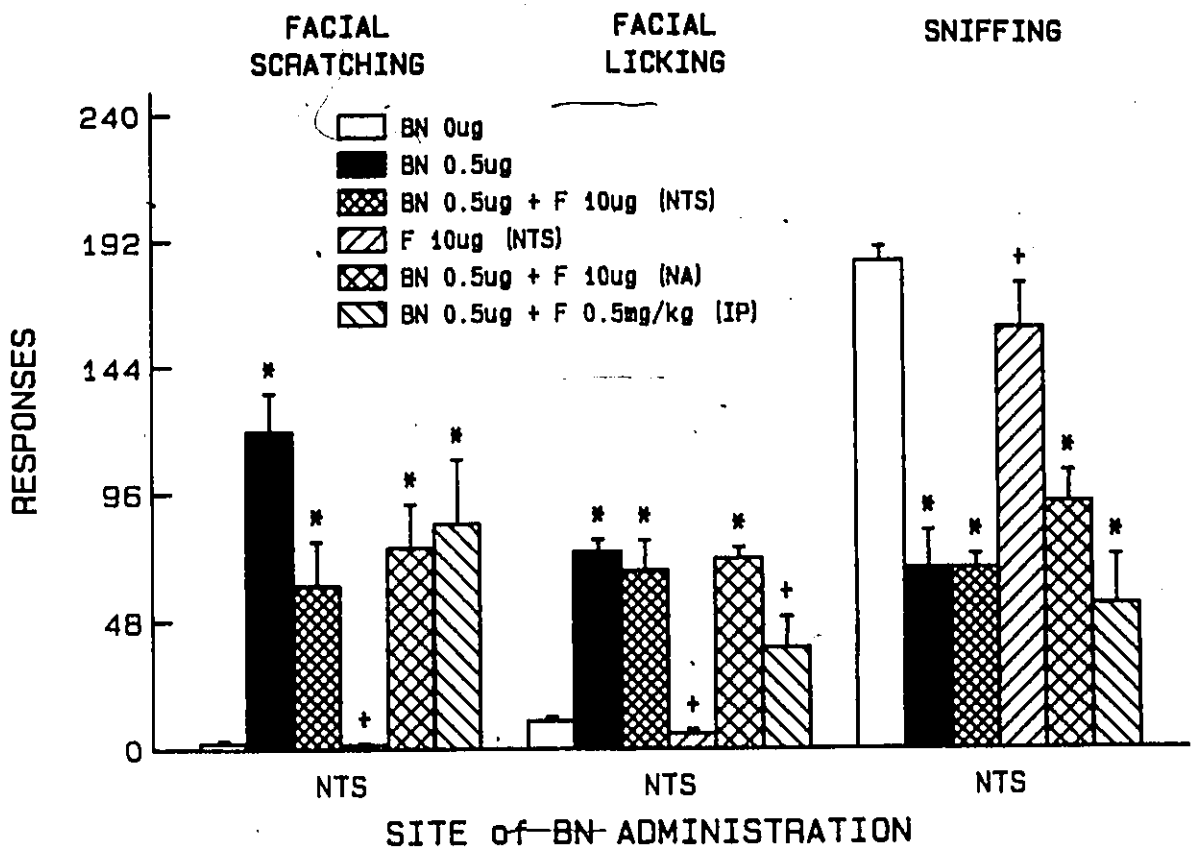

Figure 59: Effects of BN, fluphenazine (F), and BN + fluphenazine (BN + F), on the frequency of facial scratching, facial licking, and sniffing, in the satiety paradigm. On the ordinate: the number of responses, over 20 min; on the abscissa: site of BN administration: NTS (nucleus tractus solitarius). The profile of vehicle-treated controls are represented by the open columns, those of BN-alone treated groups by the solid columns, and the fluphenazine alone and fluphenazine + BN by the patterned columns. Each value represents the mean ± sem of the animals in the respective groups. *Significantly different with respect to the vehicle control at $p<0.05$. +Significantly different from the BN control condition at $p<0.05$.

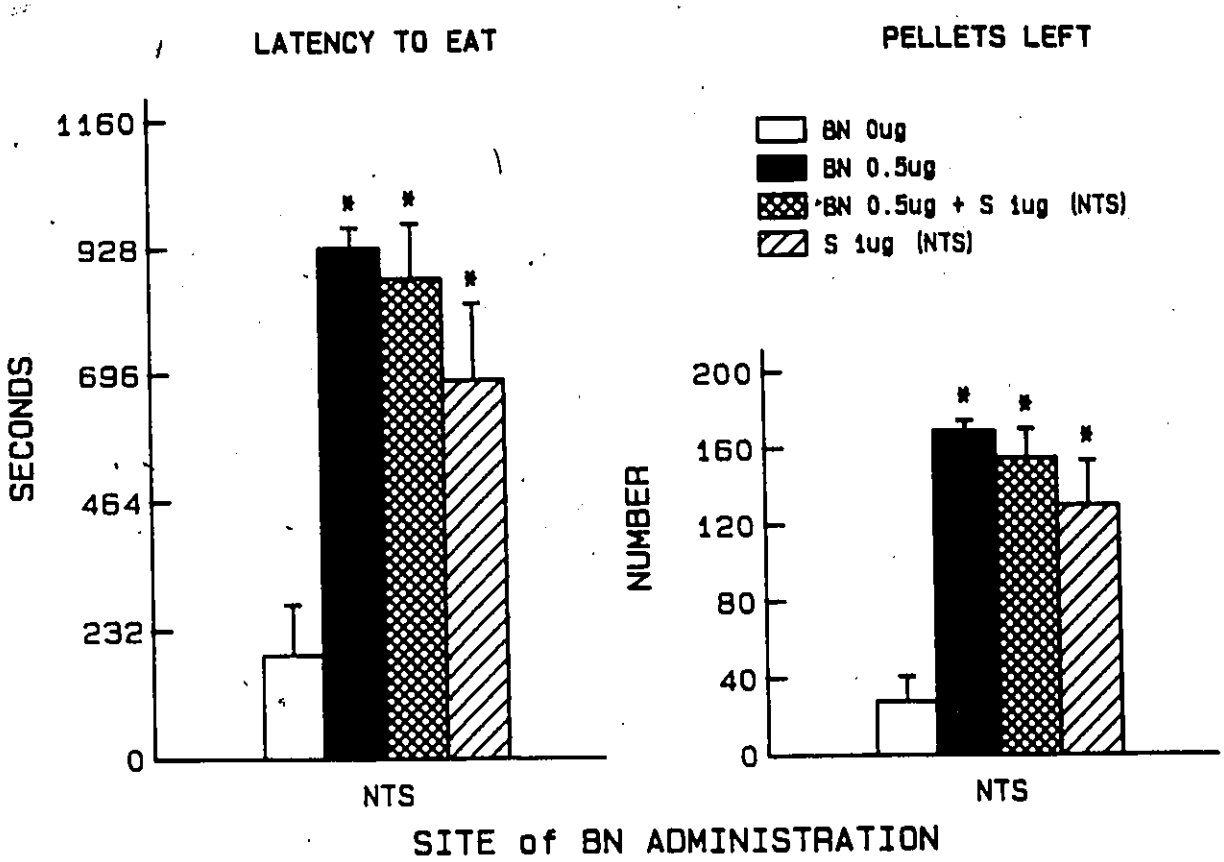

Figure 60: Effects of BN, spantide (S), and BN + spantide, (BN + S) on the latency to eat or on the number of pellets left uneaten. On the ordinate: seconds of latency to eat, over 20 min; or number of pellets left uneaten over 20 min. On the abscissa: site of BN administration: NTS (nucleus tractus solitarius. The profile of vehicle-treated controls are represented by the open columns, those of BN-alone treated groups by the solid columns, and the spantide alone and spntide + BN by the patterned columns. Each value represents the mean ± sem of the animals in the respective groups. *Significantly different with respect to the vehicle control at $p<0.05$. +Significantly different from the BN control condition at $p<0.05$.

Experiments 4.7 and 4.8: The Behavioral Effects of Peptide and/or Drug Administration at the Nucleus Accumbens and/or Peripherally, in the Satiety Paradigm.

Experiment 4.7.

Eleven animals cannulated at the NA (bilateral implants) received BN (0, 0.001, 0.01, 0.1, or 1.0 ug); followed by fluphenazine.(0, 2, 10, or 20 ug). Each drug was administered in a Latin square design.

Experiment 4.8

The twelve rats (the triple implants from Experiment 4.6) were microinjected at the NA with the following drugs: Neuropeptide Y (2 ug); GRP 1-16 (4 ug); saline; or BN (0.5 ug); in a random order. At the end of the experiment, the 6 triple implanted rats, not used for the injection of spantide at the NTS (Experiment 4.6), were microinjected at the NA with BN (0.5 ug) + spantide (2 ug), followed 3 days later with spantide (2 ug) alone.

Results Experiments 4.7 and 4.8 The Behavioral Effects of Peptide and/or Drug Administration at the Nucleus Accumbens and/or Peripherally, in the Satiety Paradigm.

The implants of all animals used in the experiments were illustrated in Figures 61 and 62.

Results Experiment 4.7

One way analysis of variance repeated over doses of BN (0, 0.001, 0.01, 0.1, or 1.0 ug) revealed a significant effect of BN administered at the NA, on duration of eating, $F(4,40) = 2.85$; sniffing, $F(4,40) = 4.43$; chewing, $F(4,40) = 2.54$; and rearing $F(4,40) = 7.89$. Comparison of means demonstrated a significant effect of BN 1 ug on rearing only. No other dose of BN had a significant effect as compared to the saline condition on any behavior monitored in the satiety paradigm. Results were illustrated for latency to eat, pellets left, facial scratching, facial licking and sniffing in Figures 44-48, respectively.

One way analysis of variance repeated over treatment (vehicle, 2 ug, 10 ug, 20 ug fluphenazine) intra NA, revealed no significant effect of fluphenazine on any behavior monitored in the satiety paradigm.

Results Experiment 4.8

One way analysis of variance repeated over treatment (vehicle; neuropeptide Y, 2 ug; GRP 1-16, 4 ug; or BN 0.5 ug) intra NA, revealed a significant effect on latency to eat, $F(3,33) = 4.21$; duration of eating, $F(3,33) = 8.14$; pellets left $F(3,33) = 4.83$; body licking, $F(3,33) = 3.0$; sniffing, $F(3,33) = 8.62$; chewing, $F(3,33) = 4.40$; and rearing, $F(3,33) = 4.87$. However comparison of means revealed no significant differences between any peptide and the vehicle condition on any behavior except sniffing after neuropeptide Y.

One way analysis of variance repeated over treatment (vehicle; BN, 0.5 ug; BN, 0.5 ug; + spantide, 2 ug; or spantide, 2ug) intra NA, revealed a significant effect on duration, $F(3,15) = 6.36$ and chewing, $F(3,15) = 6.18$. However, comparison of means did not show a significant difference between any treatment and the vehicle condition, or between BN and BN + spantide on any behavior monitored in the satiety paradigm.

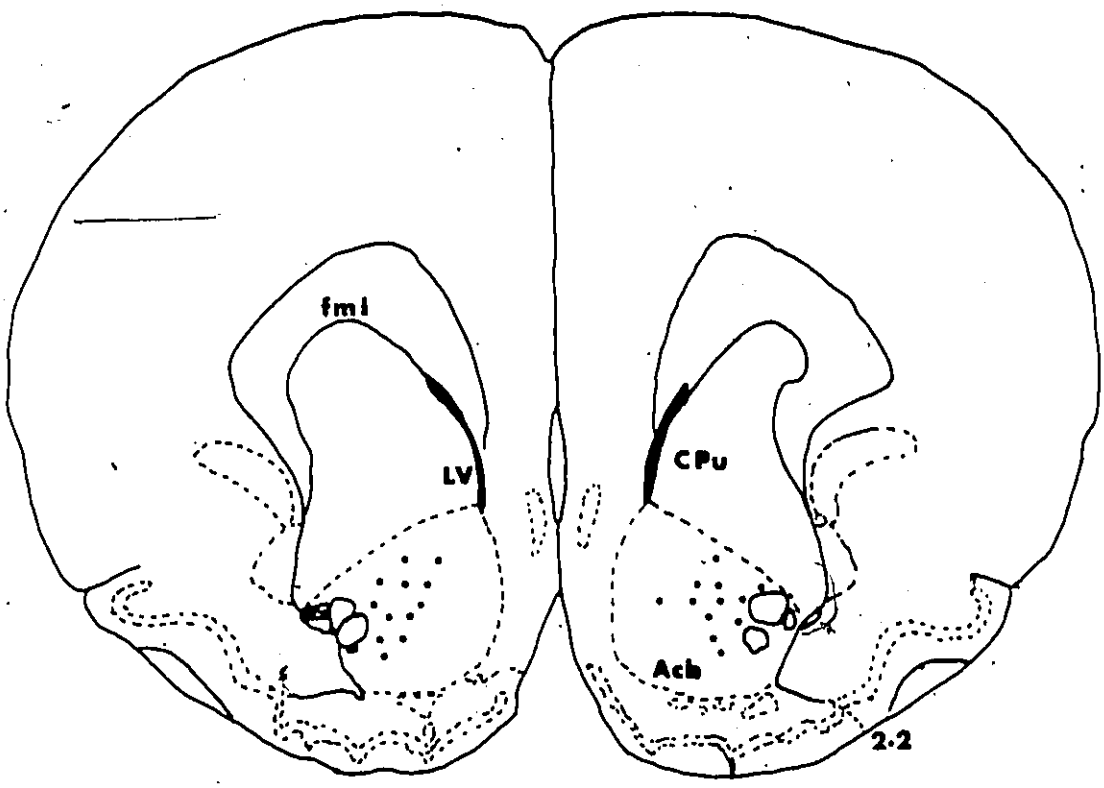

Figure 61: Verification of implantation sites after Experiment 4.7. Location of cannula implantation tip in the nucleus accumbens (NA) of the rat (n = 11) (coronal view: A-P 2.2 mm; Paxinos and Watson, 1982). Abbreviations: see Figure 40.

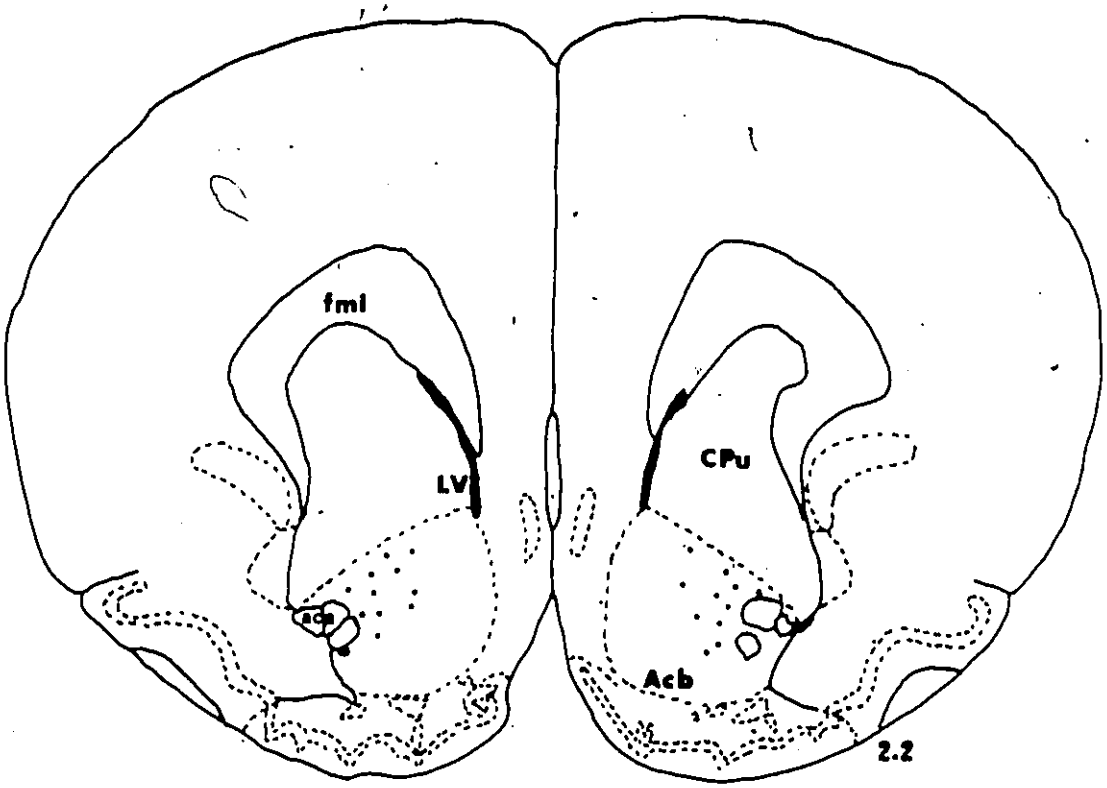

Figure 62: Verification of implantation sites after Experiment 4.8. Location of cannula implantation tip in the nucleus accumbens (NA) of the rat (n = 12) (coronal view: A-P 2.2 mm; Paxinos and Watson, 1982). Abbreviations: see Figure 40.

Discussion

In the following discussion the effects of the various drug treatments on the behaviors grooming, locomotion, satiety, sniffing, and resting were compared.

Bombesin injected at the NTS induced grooming most notably facial scratching and licking at a dose of 0.01 ug or higher in the home paradigm (as illustrated in Figures 26 and 27), and at a dose of 0.1 ug and higher in the satiety paradigm (Figures 46 and 47). The home paradigm results on behavior replicated data from Experiment 3.1 (Figures 17-19). In contrast, BN microinjected at the NA did not stimulate grooming when the rat was tested in either paradigm (Figures 26-27, 46-47). In an earlier experiment (Experiment 3.3), BN (1.0 ug) at the NA had been shown to increase facial scratching and decrease facial licking (LSD comparison of means, Figures 18-19). Actually the values for Experiment 3.3 and 4.3 were similar; reflecting a 15% increase in facial scratching over 60 min.(Figures 18 and 26). However the trend of a decrease in facial licking (LSD) observed in Experiment 3.3 was not evident in Experiment 4.3. Thus, although the NA site of Experiment 3.3 bordered on the ventral caudate putamen, whereas, the NA site for the Phase 4 NA Experiments was at the middle of the NA (0.5 mm further ventral) (Figure 11 vs Figures 40-41, 61-62) the results of the two experiments were very similar, both

showing minimal increase in facial scratching and a maximal increase in locomotion after BN (1 ug) intra NA.

Locomotion was monitored in the home paradigm only and was significantly stimulated by BN at doses of 0.5 ug or greater, microinjected at the NA (Figures 17 and 25). In contrast, although BN appeared to stimulate locomotor activity when microinjected at the NTS, it did not reach a statistically significant level.

Satiety related behaviors including latency to eat and pellets left uneaten showed a site specific response to BN as illustrated in Figures 44 and 45. At the NTS these behaviors were significantly increased at a dose of 0.01 ug. of BN and higher. However, BN microinjected at the NA did not significantly change these behaviors.

Sniffing appeared to be altered differently by BN, at both sites and in both paradigms. In the home paradigm BN at a dose of 0.5 ug or higher, intra NA (but not intra NTS) significantly stimulated sniffing (as illustrated in Figure 28). However, in the satiety paradigm, BN, at the NA had no significant effect on sniffing (Figure 48), whereas, at the NTS BN significantly decreased sniffing at a dose of 0.1 ug or higher.

The profile of behavior after injection of BN peripherally, did not resemble that seen after its administration centrally at either the NA or the NTS. In the home paradigm, systemic BN failed to affect locomotion or grooming (Figures 25-29). In the satiety paradigm, BN, (IP),

significantly altered the satiety related behaviors, but not the grooming behaviors, as previously reported (Figures 44-48) (Kulkosky et al, 1982a,b).

Resting, measured in the home paradigm only, was most significantly decreased by BN administered centrally, at the NTS (0.001 ug and higher), but was also decreased at the NA (1.0 ug) (Figure 29). Rasler (1984) has reported that infusion of 1.0 ug BN produced electroencephographic frequencies of normal waking patterns during grooming suggesting a possible physiologic function for BN in grooming and the sleep-waking cycle. In contrast, peripherally administered BN did not significantly alter resting behavior over the control value (Figure 29) (Kulkosky et al, 1982a).

An inactive fragment of GRP, GRP 1-16, administered intra NA or NTS, did not significantly affect any behaviors, in the satiety paradigm. In the home paradigm, GRP 1-16 (4 ug) microinjected at the NA, significantly increased sniffing behavior and significantly decreased resting. However, in the home paradigm, GRP 1-16 (2 ug) intra NTS, had no effect on the behaviors measured. Thus, the GRP 1-16 fragment appeared for the most part to be inactive, demonstrating that BN induced behavioral changes (except sniffing and resting at the NA, in the home paradigm) were specific to BN.

Two other peptides related to BN, neuromedin C (GRP 10) and neuromedin B were tested in the satiety paradigm, at the

NTS. Neuromedin C had mild effects on satiety scores significant with the LSD but not the Tukey test, increasing latency to eat and pellets left uneaten; however, the grooming scores were relatively unaltered with either test. In contrast, neuromedin B did not significantly change latency to eat or pellets left (either test), but significantly decreased the eating duration (Tukey) and significantly increased facial scratching but not facial licking (Tukey & LSD). Thus, there was a partial overlap between neuromedin C and BN on their effects on satiety at the NTS, and a partial overlap of neuromedin B and BN on duration of eating and facial scratching, at the NTS. The doses of BN, neuromedin B, and C were made molar equivalent at 308.64 p moles (BN 0.5 ug). Thus BN appeared to have greater potency than either neuromedin B or C on any of the overlapping significantly affected behaviors. However no check on purity and potency of the peptides was made just before injection, thus these results require replication using these control procedures, since neuromedin B and C are not as stable as BN (O'Donohue et al, 1984; Mayer et al, 1986; Bishop et al, 1986).

One further peptide tested, neuropeptide Y is from a completely different peptide family than BN. Neuropeptide Y is a member of a family which includes peptide YY and pancreatic polypeptide (Tatemoto, 1982; Tatemoto et al, 1982). Injected ICV neuropeptide Y has been reported to increase feeding in rats (Levine and Morley, 1984). In our

experiments, injected at the NA, neuropeptide Y had no significant effect on home paradigm behaviors. However, this peptide intra NA significantly decreased sniffing in the satiety paradigm. This was in contrast to BN, which in this circumstance had no significant effect on sniffing. Neuropeptide Y injected intra NTS had no significant effect on the behaviors monitored in either paradigm.

Thus overall, in comparison to GRP 1-16, neuromedin B and C, and neuropeptide Y, BN presented a unique and specific behavioral profile.

Spantide, the BN receptor antagonist, caused reduction in eating (Figure 60). Consequently, it could not be considered a true antagonist of BN-induced effects since it had biological effects of its own.

In addition, BN-induced behavioral effects were compared to the effects of dopamine agonist d-amphetamine and the dopamine antagonists fluphenazine and haloperidol since the dopaminergic system(s) were implicated in the mediation of grooming and satiety (Isaacson 1984; Leibowitz and Rossakis, 1979)

In the home paradigm, d-amphetamine microinjected at the NA (20 ug), significantly stimulated locomotor activities and sniffing, and decreased resting (Figures 30, 33 and 34), as did d-amphetamine administered peripherally (locomotion 0.5 mg/kg), (sniffing and resting 0.3 mg/kg and higher). This behavioral profile strongly resembled that observed upon administration of BN at the NA (Figures 25,

28, and 29). In addition, BN and d-amphetamine at the NA, and d-amphetamine IP, did not significantly effect grooming behaviors (Figures 26, 27 and 31, 32). On the other hand, d-amphetamine microinjected at the NTS had no significant effect on any behavior monitored (Figures 30-34), whereas, BN (intra NTS) strongly stimulated grooming and decreased resting (Figures 26, 27, 29). In review, in the home paradigm, there was a similar behavioral profile of BN intra NA and d-amphetamine administered IP and intra NA. However, BN and d-amphetamine when microinjected at the NTS, elicited very different behavioral profiles, unique to that site.

In the satiety paradigm, d-amphetamine (0.5 mg/kg) IP induced a significant increase in latency to eat and number of pellets left uneaten, and a significant increase in sniffing but no significant effect on grooming behaviors (Figures 53-57). Bombesin (2 ug/kg) IP induced satiety behavior but did not stimulate sniffing. Furthermore, in the satiety paradigm, BN injected at either the NTS or the NA had a very different behavioral profile than d-amphetamine IP (Figures 44-48 vs 53-57). Thus, the behavioral profiles of d-amphetamine (IP) and BN (IP, intra NTS or intra NA) were different in the satiety paradigm.

Haloperidol and fluphenazine, dopamine antagonists were investigated for their effects on baseline behaviors in both paradigms. Fluphenazine intra NTS (0-10 ug), intra NA (0-20 ug) or IP (0-0.5 mg/kg) had no significant effect on behaviors monitored in either paradigm (Figures 35-39, 58-

59). Haloperidol IP, in the home paradigm had no significant effect on behaviors monitored. However, haloperidol IP (0.5 mg/kg) in the satiety paradigm significantly increased latency to eat and pellets left and significantly decreased duration of eating, facial licking and sniffing (Figures 53-57). Thus haloperidol appeared to have a suppressive effect on feeding and grooming behaviors in the satiety paradigm. At the dose of 0.5 mg/kg the rats showed rigid posture suggesting extrapyramidal effects of the drug.

Since fluphenazine at the doses tested had no significant effect on baseline behaviors in either paradigm, it was chosen to test for antagonism of BN-induced effects. In the home paradigm, fluphenazine (10 ug) intra NA significantly decreased the effects of BN (1 ug) intra NA, on locomotor activities and sniffing and significantly increased resting at the fluphenazine dose of 20 ug + 1 ug BN (Figures 35-39). Thus, in compliment to the similarity of d-amphetamine and BN-induced effects on behavior at the NA, there was also an antagonism of BN induced effects by fluphenazine, at the NA, in the home paradigm. These two complimentary results with a dopaminergic agonist and antagonist were consistent with the hypothesis that BN-induced behavioral effects on locomotion, sniffing and resting were mediated through dopaminergic systems at the terminal area of the NA.

However, in the home paradigm, at the NTS, the fluphenazine antagonism of BN-induced effects was not

significant with the Tukey test, but, was significant with the LSD test. Fluphenazine (10 ug) administered at the NTS, antagonized (LSD, only) BN-induced (0.5 ug intra NTS), stimulation of facial scratching and facial licking, but did not antagonize BN-induced decrease in resting (Figures 36, 37, and 39).

In the satiety paradigm, fluphenazine (10 ug), intra NTS, intra NA and (0.5 mg/kg) IP significantly (LSD only) decreased BN-induced (0.5 ug, intra NTS) facial scratching (Figure 59). Similarly, fluphenazine (0.5 mg/kg) IP significantly (LSD only) reduced BN-induced (0.5 ug, intra NTS) facial licking. Thus, in the satiety paradigm, a rather consistent effect of fluphenazine (IP, intra NTS or NA), was to partially block BN-induced (NTS) grooming scores but not the eating scores (Figures 58 and 59). In line with this, in the home paradigm fluphenazine, also partially blocked BN (intra NTS)-induced grooming, but did not effect the BN-induced decrease in resting (Figures 36, 37 and 39). These results taken in consideration with the lack of effect on grooming of d-amphetamine at the NTS (home paradigm), suggested that dopaminergic modulation of BN-induced grooming, did not occur at the NTS, but rather further up in the midbrain or possibly the limbic striatal region. Furthermore the role of dopaminergic transmission in BN-induced grooming behavior appeared to be secondary to initiation of the behavior by BN administered at the NTS. However, dopaminergic neural transmission appeared to be of

primary importance in the induction of increased locomotor activity since d-amphetamine IP or intra NA potently stimulated locomotor activities. Moreover, dopaminergic initiation of locomotion appeared to be specific to the NA, but, not the NTS (Figure 30).

CHAPTER 6

GENERAL DISCUSSION

Initial studies of the thesis research characterized the stimulation of central (ICV) effects of BN on locomotor activities and the antagonism of these effects by dopamine antagonists. Thereafter, by microinjecting BN into various brain loci which had a high density of BN binding sites, we identified which of the high density binding sites resulted in eliciting locomotion and/or grooming. Comparatively, the NA and NTS proved to be the most responsive sites (of those tested) to locomotion and grooming respectively. Additional experiments replicated the site specific effects of BN at the NA and NTS in regard to locomotion and grooming and in addition concomitantly explored the site specific effects of BN on satiety and grooming at the NTS and NA. As well, the behavioral effects of peripherally administered BN were characterized; they included satiety only, neither increases in grooming nor locomotion were evident. Bombesin microinjected at the NA specifically stimulated locomotion, but did not significantly change grooming or feeding behaviors, whereas at the NTS grooming was stimulated and eating was reduced but locomotion was not significantly changed. Moreover through the use of dopamine agonists and antagonists it was determined that BN-induced locomotion appeared to be significantly mediated through dopaminergic

system(s) but BN-induced grooming and satiety did not appear to be mediated through dopaminergic systems. In addition, it was possible to dissociate grooming and satiety effects of BN at the NTS on the basis of time-course and dose-effect.

Our results of BN injection at the NTS, demonstrated a behavioral effect of BN which included increased grooming and satiety and decreased rest but satiety occurred at a dose of BN an order of magnitude lower than grooming (0.01 vs 0.1 ug; one way anova). Other studies have indicated that bombesin administered ICV reduced feeding and increased grooming at the same order of magnitude (Kulkosky et al, 1982a). The increased potency of BN intra NTS for inducing satiety over grooming suggested that perhaps BN at the NTS may have a specific role in BN-induced satiety.

Further comparison of means after two way analysis of variance (repeated over time and dose) revealed a very similar time-course dose-effect profile for BN intra NTS (0.0001-0.1 ug) and BN IP (1-8 ug/kg) on latency to eat and pellets left (Figures 49-50). This suggested that BN at the NTS may be critical for inducing satiety since reduction in eating was most pronounced, at the terminal part of a meal. Furthermore, at the NTS, satiety occurred at a lower dose of BN than changes in grooming thus suggesting the independence of satiety from grooming at least at the lower doses of BN (0.0001 and 0.001 ug) (Figures 49-52). As well, BN appeared to have its maximal effect on satiety at 20 min, whereas its effects on facial scratching and licking were maximal (or

almost maximal) at the first 5 min and continued rather constantly throughout the 20 min. Thus, both time-course and dose-effect were different for satiety and grooming after BN administration at the NTS.

It would be useful to map the distribution of BN autoradiographically (Johnson and Epstein, 1975) after injection of 0.5 ul administration at the NA and NTS in order to identify more precisely the site of BN-binding. Johnson and Epstein (1975) did extensive injection of angiotensin in the brain. They found that angiotensin had maximal effect when injected ventricularly. Moreover if the path of the cannula directed at various brain sites crossed the ventricle they found similar dose-effects to those of ventricular injection. Thus they demonstrated autoradiographically that this was due to the diffusion of the injection up the guide to the ventricle. However they found when they implanted in a brain structure without crossing the ventricle their results were site specific. Consequently the sites implanted at the NA and NTS in these experiments were chosen in a path that avoided the ventricles. Furthermore the volume chosen of 0.5 ul was injected to insure that the peptide did indeed reach the brain tissue. Smaller injection volumes would increase the chance that a small fragment of tissue might block the injection completely, or that a tiny air bubble might displace the injection volume. Moreover, the 0.5 ul volume has been employed in previous experiments at both the NTS

and NA as well as other specific sites (Crawley, 1985; Johnston et al, 1986; Gmerek and Cowan, 1983; Spruijt et al, 1986; Stuckey and Gibbs, 1982). Additionally, this volume is an experimentally acceptable volume (Myers, 1974). Both the NTS (Kalia and Sullivan, 1982) and the NA site (Chronister et al, 1981) have dense cell distributions where diffusion of dye remained in a small radius around the tip. However, it would be more relevant to demonstrate through autoradiography the exact dispersion of peptide around the site. Certainly if the dispersion was large and involved more than the nucleus specified smaller volumes possibly in the nl range could be considered.

However the behavioral data showed general stimulation of locomotion, grooming and satiety after BN ICV (Johnston et al, 1986) and specific effects of BN on each behavior after brain site injection. This was a clear behavioral verification that BN at the NA and NTS did not appear to be escaping into the ventricular system. Bombesin injected in the 4th ventricle also resulted in satiety effects (0.01 ug) at a lower dose than grooming (0.05 ug) (Flynn, 1986). Thus, both the 4th ventricle and the NTS demonstrated satiety at a lower dose of BN than grooming. However, at the NTS site behavioral response to BN (grooming at 0.01 ug and satiety at 0.0001 ug) administration was significant at lower doses than intra the 4th ventricle. The behavioral effects of BN at specific sites appeared to be distinct from the effects of intraventricular BN.

Further testing of BN-induced satiety at the NTS, to demonstrate whether it reduced sham-feeding in rats, and whether it reduced drinking as well as eating would further elucidate the nature of BN-induced (NTS) reduction in eating (Kulkosky, 1985; Smith, 1982). It appeared that BN, did not cross the blood-brain barrier since BN, when administered intra NTS, but not when injected peripherally, induced increased grooming. Kulkosky et al (1982b) also noted the separation of effects of BN peripherally and ICV since grooming and reduction in drinking occurred after ICV injection but not after peripheral injection of BN. Presently the answer to the question of where peripheral BN induces satiety remains unknown. In relation to the neuroanatomical pathways as illustrated in Figure 20 the caudal medial NTS is the sensory visceral nucleus receiving visceral afferents from the gut (Kalia and Sullivan, 1982; Hamilton and Norgren, 1984). Consequently, the NTS must be a candidate for the critical site for BN (IP)-induced satiety because the NTS is the brain site most effective in inducing reduction in eating (70% after 0.1 ug) with a threshold at the lowest dose thus far reported (0.0001 ug BN) for either a central or peripheral site. Moreover, the effect of BN at the NTS, fulfilled at least two of the criteria for short term satiety agents: BN induced reduction in eating in the later part of the meal, not initially (0.0001-0.01 BN; Figures 49-50) and it mediated its effects during the meal over 20 min (Smith, 1982; Kulkosky, 1985). Thus, it would be

interesting to investigate whether the lesion of this NTS site blocks the satiety effects of BN IP or ICV.

In a recent study dopaminergic neurons were unilaterally lesioned (75- 85% depletion) and BN receptor autoradiography carried out to see whether loss of DA neurons resulted in loss of BN-binding sites. The results indicated that the majority of binding sites for BN-like peptides were not present on dopamine containing neurons in the nucleus accumbens, caudate putamen or olfactory tubercle. In contrast the density of BN receptors decreased by 20% in the CE where dopamine levels decreased by 60% (Moody et al, 1987a). The significance of this overlap in the CE of DA terminal areas and BN receptors remains to be interpreted in relation to the possible modulation of grooming or locomotion since the CE did not seem to be a critical site for the mediation of either BN-induced behavior. The CE has reciprocal projections to brainstem nuclei such as the NTS (Ottersen, 1981; Price, 1981); thus, perhaps CE dopamine modulates the grooming activity induced by BN intra NTS. Since the CE does not project to the NA, it seems unlikely that there is any effect of the CE, dopamine-BN overlap on locomotion induced by BN intra NA. The mechanism whereby dopamine antagonists blocked BN-induced locomotion at the NA remains to be elucidated, as well as the mechanism whereby BN induced locomotor activity at the NA. From the results of Experiment 2.2, it has been shown that BN-induced locomotor stimulation requires intact

dopaminergic neurons. The small proportion of BN receptors on dopamine terminals may be functionally important for elicitation of the BN effects. However, BN may act directly through BN receptors on neurons which also have dopamine receptors and BN may be partly dependent on dopamine neural transmission (intra neuronally) to mediate its effects on locomotion. Thus, the antagonism by dopamine antagonists of BN-induced locomotor stimulation may be physiological.

The role of the dopaminergic system(s) in satiety, appeared to involve motor side effects such as the rigidity induced by haloperidol. It was likely that d-amphetamine effects on reduction in eating were motor side effects especially when taken in relation to the above results in the satiety paradigm, where d-amphetamine (IP or intra NA) increased sniffing. Increased sniffing was associated with increased locomotor activities, but, was not associated with the satiety effects of BN IP or intra NTS. The conclusion that motor side effects were the cause of dopaminergic agonist and antagonist effects on the reduction in eating has been supported by a recent review of the lack of a role of dopaminergic systems in satiety (White, 1986). In addition, Fibiger and Phillips (1986) stated that it appeared unlikely that the mesolimbic dopamine system was involved critically in mediating the primary reinforcement or hedonic properties of food, since 6-OHDA lesions of either the VTA or the NA did not disrupt food intake in the rat (Koob et al, 1978, 1981). Our results, in concurrence

with Stricker and Zigmond (1986), suggested that catecholamine (dopamine) agonists and antagonists both decrease feeding. This inverted U-shaped curve appeared to relate dopamine activity to the expression of motivated behavior. Thus, optimal levels of dopamine were necessary for normal food intake and either decreases or increases resulted in reduced feeding (Heffner et al, 1977).

There remains the possibility that dopamine may mediate reduced food intake specifically. The most sensitive region for a reduction of feeding by dopamine was the perifornical area (Leibowitz and Rossakis, 1979). In our results d-amphetamine IP affected satiety mildly (20% inhibition at 0.5 mg/kg) reducing eating possibly indirectly, by disinhibiting the animal or possibly actually causing a direct satiety effect. However, there was no antagonism of BN-induced (NTS) satiety by the dopamine receptor antagonist fluphenazine whether it was administered IP, intra NTS or NA. Thus, BN-induced (intra NTS) satiety did not appear to be modulated through dopaminergic neural transmission either directly at the NTS, or indirectly at the NA or along the gustatory pathway.

The facial scratching significantly increased by BN intra NTS occurred in either paradigm as illustrated in the two pie charts (Figures 63-64). The behavioral profile after saline injection was different in the home paradigm as compared to the satiety paradigm with facial licking predominating in the satiety paradigm and body licking

predominating in the home paradigm. After BN (intra NTS), the grooming as monitored immediately for 20 min in the satiety paradigm, or as monitored for 60 min starting at 20 min after injection as in the home paradigm resulted in a grooming profile of approximately 50% facial scratching and 30% facial licking. These results were consistent across two replications of the experiments. The independence of the BN-induced grooming profile from the home and satiety paradigms was further proof that BN-induced grooming did not appear to be specific to the postprandial behavioral sequence. This dissociation was further characterized by demonstrating that BN-induced grooming was elicited in a similar fashion in both the satiety and the home paradigm suggesting that BN-induced grooming may be a reflexive behavior which occurs independent of the external situation and the internal state of food deprivation or free feeeding.

Fluphenazine (10 ug NTS) appeared to partially shift the grooming profile to the control condition by reducing the overall grooming score and proportionately changing the value of each grooming element as illustrated in Figures 63 and 64. However, this effect was mild and not significant with the Tukey test. The dopamine antagonist haloperidol has been reported to suppress BN-induced grooming. Van Wimersma Greidanus et al, (1985b) reported a general reduction in BN-induced excessive grooming (BN 30 ng + haloperidol 0.05 mg/kg subcutaneous) without changing the composition of grooming behavior. However, their dose of haloperidol

suppressed baseline total grooming, thus the effect of haloperidol may be nonspecific. Haloperidol administered in Experiment 1.2 resulted in a suppression of baseline behavior which BN (1ug) could not reverse even at the lower doses of haloperidol. Similarly in Experiment 4.5 haloperidol (IP) suppressed behaviors causing a waxy rigidity in posture. Haloperidol thus appeared to effect a variety of behaviors such as locomotion, grooming and satiety possibly inhibiting all movement nonspecifically. Gmerek and Cowan (1983) reported that haloperidol, morphine, naloxone and neurotensin were unable to affect BN-induced grooming at behaviorally non depressant doses. In line with these results, neurotensin and naloxone have also been reported to reduce BN-induced scratching (Van Wimersma Greidanus, 1985b). However, their doses of neurotensin (1 ug, ICV) and naloxone (1 ug, ICV) also reduced baseline total grooming scores, thus neurotensin and naloxone may act nonspecifically to reduce grooming.

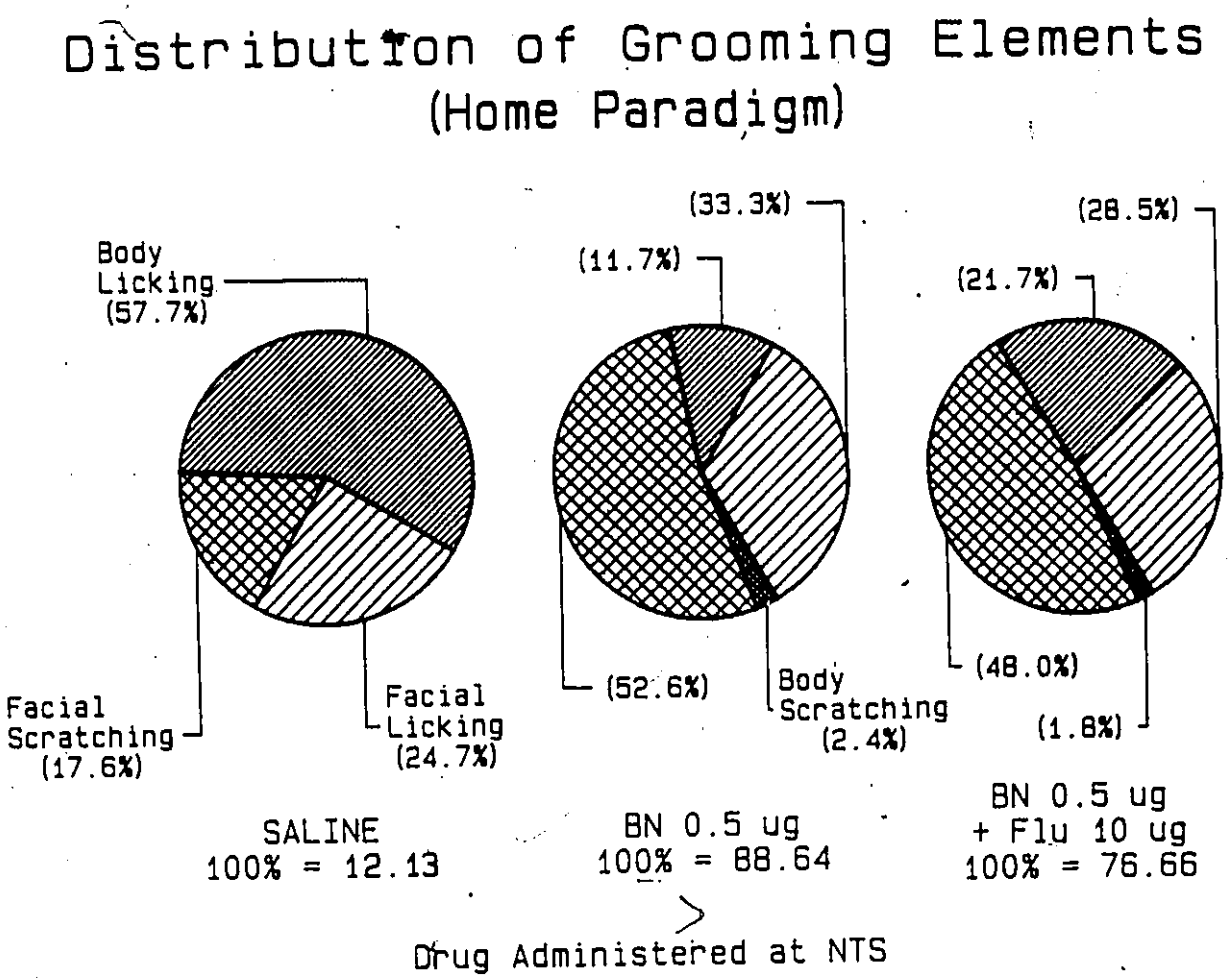

Figure 63. Distribution of the grooming elements, facial scratching, facial licking, body scratching and body licking, in the home paradigm after administration of saline, BN (0.5 ug) or BN (0.5) ug + fluphenazine (10 ug) intra NTS (n=8). The values are the mean score for each element as compared to the total mean grooming score expressed as a per cent, for each drug treatment.

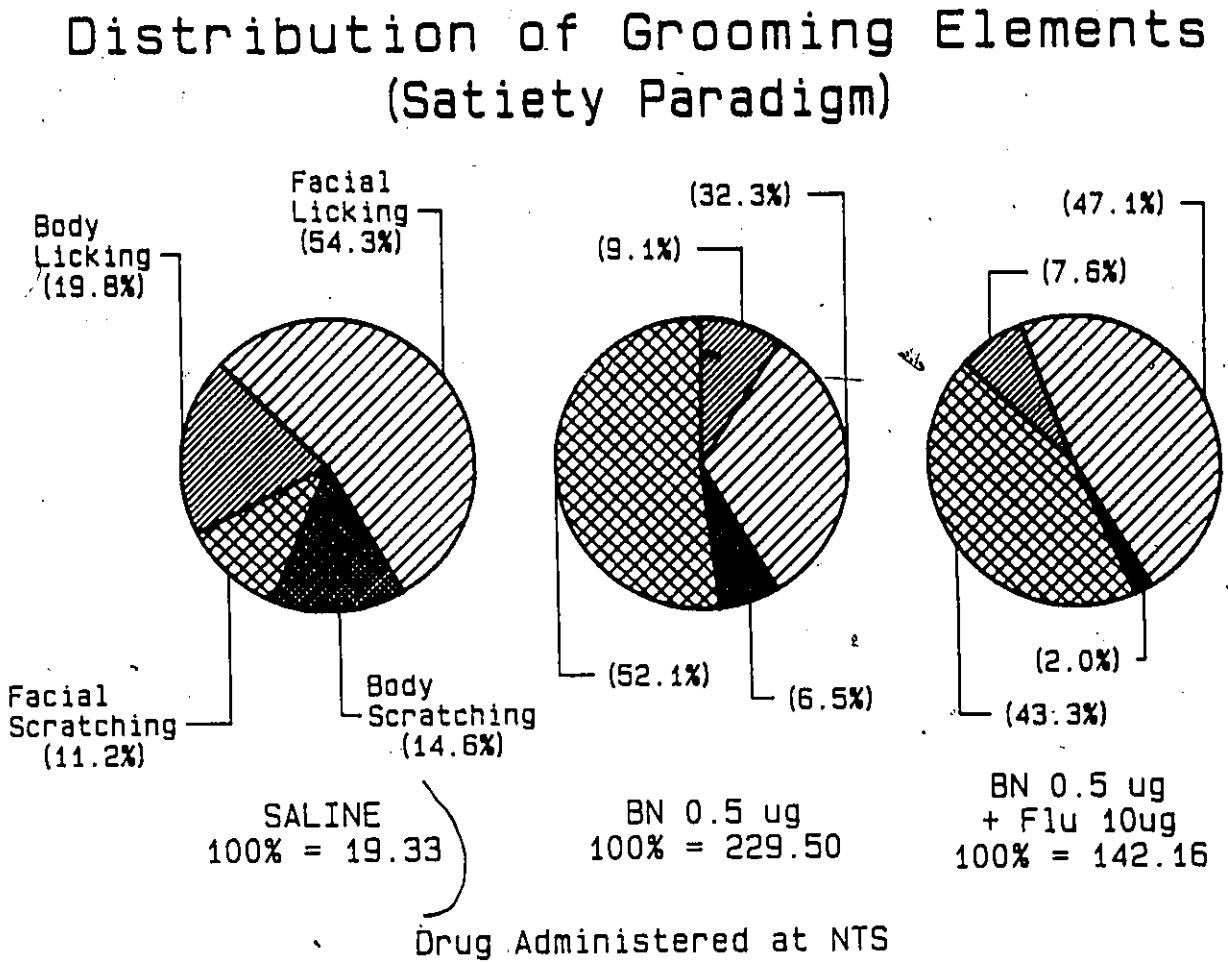

Figure 64: Distribution of the grooming elements, facial scratching, facial licking, body scratching and body licking, in the satiety paradigm after administration of saline, BN (0.5 ug) or BN (0.5) ug + fluphenazine (10 ug) intra NTS (n=12). The values are the mean score for each element as compared to the total mean grooming score expressed as a per cent, for each drug treatment.

Although naloxone results were negative, for Gmerek and Cowan (1983; 1986) they found that a kappa (opiate) receptor agonist antagonized BN induced scratching. Tolerance developed to the inhibition of BN-induced grooming by kappa opioids, suggesting that although there was an opioid link in this behavior it likely was physiological antagonism rather than receptor antagonism (Gmerek and Cowan, 1986). Neither tolerance to a repeated dose of BN (Kulkosky et al, 1982b; Gmerek and Cowan, 1983) or cross tolerance between BN and ACTH, or somatostatin (ODT8-SS) has been demonstrated (Gmerek and Cowan, 1983; Van Wimersma Greidanus et al, 1985a). Interestingly, one of these kappa agonists (U-50,488H) has also been reported to increase feeding duration in partially pre-sated rats (Jackson and Cooper, 1986). Thus, there may be an association between effects of kappa agonists on decreasing BN-induced grooming and increasing eating, suggesting the possibility that kappa agonists may also decrease the satiety induced by BN.

In addition, physiological antagonism of BN-induced grooming has been reported with diazepam and chlordiazepoxide (Crawley and Moody, 1983; Moody et al, 1987).

Several explanations for BN-induced increased scratching have been investigated. It was clearly a CNS mediated effect since scratching did not occur after peripheral injections (Kulkosky et al, 1982a, b). Furthermore it was independent of the pituitary adrenal axis

(Gmerek and Cowan, 1983). Scratching did not occur after BN microinjection at the lateral hypothalamus or the paraventricular nucleus of the hypothalamus (Stuckey and Gibbs, 1982; Willis et al, 1984). To date, the most sensitive CNS site is the NTS where there was a dose-dependent increase in scratching, with 0.01 ug being the threshold dose in the home paradigm (Figure 26) with a frequency of approximately 35% of the total possible score. At a dose of 1 ug BN-induced a frequency of scratching of approximately 60% of the possible total score (Figure 18). In addition BN intra NTS increased the frequency of facial licking to approximately 25-30% of possible total score at 1.0 and 0.5 ug (Figures 19, 27, 37 and 59). Pert et al (1980) injected BN intra PAG and found BN resulted in analgesia to hot plate suggesting a role in pain perception for BN, which is feasible since BLI and BN receptors are present in the PAG (Pert et al, 1979, 1980). The possibility that BN-induced scratching was mediated directly through the PAG was ruled out (Gmerek and Cowan, 1983; Gmerek et al, 1983), since BN intra PAG (A_{50} value 0.78 ug) has less effect than BN ICV (0.013 ug) or intrathecally (0.004 ug) to induce scratching. Furthermore Spruijt et al (1986), found BN ICV to induce more scratching behavior than BN intra PAG. In addition lesioning the PAG did not significantly reduce BN (ICV) -induced grooming suggesting that BN probably did not mediate its effects on grooming through the dorsal PAG. Thus it appeared that BN-induced grooming was not mediated

directly through the PAG. However, ACTH-induced grooming which resulted in a normal sequence of elements of grooming with increased bouts of grooming appeared to be mediated through the striato-nigro-collicular pathway. One component of this pathway is the striato-nigral GABAergic pathway. Thus, ACTH-induced grooming was not mediated solely through the substantia nigra. They found that the periaquaductal gray appeared to play a primary role in ACTH-induced grooming (Spriujt et al, 1986a,b). BN-induced grooming is clearly distinguished from ACTH-induced grooming neurochemically, neuroanatomically and behaviorally.

Bombesin has been found in dorsal root ganglia (O'Donohue et al, 1984, Panula et al, 1983; Fuxe et al, 1983) and in cranial sensory nuclei (Moody et al, 1981) suggesting BN may mediate its effects on grooming by influencing sensory perception at primary and/or secondary sensory neuronal synapses (O'Donohue et al, 1984). Moreover, the mammalian counterpart to BN, GRP-27 (injected intrathecally), has also been reported to induce scratching, with the major difference in the effect, between BN and GRP-27 being temporal. Scratching was induced by GRP-27 in half the mice for 14 min and by BN for 120 min (O'Donohue et al, 1984). O'Donohue et al, (1984), postulate that BN, ICV, may cause grooming and scratching by stimulating second order cranial sensory neurons which mimic a painful or irritating stimulation of the skin of the face and neck region. Spinal cord lamina I, II contain BLI, and BN-like-immunoreactive

fibers. Bombesin binding sites were observed in laminae I-III and perhaps IV of the dorsal horn from the cervical to the lumbar cord of cat, rat and mouse. Although the bite/scratch actions of substance P, BN and GRP-27, appeared similar initially, being directed to the abdominal region, the response to BN and GRP-27 soon generalized to other parts of the body and came to resemble a vigorous grooming behavior. Although cells in lamina I respond specifically to noxious and thermal stimuli and contribute fibers to the contralateral spinothalamic tract, lamina II (substantia gelatinosa) neurons appear organized to exert influences upon larger cells in lamina III and IV (Carpenter, 1985), possibly playing a modulatory role in transmission of sensory information (Wall, 1978). Thus, BN, in the dorsal horn of the spinal cord may cause increases in grooming, mostly directed at the head, ears and neck by potently modulating, pain and temperature and/or somatosensory perception of these areas.

Bombesin appeared to activate a facial grooming reflex, in the CNS, that was a closed sensory-motor circuit which once activated was very strong and repetitive and not environmentally dependent, either in relation to the satiety or home paradigm or in relation to denuded condition or anesthesia to the skin or irritation of the skin due to persistent scratching (Gmerek and Cowan, 1983). Fentress (1973; 1977) has observed that grooming proceeded independently of its effects on the organism and implied the

existence of a central nervous system generator for behavioral programs that can operate without the normal flow of sensory information.

Intrathecal administration of BN initially elicited body biting and scratching which quickly changed to facial scratching and licking (O'Donohue et al, 1984; Bishop et al, 1986). This facial grooming "reflex" appeared to be triggered by primary sensory spinal neurons and secondary sensory neurons projecting to supraspinal sensory nuclei such as the NTS and the spinal trigeminal nucleus, sensory nuclei of the face and neck. Several researchers have reported eliciting the scratch reflex with the same components (as BN) of increased facial scratching which appeared to be directed at the ear and just behind the ear as well as facial licking (Koenigstein, 1948; Feldberg and Fleischhauer, 1960; Domer and Feldberg, 1960). They elicited the scratch reflex with substances injected intracisternally (Koenigstein, 1948) or at the exposed dorsal surface of the lower part of the medulla oblongata and at the upper cervical cord in several species of mammal (Feldberg and Fleischhauer, 1960). The scratching movement suggested a selective activation of the long descending propriospinal neurons in the lateral column of the spinal cord which on stimulation of the skin (of the ear) mediate the scratching movements of the hind legs. The substances must act where the sensory fibres from the skin impinge either directly or through internuncials on large descending propriospinal

neurons (Feldberg and Fleischhauer, 1960). Transection of the medulla below the obex greatly reduced scratching (Domer and Feldberg, 1960). The results are in accord with the assumption that the drugs act at the afferent filaments of these nerve roots (possibly the trigeminal, the vagus and the upper cervical nerves) (Koenigstein, 1948) which are activated in the scratch reflex (Domer and Feldberg, 1960).

Recent studies comparing the A_{50} values of BN ICV or intrathecal, to induce scratching, reported very similar potencies (0.010 and 0.019 ug respectively) (Cowan et al, 1985). Bombesin intra NTS appeared to fall within this range thus it is questionable whether it is the most sensitive site possible. However of the brain sites tested the NTS is the most sensitive tested to date as compared to the CA4, NA, CE, FST, AON, (Johnston et al, 1986) and PAG (Gmerek and Cowan, 1983; Spriujt et al, 1986).

The involvement of the spinal trigeminal nucleus in sensory transmission in the medulla (Carpenter, 1985) as well as the presence of dense BN binding sites at this nucleus (Zarbin et al, 1985) suggest that it may be a important site for BN-induced scratching. Therefore it would be interesting to investigate behaviors after BN administration at the spinal trigeminal nucleus in the medulla. Further experiments could also be undertaken in order to identify more exactly the sensory neurons or interneurons (involved in the scratch reflex) which project

either directly or through internuncials on large descending propriospinal neurons.

The motor area involved in this grooming reflex may be the brain stem reticular formation (Norgren, 1983a). Recent experiments in our laboratory with cholinergic antagonists scopolamine and hemicholium that resulted in a decrease in grooming suggested that the cholinergic system may play a role in the mediation of grooming possibly in this area of the brain stem since injections were in the third ventricle.

Moreover, the CNS effects of BN include a significant decrease in resting which may involve the ascending reticular activating system rather than the ascending sensory pathways and/or pain and temperature pathway in the brain stem (Carpenter, 1985). Thus, BN may modulate arousal possibly through modulation of the noradrenergic system. Bombesin may effect adrenergic and/or noradrenergic neurons at the NTS where heavy innervation is evident (Hokfelt et al, 1984) at the location of our microinjection of BN (Paxinos and Watson -13.8 mm from bregma) in the lateral and medial caudal NTS to elicit its satiety and grooming effects. Hokfelt et al, (1984) have demonstrated the co-existence of adrenaline and neurotensin in the lateral caudal NTS and noradrenaline and neurotensin in the medial caudal NTS. In addition, the distribution of substance P, enkephalin and somatostatin and neurophysin immunoreactive nerve terminals and preterminal processes in the caudal part of the NTS was examined by the indirect immunofluorescence

method for immunocytochemistry combined with cytoarchitectural identification of nuclear subgroups in the same tissue. The medial subnucleus (our medial caudal area), the primary site for the termination of gastrointestinal afferents, showed substance P immunoreactivity in moderate amounts and weak immunoreactivity for the three other peptides studied (Kalia et al, 1984). Although several immunohistochemical studies have been carried out on the location of BN cell bodies, fibers and terminal areas, throughout the rat brain (Panula, 1986), none have concentrated on the subgroups of the caudal NTS checking for co-existence of BN with other neuropeptides and noradrenaline and/or adrenaline. Such a study would be most helpful in understanding the neuroanatomical location of BN in specific subnuclei since each area has been identified with certain neurophysiological functions such as respiration, heart rate, gustatory information and so on (Kalia et al, 1984; Hamilton and Norgren, 1984). In addition co-existence of BN with other neuropeptides and/or neurotransmitters such as adrenaline and noradrenaline, at the NTS may result in neurochemical co-modulation by these transmitters and BN of behaviors such as grooming and satiety which have been reported here.

CONCLUSION

In conclusion, locomotion grooming and satiety were induced by BN by different neuroanatomical and possibly neurochemical pathways. The stimulation of locomotion occurred site specifically at the NA (not at the NTS) and appeared to be to a significant degree dependent on the dopaminergic neuronal transmission both presynaptically (6-OHDA lesion, amphetamine) and postsynaptically (fluphenazine). It is possible that the small portion of BN receptors on dopamine terminals may modulate dopaminergic transmission. However, the relationship between dopamine and BN in the transmission of locomotion may be physiological, with both transmitters binding to their own receptor on the same neurons mediating locomotor activity in such a way that BN requires dopamine neurotransmission (receptor binding) to trigger a stimulation of locomotion. Bombesin administered, intra NA, did not significantly effect grooming or satiety behaviors.

In contrast, both grooming and satiety were markedly increased by BN microinjected specifically at the NTS. This BN-induced grooming appeared to trigger a reflex pattern of grooming that included facial scratching and facial licking which occurred independent of paradigm. In addition, fluphenazine injected either intra NTS, NA or administered systemically (IP) did not significantly effect (intra NTS), BN-induced satiety. Fluphenazine similarly administered

appeared to have a marginal effect on reducing this BN-induced grooming. Amphetamine microinjected at the NTS did not effect either grooming or locomotion. Thus, BN-induced grooming and satiety appeared to be mediated neuroanatomically and neurochemically by different mechanisms than locomotion.

Most interestingly, in the satiety paradigm, there was the dissociation of time-course and dose-effect of BN intra NTS on the grooming behaviors and satiety behaviors. This dissociation implies a different neuroanatomical substrate for BN-induced grooming and satiety. Bombesin intra NTS and IP demonstrated a similar time-course of effects on latency to eat and pellets left, suggesting that BN intra NTS may mediate a physiological satiety signal similar to that of BN IP. The neuroanatomical connections of gut visceral afferents to the caudal medial NTS indicate that the NTS may be the critical site where BN-IP induces its satiety effects. Furthermore, the greater efficacy and potency of BN intra NTS than IP in reducing eating support this hypothesis. Whether the caudal medial NTS is a critical site where peripheral and centrally (ICV) administered BN induce satiety remains to be tested.

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