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THE SHORT- AND LONG-TERM EFFECTS OF NEONATAL EXPOSURE TO BOMBESIN AND/OR [D-Phe^{6},ΨLeu^{13}-Cpa^{14}]BN(6-14)A BOMBESIN RECEPTOR ANTAGONIST.

A Doctoral Dissertation

by

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DEDICATION

This thesis is dedicated to my father David Piggins, and my mother, Susan Piggins (1937-1971).

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The nature of doctoral research dictates that it cover such a broad range of experiments and associated techniques that no one person can claim to have single-handedly conceived and executed his or hers thesis. This doctoral thesis is no exception; numerous people have contributed in a multitude of ways to its genesis and completion. Acknowledging them all is a tall order, particularly when one cannot be said to have the most reliable of memories, but here goes.

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SUMMARY

Bombesin (BN) is a tetradecapeptide originally isolated from the skin of the European anuran, Bombina bombina. BN-like peptides (and their receptors) have been found in the mammalian central nervous system (CNS) and peripheral nervous system (PNS). Central and peripheral administration of BN can elicit behavioural and physiological responses in adult rats. The overall purpose of these studies was to gain some insight into the possible physiological role(s) of BN-like peptides during ontogeny. The specific objectives of these experiments were: (1) to characterize the behavioural response(s) of developing rats to centrally and peripherally administered BN; and (2) to examine the short- and long-term consequences of neonatal exposure to BN and/or [D-Phe^{6},ΨLeu^{13}-Cpa^{14}]BN(6-14), a BN receptor antagonist.

Subcutaneous (s.c.) administration of BN (1-10 mg/kg) elicited grooming in rat pups of 1-10 days of age and the magnitude of this response decreased as a function of age. The form of grooming induced was qualitatively different from that seen following central injection of BN to adult rats. The decrease in behavioural sensitivity to BN probably reflected the declining permeability of the blood-brain barrier to BN. Indeed, intracerebroventricular (i.c.v.) injection of BN (0.01-1.0 ug) dose-dependently induced grooming in rat pups up to 20 days of age.

Furthermore, the 20 day old rat pups were the most and the 1 day olds the least sensitive to i.c.v. BN. Non-contact scratching activity was prevalent from postnatal day 1 and was replaced in the sequence of grooming behaviour by contact scratching activities by postnatal day 20. Scratching activities appeared to form a subsystem connected to but nonetheless separate from washing behaviours and this was particularly distinguishable at postnatal day 20. Changes in BN-induced grooming reflected the maturation of the motor capabilities of the developing rat.

Subchronic neonatal exposure to BN (5 or 10 mg/kg; s.c., twice daily for the first 8 postnatal days) had no effect on later behaviour displayed under mildly stressful or novel conditions or activity elicited by the open field or elevated plus maze. However, both saline and the high dose of BN (10 mg/kg) pretreatments increased adult sensitivity to central BN (0.1 ug; i.c.v.) as compared to non-injected but neonatally handled controls or those rats neonatally pretreated with the lower dose of BN (5 mg/kg). Neonatal pretreatments had no effect on later adult sensitivity to BN injected intraperitoneally (i.p.). Thus the observed effects appear to be due to long-term changes in central BN-receptor based mechanisms.

Subchronic neonatal exposure to [D-Phe^{6},ΨLeu^{13}-Cpa^{14}]BN(6-14), a BN receptor antagonist, under a regime of 5 and 10 mg/kg; s.c. twice daily for the first 8 postnatal

days, had no effect on the later expression of behaviour under mildly stressful or novel conditions or activity elicited on the open field test. However, neonatal pretreatment with the higher dose of [D-Phe^{6},ΨLeu^{13}-Cpa^{14}]BN(6-14) (10 mg/kg), had an apparent anxiolytic effect on later behaviour in the elevated plus maze. In contrast, the neonatal pretreatments failed to significantly alter the sensitivity to BN (i.c.v. or i.p.) in adulthood.

In conclusion, these data indicate that BN receptors in the rat c.n.s. are pharmacologically functional early in ontogeny, prior to the availability of measurable amounts of BN-like peptides. Behaviour induced by BN (s.c. or i.c.v.) reflects the status of motor capabilities of the developing organism and BN may serve as a useful pharmacological tool for investigating the ontogeny of grooming/scratching behaviour(s). Systems utilizing BN-like peptides are, to a degree, plastic early in ontogeny and altered adult sensitivity to BN i.c.v. can be achieved via subchronic exposure to BN during infancy. Endogenous BN-based mechanisms did not appear to play a role in the development and/or expression of behaviour(s) elicited under mildly stressful or novel conditions but may influence the behavioural regulation of anxiety-like responses (as measured by the elevated plus maze). The mechanisms subserving the above effects remain to be fully elucidated and warrant further research.

I. INTRODUCTION

A. Discovery and Isolation of Bombesin

Bombesin (BN) is one of a number of oligopeptides found in extracts of amphibian skin (see Erspamer (1984) for comprehensive review). Alytesin and BN, both tetradecapeptides, were first isolated from the skin of the European dicoglossid frogs Alytes obstetricus and Bombina bombina, respectively (Anastasi et al., 1971; Erspamer et al., 1972). Previously, Nakajima, Tanimura and Pisano (1970) had described the structure of a novel vasoactive decapeptide ranatensin from the skin of the American frog Rana pipiens and BN was found to show a similar heptapeptide sequence at the carboxy (or C-)terminal. Pharmacological studies indicated that both BN and ranatensin had similar actions in a number of intestinal and uterine preparations (Erspamer, Falconieri Erspamer and Inselvini, 1970; Erspamer et al., 1972; Geller et al., 1970; Van Clinesmidt et al., 1971) and thus these peptides were initially classified as belonging to the same family.

Subsequent research identified a number of other structurally related peptides of amphibian skin origin and

these include litorin (Anastasi, Erspamer and Endean, 1975) and phylollitorin (Yasuhara et al., 1983). The term "bombesin-like peptides" (BN-like peptides) was given to those peptides which shared a structural homology with the C-terminal heptapeptide of BN (International Meeting on Bombesin, Rome, Italy, 1987). To date, there are 13 known BN-like peptides of frog skin origin which can be divided into 3 subfamilies on the basis of structure and these are the bombesins, litorins and phyllotirins (see table 1 for sequences).

Erspamer and Melchiorri (1975; 1976) reported that bioassay and radioimmunoassay (RIA) studies indicated the presence of BN-like immunoreactivity in the mammalian gastrointestinal (GI) tract and chicken proventriculus (the avian equivalent of the GI tract). McDonald and colleagues (1979) isolated a 27 amino acid peptide from porcine non-antral gastric tissue. This peptide was coined gastrin releasing peptide (GRP_{1-27}) for its potent effects on gastrin secretion and was found to share C-terminal homology with BN. This was the first mammalian BN-like peptide to be discovered and further research indicated that structurally similar GRP was to be found in human lung tumours (Orloff et al., 1984), guinea pig gastric tissue (Shaw, Thim and Conlon, 1987) and avian proventriculus (McDonald et al., 1980). From the dog small intestine, three forms of GRP

Table 1. --The Amino Acid Sequences of Some Naturally Occurring Bombesin-like Peptides

Substance	Name	M.W.
Bombesin subfamily		
pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2	Bombesin	1620
pGlu-Gly-Arg-Leu-Gly-Thr-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2	Alytesin	1536
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	$pGRP_{1-27}$	2805
Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH_2	GRP_{18-27}	1120
Litorin/ranatensin subfamily		
pGlu-Gln-Trp-Ala-Val-Gly-His-Phe-Met-NH_2	Litorin	1085
pGlu-Val-Pro-Gln-Trp-Ala-Val-Gly-His-Phe-Met-NH_2	Ranatensin	1282
Gly-Asn-Leu-Trp-Ala-Thr-Gly-His-Phe-Met-NH_2	Neuromedin B	1132
Phyllolitirin subfamily		
pGlu-Leu-Trp-Ala-Val-Gly-Ser-Phe-Met-NH_2	Phyllolitorin	1020
pGlu-Leu-Trp-Ala-Val-Gly-Ser-Leu-Met-NH_2	[Leu^6]-Phyllolitorin	986

Underlined sequences demonstrate the degree of structural homology of the C-terminal heptapeptide. M.W. abbreviates molecular weight.
Amino acid abbreviations: Ala=alanine, Arg=arginine, Asn=asparagine, Gln=glutamine, Gly=glycine, His=histidine, Leu=leucine, Lys=lysine, Met=methionine, Phe=phenylalanine, Pro=proline, pGlu=pyroglutamic acid, Ser=serine, Thr=threonine, Trp=trptophan, Tyr=tyrosine, Val=valine.

consisting of 27, 23 and 10 amino acids were isolated (Reeveet al., 1983). The shortest one was labelled GRP_{18-27} (as its sequence matched that of the last 10 amino acids of the largest GRP) and its origin could not be attributed to the metabolism of the larger, 27 amino acid form.

Minamino, Kangawa and Matsuo (1983) isolated and characterized a decapeptide from porcine spinal cord which had structural and pharmacological properties similar to BN-like peptides. This peptide was named neuromedin B. Subsequent research indicated the presence of another decapeptide from porcine spinal cord which they called neuromedin C (Minamino, Kangawa and Matsuo, 1984). However, this peptide was found to be identical to GRP_{18-27} and the name neuromedin C has been abandoned in favour of the original GRP_{18-27} (International Meeting on Bombesin, Rome, Italy, 1987). More recently, larger 30 and 32 amino acid BN-like peptides were identified and characterized from porcine brain using antiserum against neuromedin B (Minamino et al., 1985). These two peptides contracted rat uterine preparations similarly to neuromedin B and were designated as precursors to the smaller neuromedin B. They have hence been named neuromedin B_{1-30} and neuromedin B_{1-32}.

Molecular biology techniques have been particularly fruitful in the identification of the origins and sequences of BN-like peptides (see Battey et al., 1988; Lebacq-Verheyden et al., 1990; Spindel and Krane, 1988 for recent

reviews). The gene encoding human GRP has been localized to chromosome 18 (Naylor et al., 1987) and the expression of this gene has been identified in large cell undifferentiated carcinoma of the human lung (Hamid et al., 1990). The processing of human prepro-GRP into GRP_{1-27} and related peptides (GRP_{1-17} and GRP_{18-27}) has also been characterized (see Reeve et al., 1988 for review). Similarly, the use of complementary deoxyribonucleic acid probes has allowed for the sequence of human neuromedin B propeptide and amphibian ranatensin propeptide to be characterized (Krane et al., 1988). Cross-species comparisons of these prohormones can indicate the evolutionary origins of the peptides (Spindel and Krane, 1988).

B. Distribution of BN-like Peptides

A variety of techniques have been employed to demonstrate the presence of BN-like peptides in the mammalian central nervous system (CNS). Brown and colleagues (1978) were the first to develop a RIA procedure to measure BN-like peptides in biological tissue and found high concentrations in the hypothalamus and GI tract. In the rat brain, Moody and Pert (1979) using rabbit antisera directed against BN/GRP found endogenous BN-like peptides to be of highest concentration in the hypothalamus with moderate levels in the thalamus, midbrain, pons/medulla,

striatum, cortex, and hippocampus. Low levels were observed in the olfactory bulb and spinal cord with no measurable amount in the cerebellum. With a combination of micropunch and RIA, a greater resolution in the neuroanatomical localization of BN-like peptides was achieved (Moody, O'Donohue and Jacobowitz, 1981). The highest levels of BN-like peptides were found in the hypothalamic nuclei (periventricular nucleus (PeriVN), paraventricular nucleus (PVN) and the suprachiasmatic nucleus (SCN)) as well as the central gray tissue of the midbrain, the nucleus tractus solitarius (NTS) and the substantia gelatinosa of the spinal cord. Considerably lower amounts were detected in the caudate nucleus, hippocampus, and cingulate cortex.

Panula and colleagues (Panula, Yang and Costa, 1982; 1984; and for review see Panula, 1986; Panula et al., 1988) have used immunocytochemical procedures to localize peptide immunoreactivity within CNS neurons. Using a colchicine pretreatment, neurons containing BN-like peptides were demonstrated throughout the hypothalamus, and pons/medulla (Roth, Weber and Barchas, 1982; Panula, Yang and Costa, 1982; 1984). In particular, immunoreactive cell bodies were found in the preoptic area of the hypothalamus (POAH), PVN, and SCN as well as the NTS and laterodorsal tegmental nucleus of the pons/medulla. High densities of BN-like peptide immunoreactive nerve fibres and nerve terminals have similarly been identified in a

variety of hypothalamic nuclei (POAH, SCN, and arcuate nucleus) as well as the parabrachial nucleus, NTS and trigeminal complex (Panula, 1986; Fuxe et al., 1983). Tracing studies indicate that medullary BN-like peptide immunoreactive nerve fibres could originate in the PVN (Panula et al., 1988). In the spinal cord, BN-like immunoreactivity was found mainly in the dorsal horn, particularly in the superficial laminae (layers 1 and 2) (Moody et al., 1981). The identification of messenger ribonucleic acids (mRNA) encoding the sequence of rat GRP (Lebacq-Verheyden et al., 1989) has allowed for the identification of neurons containing GRP mRNAs in the rat brain. Zoeller, Lebacq-Verheyden and Battey (1990) using in situ hybridization techniques identified mRNA for GRP in most of the above mentioned areas that show BN-like immunoreactivity although some areas such as the parvocellular layer of the PVN did not have GRP mRNA. Also, some areas were found to have GRP mRNA (e.g. ventral pallidum) which do not have BN-like immunoreactivity indicating that the prepro-GRP may not be processed to GRP or related peptides in these areas. In this regard, it is of interest that Wada and colleagues (1990) recently reported that mRNAs for GRP and neuromedin B were differentially distributed in the rat brain. Specifically, they found that mRNA for GRP was most prominent in forebrain structures (isocortex, hippocampus and amygdala) whereas the

highest levels of mRNA for neuromedin B were found in the olfactory bulb, dentate gyrus and dorsal root ganglion. These data raise the possibility that these BN-like peptides may be functionally distinct.

BN-like immunoreactivity was found in isolated rat brain synaptosomes and the release of BN-like peptides in a calcium-dependent manner was demonstrated in both hypothalamic and spinal cord slices (Moody et al., 1980; Moody et al., 1981; Moody, Korman and O'Donohue, 1986). Thus, BN-like peptides are found throughout the CNS of the laboratory rat and many other mammalian species such as man (Ghatei et al., 1984; Namba et al., 1985a) and guinea pig (Namba et al., 1985b) and have characteristics similar to those of known classical neurotransmitters.

In the mammalian GI tract, BN-like peptides are found only in enteric nervous tissue (see Furness, Miller and Costa, 1988 for review). Initial studies with RIA techniques indicated that the highest concentrations of endogenous BN-like peptides in the rat GI tract were in the stomach with moderate levels in the jejenum and ileum and lower amounts in the duodenum (Brown et al., 1978). Within the stomach, BN-like peptides were found in the nerve cell bodies and fibres of the myenteric ganglia, in the smooth muscle layers and in the mucosa of the non-antral and antral regions (Dockray, Vaillant and Walsh, 1979; Kuwahara et al., 1983, Moghimzadeh et al., 1983; Iwanga, 1985). In the small

intestine, BN-like immunoreactivity is found within the nerve cell bodies of the myenteric ganglia and immunoreactive fibres are found within both myenteric and submucous plexeses (Dockray, Vaillant and Walsh, 1979; Iwanga, Fujita and Yanahaira, 1983). The large intestine shows a similar pattern of innervation with the exception that in the rat, immunoreactive fibres are also found within the lamina propria of the mucosa (Moghimzadeh et al., 1983).

C. Binding Sites for BN-like Peptides

Specific, high affinity binding sites for BN-like peptides have been demonstrated within the mammalian c.n.s by both radioreceptor assay (Moody et al., 1978; 1980) and autoradiographic (Wolf et al., 1983; Wolf and Moody, 1985; Zarbin et al., 1985) techniques. Using rat brain homogenates, Pert and colleagues (1980) demonstrated that [^{125}I-Tyr^{4}]-BN bound specifically to high affinity binding sites (hereafter to be called BN receptors) which were highest in concentration in the hypothalamus and hippocampus. The specificity of this receptor site was suggested by the apparent necessity for structural homology of the carboxy terminal end of the peptide to ensure high affinity binding. Autoradiography studies employing [^{125}I-Tyr^{4}]-BN as a ligand allowed for more precise localization of BN-like peptide receptors in the rat CNS (Wolf et al.,

1983; Wolf and Moody, 1985; Zarbin et al., 1985). Collectively (as reviewed by Moody et al., 1988), these studies indicated high densities of these receptors in a variety of hypothalamic (SCN, PVN, and PeriVN), amygdaloid (lateral, central, medial and posterior cortical) and thalamic (centrolateral and paraventricular) nuclei as well as the dentate gyrus, CA4 region of the hippocampus, nucleus accumbens, anterior olfactory nucleus, and olfactory tubercle. Moderate densities of BN-like peptide receptors were identified in the frontal cortex, basal caudate-putamen, bed nucleus of the stria terminalis, zona incerta, locus coerulus, NTS as well as certain hypothalamic (anterior, medial preoptic and arcuate), amygdaloid (basolateral and basomedial) and thalamic (rhomboid) nuclei. Low densities were reported in a variety of hypothalamic regions (lateral, ventromedial, and dorsomedial), cingulate and parietal cortices, central gray area and substantia nigra pars reticulata. Grains were absent in the corpus callosum, globus pallidus, red nucleus, medial and dorsolateral geniculate nuclei as well as a number of thalamic (ventrolateral and anterocentral) nuclei. Recently, Ladenheim and colleagues (1990) differentiated BN receptor subtypes in the rat CNS by using various antagonists and [^{125}I-Tyr^{4}]-BN and [^{125}I-Tyr^{0}]-neuromedin B as ligands. Their studies suggest that the effect of BN-like peptides may be mediated by two different classes of

receptors: (1) one class with high affinity for BN or GRP and low affinity for neuromedin B, and (2) a second class with high affinity for neuromedin B and low affinity for BN or GRP. Their data indicate that the neuromedin B-type receptors were present within certain forebrain and thalamic nuclei (particularly the olfactory bulb and the central medial nucleus of the thalamus). There was a greater preponderance of the BN/GRP-type receptors in the nucleus accumbens, hippocampus, and hypothalamic nuclei. A distinction between receptor subtypes remains to be characterized in certain hindbrain structures. Thus, within the rat CNS, receptors for BN-like peptides appear most prevalent in limbic forebrain and midbrain areas as well as the neostriatum whilst pons/medulla and cerebellar regions have few if any measurable binding sites.

Within the mammalian GI tract, initial evidence for specific high affinity binding sites was demonstrated on dispersed guinea pig and human pancreatic acini utilising [^{125}I-Tyr^{4}]-BN as a ligand (Jensen et al., 1978; Scemama et al., 1986). Recently, autoradiographic studies have evidenced a high density of BN receptors on the circular muscles of the fundus and antrum with less dense grains on the circular muscles and submucosal layers of the small intestine (Moran et al., 1988). Specificity of binding relied on the carboxy terminal of the peptide and Nakamura and colleagues (1988) using [^{125}I]-GRP found cytoplasmic

receptors for BN-like peptides within parietal cells. Porcine gut has also been reported to show a similar distribution of BN receptors (Seybold et al., 1990). As with the CNS, recent studies indicate that the receptors for BN-like peptides in the GI tract may also be subdivided according to their affinity for BN/GRP or neuromedin B (Von Schrenk et al., 1989; 1990).

In this context, the recent cloning of the BN receptor gene from Swiss 3T3 cells is of considerable importance (Giladi et al., 1990; Segerson et al., 1990). These studies indicate that the protein encoded (receptor) has a structure characteristic of a G protein-coupled complex. This research should provide much needed insight into the possible mechanism(s) of action of BN-like peptides.

D. Effects on Food and Water Intake

Peripherally administered BN reduces food intake in a variety of species including the rat (Gibbs et al., 1979), turkey (Denbow, 1989), baboon (Figlewicz et al., 1985) and man (Muurhainen et al., 1983). Systemically injected BN was found to reduce the consumption of both solid and liquid food but not water in fasted rats (Gibbs et al., 1979). This reduction in food intake was followed by a normal, postprandial behavioural sequence of grooming, exploratory activity and apparent sleep. BN has thus been labelled a

putative satiety agent by some (Hsiao and Spencer, 1983; Kulkosky et al., 1981) but others have indicated that BN could have aversive effects (Deutsch and Parsons, 1981) and hence could be reducing food intake by inducing a state of general malaise (Deutsch, 1980).

Nonetheless, a considerable body of research indicates that BN could be a satiety agent (see McCoy and Avery, 1990; Gibbs, 1985; Gibbs and Smith, 1988 for recent reviews). Systemic BN does not affect the initiation of a meal or the ingestive motor act but rather reduces meal size and increases the intermeal interval (Gibbs et al., 1979; Hsaio and Spencer, 1983; Mindell et al., 1985; Weiner, Gibbs and Smith, 1984). BN elicited satiety does not require gastric distension (Martin and Gibbs, 1980; but see also Ewarts, Jones and Primi, 1990), is similar to satiety induced by the presence of food in the stomach (Gibbs and Smith, 1982) and is independent of BN's apparent inhibitory action on gastric motility (Hostetler, McHugh and Moran, 1989). Further, data from conditioned taste aversion studies are not conclusive as positive and negative results have been reported (Deutsch and Parsons, 1981; Kulkosky et al., 1981) and as indicated by Smith, Gibbs and Kulkosky (1982), conditioned taste aversion is not necessarily indicative of malaise per se.

The satiety properties of systemically administered BN are independent of adrenal (Gibbs, Kulkosky and Smith, 1981) or pituitary function (Stuckey, Gibbs and Smith, 1982). BN

induced satiety is unaffected by spinal cord transection at the level of sixth thoracic vertebra (Stuckey, Gibbs and Smith, 1982) or total subdiaphragmatic vagotomy (Smith, Jerome and Gibbs, 1981). These can be interpreted to indicate that BN induces satiety independent of its secretagog effects on cholecystokinin (CCK) release or gastric distension as vagotomy abolishes satiety induced by either means and points to the possibility that endogenous BN acting humorally may play a role in satiety.

Electrolytic lesions of the ventromedial hypothalamus (West et al., 1982; Geary, Smith and Gibbs, 1986) or dorsomedial hypothalamus (Bellinger and Beranardis, 1984) had no effect on systemic BN's satiety inducing action, further supporting a peripheral site of action. However, lesions of the NTS inhibit food intake suppression by systemic BN (Ladenheim and Ritter, 1989) indicating a possible gut-brain interaction in the modulation of these effects although BN does not appear to readily cross the blood-brain barrier. Central injection of a specific BN receptor antagonist blocks the satiety effect of systemically administered BN (Merali et al., 1988). Total neural disconnection of the gut from the brain does inhibit the satiety action of systemically administered BN implying some degree of CNS involvement in these effects (Stuckey, Gibbs and Smith, 1985). However, the effect of systemic BN on intermeal interval was still apparent following these

treatments thus implying that this aspect of satiety could be peripherally modulated.

Other BN-like peptides have been found to inhibit food intake when peripherally administered and these include GRP_{1-27} (Stein and Woods, 1982) and GRP_{18-27} (DiPaola and Gibbs, 1985). The mechanisms of action of these peptides remains to be studied but thus far, they appear to have similar if less potent effects than BN in numerous bioassays.

Centrally administered BN also inhibits food intake in a number of species including rat (Kulkosky, Gibbs and Smith, 1982a,b), baboon (Figlewicz et al., 1986) and turkey (Denbow, 1989). Early investigations indicated that BN infused into the lateral ventricles of food deprived rats reduced the amount of food consumed in the ensuing test period (Kulkosky, Gibbs and Smith, 1982a). However, this form of treatment also decreased water intake and elicited increases in other possibly competitive behaviours such as grooming. This effect on water intake was confirmed by further studies (de Caro et al., 1985). Avery and Calisher (1982) reported that intracerebroventricularly (i.c.v.) administered BN only affected water intake when tested in the presence of food; an effect corroborated recently by Flynn (1989). Hence, controversy exists in terms of the specificity of effect of i.c.v. BN on water intake.

However, numerous researchers have examined specific neural sites of action as well as extended dose-response curves to deal with this problem. Stuckey and Gibbs (1982) indicated that injections of BN into the lateral hypothalamus decreased food intake but not water consumption and did not significantly affect ongoing behaviour. Similarly, Willis et al (1984) reported that injections of BN into the PVN were effective at reducing food but not water intake (although Calisher and Avery, 1984 failed to confirm this).

Many studies point to a caudal brain stem site of action for BN's effect on feeding. Johnston and Merali (1988a) reported that low doses of BN injected intra-NTS reduced food intake with only higher doses eliciting other, possibly competing behaviours (e.g. grooming). De Beaupaire and Suandeau (1988) found both effects to be elicited at the same doses. Ladenheim and Ritter (1988) found that both food and water intake could be reduced by 4th ventricular injection of BN without affecting baseline activity. Flynn (1989) however indicated that the effect on water intake was contingent on the presence of food and that the reduction of food intake following 4th ventricular infusion of BN occurred without changes in ongoing activity. And further, area postrema and NTS lesions inhibit the food intake suppressive effect of 4th ventricular BN (Ladenheim and Ritter, 1989). Similarly, LaCour et al (1986) reported that

thermal lesions of the area postrema block the inhibition of food intake induced by BN infused into the lateral ventricles. Hence, these studies indicate that centrally administered BN can act to specifically reduce food intake and that the possible site(s) of action could be in the caudal brain stem or hypothalamus.

In the baboon, it has been found that intraventricularly infused BN reduces food intake without inducing grooming (Figlewicz et al., 1986). Other BN-like peptides have been found to influence food and water intake when administered centrally and these include neuromedin B_{23-32} and neuromedin B_{1-32} (Piggins, Lafreniere and Merali, 1989).

E. Effects on Grooming

Central but not systemically injected BN elicits vigorous grooming in the mouse (Katz, 1980) and rat (Brown, Rivier and Vale, 1977a). This response can be elicited following intrathecal (i.t.) injection (O'Donohue et al., 1984; Cowan et al., 1985) and via a variety of i.c.v. injection sites including: the lateral ventricles (Kulkosky, Gibbs and Smith, 1982), the 3rd ventricle (Merali, Johnston and Zalcman, 1983), the 4th ventricle (Ladenheim and Ritter, 1988) and intracisternally (Brown, Rivier and Vale, 1977a). The form of grooming elicited by central injection of BN is

characterized by vigorous scratching of the face, neck, and body flanks by the hindlimbs (Katz, 1980; O'Donohue et al., 1984; Merali and Piggins, 1990). This differs from that form of grooming elicited by central injection of adrenocorticotrophic hormone (ACTH) where the bout duration and frequency of expression of all grooming elements (washing, scratching, and licking) are enhanced (Gispen and Isaacson, 1981). Further, it differs from the scratching activity induced by substance P and somatostatin in terms of duration of behavioural activation (Gmerek and Cowan, 1983; Meisenberg and Simmons, 1986; O'Donohue et al., 1984). Tolerance to these effects does not appear to occur with repeated injection of BN (Gmerek and Cowan, 1983; Merali, Johnston and Zalcman, 1983).

A number of neurochemical systems have been implicated in the mediation/modulation of BN-induced scratching. These include kappa-opioid receptor based mechanisms (Gmerek and Cowan, 1982), cholinergic mechanisms (Merali, Kateb and Kateb, 1988) and dopaminergic systems (Merali, Johnston and Zalcman, 1983; Piggins and Merali, 1989; Van Wimersma et al., 1989). Neither adrenalectomy or hypophysectomy (Gmerek and Cowan, 1983) affect BN-induced grooming but central 6-hydroxydopamine lesions attenuate its expression (Merali, Johnston and Sistek, 1985).

In terms of sites of action, caudal brain sites appear to be the most sensitive to the effects of BN. Injections

of BN into several brain sites including the hippocampus, anterior olfactory nucleus, nucleus accumbens, lateral ventricle, and NTS indicated that the nucleus accumbens and NTS needed the lowest thresholds doses (Johnston, Parmashwar and Merali, 1986). Follow up studies revealed that the NTS was the most sensitive site for the elicitation of BN-induced grooming (Johnston and Merali, 1988b). Other researchers have also found the 4th ventricle to be a sensitive site for BN induced effects (Flynn, 1989; Ladenheim and Ritter, 1988; 1989) and the presence of BN-like peptides and BN binding sites in the spinal cord has led to the hypothesis that BN may modulate sensory processing in the spinal cord and brain (O'Donohue et al., 1984).

F. Effects on Locomotory Activity

Central but not peripheral administration of BN to laboratory rats elicits, in a dose-dependent fashion, non-stereotypic locomotory activity (Pert et al., 1980; Merali, Johnston and Zalcman, 1983; Merali and Piggins, 1990; Shulz et al., 1984). This effect appears to be dependent on both the degree of habituation and shape of the test cage employed (rectangular vs. circular) as one group of researchers have had difficulty reproducing this phenomena (Van Wimersma et al., 1984; 1989).

Using well-acclimatised animals in rectangular testing cages, dopaminergic mechanisms have been implicated in the mediation/modulation of BN-induced locomotory activity. Neuroleptics, haloperidol and fluphenazine, block BN-induced locomotory activity (Merali, Johnston and Zalcman, 1983) and central dopaminergic lesions attenuate its expression (Merali, Johnston and Sistek, 1985). Pretreatment with agents specific for the dopamine receptor subtypes indicate that although both subtypes are involved, the dopamine D_2 receptor subtype appears to play a larger role in the mediation of BN-induced locomotion (Piggins and Merali, 1989). However, unlike the stereotypic ambulatory activity induced by selective dopamine D_2 receptor agonists, BN elicited activity is non-stereotypic (Merali and Piggins, 1990), suggesting an indirect role for dopaminergic system(s) and the possible involvement of other neurochemical system(s).

In this regard, research on the sites of action indicated that when BN was injected into various brain sites, the nucleus accumbens had the lowest threshold for the elicitation of locomotory activity (Johnston and Merali, 1988b). Pretreatment with haloperidol blocked this rise in locomotory activity and microdialysis experiments showed that BN can elicit the release of dopamine at the level of the nucleus accumbens and caudate putamen (Merali et al., 1989).

G. Effects on Thermoregulation

Central but not peripheral injection of BN to adult laboratory rats has a multitude of effects on thermoregulatory mechanisms (Brown, Carver and Fisher, 1988). A variety of testing situations and parameters have been employed and a distinction between ambient temperature (Ta) and level of food deprivation roughly divides the methods of research.

In the cold-exposed rat (Ta=4°C), BN (minimal effective dose 1 ng) injected intracisternally induces a drop in core body temperature (i.e. hypothermia) (Brown, Rivier and Vale, 1977a). At Ta=24°C, BN also induces hypothermia whereas at Ta=36°C, BN elicits an elevation of core body temperature (Tache, Pittman and Brown, 1980). BN may also alter the set point around which behavioural activity is regulated (Stump, McCoy and Avery, 1990). Hence, BN induces poikilothermy in the laboratory rat and these effects appear unrelated to its known action on pituitary (Rasler, 1983) or adrenal (Hawkins and Avery, 1983) function.

In terms of mechanisms, BN reduces metabolism and reduces sympathetic activation of brown adipose tissue (BAT) leading Brown and colleagues to suggest that hypothermia elicited by BN may be secondary to its known action on cardiovascular function (Fisher, Cave and Brown, 1985a). At Ta near room temperature (18-24°C), BN reduces metabolism,

decreases BAT thermogenesis and may facilitate peripheral heat loss mechanisms (Lin and Lin, 1986; Shido, Noda and Nagasaka, 1987; Tache, Pittman and Brown, 1980). Injections of BN into specific brain sites indicates that at Ta=4°C, the POAH is a potential site of action (Pittman, Tache and Brown, 1980; Wunder et al., 1980). This site is implicated in the central mechanisms of thermoregulation and contains neurons responsive to changes in body temperature (Crawshaw, 1985; Gordon, 1990). In anaesthetized rats whose rectal temperature was maintained at 37°C, BN was found to suppress the activity of cold-responsive neurons and to facilitate the activity of warm-responsive neurons whilst having no effect on most thermoneutral neurons (Lin and Lin, 1986). Thus, BN may act at the level of the POAH to mediate heat production and heat loss mechanisms.

In the food-deprived rat, BN induces a naloxone reversible hypothermia at Ta=23°C (Avery and Calisher, 1982; Babcock, Barton and Keene, 1989) which is not attributable to changes in metabolism (Babcock and Wunder, 1984). These results suggest a different mechanism of action from BN's hypothermic effect on food-sated rats. Indeed, cold-exposure (Ta=11°C) will accentuate the hypothermic effect of BN in food-deprived rats (Babcock, Barton and Keene, 1989). BN also elicits hypothermia in food-sated, insulin-treated rats at Ta=23°C (Babcock et al., 1989), an effect unaccompanied by changes in peripheral heat loss (Babcock

and Barton, 1989). Refeeding can attenuate BN-induced hypothermia in either insulin-treated or food deprived rats (Barton and Babcock, in press). In both situations, the POAH may be the site of BN's action (Babcock, Barton and Keene, 1989; Babcock and Barton, 1989; Barton and Babcock, in press) although the substantia nigra and PVN have also been implicated (Babcock and Barton, 1990; Calisher and Avery, 1984). Therefore, in the case of food-deprived and insulin-treated rats, the mechanisms subserving BN elicited hypothermia remain to be elucidated.

H. Effects on Cardiovascular Function

Central but not peripheral injection of BN influences cardiorespiratory mechanisms (Brown, Carver and Fisher, 1988). Central injection of BN to rats at room temperature decreases heart rate and increases mean arterial pressure (Fisher and Brown, 1984; Fisher, Cave and Brown, 1985a). Adrenalectomy does not affect the decrease in heart rate following i.c.v. BN but prevents the rise in mean arterial pressure indicating that the change in heart rate is not a baroreflex-mediated effect (Fisher, Cave and Brown, 1985a). This bradycardia effect of central BN could well be related to the known effects of central BN on adrenomedullary epinephrine secretion (see below) as phentolamine, an α-adrenergic receptor antagonist prevents BN-induced

elevations of mean arterial pressure. BN-induces bradycardia partly through apparent increases in the parasympathetic-cholinergic outflow to the heart as methylatropine partially reverses this effect (Fisher, Cave and Brown, 1985a).

In cold-exposed rats, central injection of BN prevents cold-induced tachycardia but has no effects on cold-induced elevations of arterial pressure (Fisher, Cave and Brown, 1985b). Central BN also reduces sympathetic outflow to brown adipose tissue (see above) and reduces oxygen consumption in cold-exposed rats (Brown, 1982; Brown, Allen and Fisher, 1987). These researchers point to decreased activation of the sympathetic nervous system by BN as a probable explanation for some of the effects of central BN on thermoregulation.

I. Endocrine and Exocrine Effects

1. Gastrointestinal Secretions

Systemic administration of BN has a number of effects on GI secretions. These include: stimulation of gastric acid and gastrin release (Bertaccini, Erspamer and Impicciatore, 1973; Tache et al., 1981; Walsh et al., 1981), somatostatin release (Bloom, Edwards and Ghatei, 1983), CCK release (Erspamer et al., 1974; Ghatei et al., 1982; Namba et al., 1984), enteroglucagon release (Namba et al., 1984), neurotensin and vasoactive intestinal polypeptide (VIP)

release (Ghatei et al., 1982). Central injection of BN also influences the release of a number of gastric secretions. These include inhibiting gastric acid secretion and stimulating gastrin release (Tache et al., 1981; Tache and Collu, 1982).

2. Pancreatic Secretions

Systemic injection of BN influences the release of a number of pancreatic secretions. These include stimulating amylase release (Erspamer et al., 1974), pancreatic polypeptide release (Bloom, Edwards and Ghatei, 1983), insulin and glucagon release (Ghatei et al., 1982; Greeley and Thompson, 1984). The effect of BN on dispersed pancreatic acini has been used as a bioassay for the measurement of the binding potencies of recently developed BN receptor antagonists (Von Schrenk et al., 1990).

3. Adrenal and Pituitary Secretions

Systemic administration of BN induces adrenal corticosterone release (Sander and Porter, 1988). Central injection of BN stimulates adrenal epinephrine release which secondarily increases plasma glucose levels, stimulates glucagon release and causes a relative decrease in insulin secretion (Brown, Rivier and Vale, 1977c). Adrenalectomy abolishes these responses (ibid, 1977c). Similarly, lateral hypothalamic lesions abolish the hyperglycaemic response to BN i.c.v. indicating that the hypothalamus could be mediating this effect (Gunion et al., 1984). Further,

injections of BN into hypothalamic areas indicated that the VMH and LH were possible mediatory sites for the hyperglycaemic effect (Iguchi et al., 1984). Recently, intra-PVN injections of BN were found to result in elevated blood glucose levels as well as increased concentrations of blood free fatty acids and corticosterone (Gunion et al., 1989). Hence, a number of hypothalamic sites could mediate the endocrinological responses to centrally injected BN.

J. Gastrointestinal Motility Effects

Peripherally administered BN affects GI motility. Systemic BN increases stomach antrum motility (Bertaccini and Impicciatore, 1975; Fox and McDonald, 1984), decreases gastric emptying (Chiba et al., 1980; Martindale et al., 1982) and inhibits small intestinal motility (Bertaccini and Impicciatore, 1975; Fox and McDonald, 1984). In vitro, locally applied BN increases antrum motility (Kantoh et al., 1985), as well as small and large intestinal motility (Erspamer et al., 1972). BN injected centrally increases antrum (Spencer and Talman, 1987) and small intestinal motility (Fulginiti et al., 1984) and decreases gastric emptying (Porreca and Burke, 1983; Gmerek and Cowan, 1984).

The relation between BN's effects on the GI tract and its anorexogenic action has yet to be fully examined. Adrenalectomy, which abolishes the hyperglycaemic response

to central BN has no effect on i.c.v. BN's food intake effect (Gibbs, Kulkosky and Smith, 1981). Peripherally administered BN's satiety appears different from that induced by systemically administered CCK or glucagon in that it is not abolished by vagotomy (Smith, Jerome and Gibbs, 21981). Further, it is also unrelated to BN's action on gastric motility (Hostetler et al., 1989). Thus, provisional evidence suggests that BN's effects on ingestive behaviour may be unrelated to its action on gastric and pancreatic functions.

K. Trophic Effects

BN-like peptides have been implicated as potential growth factors in the regulation of cellular growth and division (see Lebacq-Verheyden et al., 1990 for recent review). In vitro, exogenously applied BN or GRP stimulated cell division and DNA synthesis in the non-tumourigenic murine Swiss 3T3 cell line in a dose-dependent manner (Rozengurt and Sinnett-Smith, 1983). This effect was associated with a high affinity cell surface binding site and a largely intact C-terminal heptapeptide sequence was required for the mitogenic effect of BN (see Rozengurt and Sinnett-Smith, 1990 for review). BN was also found to stimulate the growth and development of embryonic avian otic vesicle cells in vitro (Represa et al., 1985). These

phenomena coupled with the potential role of BN in the development of the human lung and lung disease (Johnson et al; 1982) have lead to the interesting notion that BN-like peptides may be of considerable importance in cellular growth and development.

In vivo, chronically administered BN leads to the proliferation of gastrin cells in rat antral mucosa (Lehy et al., 1983). Further, chronic administration of BN or GRP results in pancreatic cell hypertrophy in both rat and mouse (Damge et al., 1988; Dembinski et al., 1990; Lhoste et al., 1985a,b; 1989). These experiments indicate that BN-like peptides can act directly on target tissue cells and effect changes in cellular growth and division mechanisms. In this context, Getz, Moody and Rosentein (1987) found increased binding sites for BN-like peptides in fetal neocortical tissue transplanted into the 4th ventricle of an adult rat, implying that BN-like peptides in the host CNS induced receptor proliferation. Hence, BN-like peptides have been implicated as possible growth factors in a variety of biological tissues.

II. OBJECTIVES

The preceding literature survey clearly illustrates that BN-like peptides (and their receptors) are found within the CNS and PNS of a number of mammalian species. Further,

it is quite apparent that central or peripheral injection of BN-like peptides can elicit behaviour and can influence a number of physiological processes. However, the physiological roles of BN-like peptides have yet to be elucidated. In this regard, it is of considerable interest that the vast majority of studies surveyed in the preceding sections employed adult animals only, the actual development of behavioural and physiological responses to BN-like peptides has yet to be examined. Gillati and Moody (1984) established that BN receptors were present in the CNS of the developing rat prior to the availability of measurable amounts of the peptide. The first series of experiments examined if these receptors were pharmacologically functional in a behavioural sense. The second experiments attempted to ascertain the long-term outcome of activating or inactivating these receptors early in ontogeny.

The overall objective of these studies was to gain some insight into the possible physiological role(s) of BN-like peptides during ontogeny. The specific objectives were: (1) to characterize the behavioural response(s) of developing rats to centrally and peripherally administered BN; and (2) to examine the short- and long-term consequences of neonatal exposure to BN and/or [D-Phe^{6},ΨLeu^{13}-Cpa^{14}]BN(6-14), a BN receptor antagonist.

III. SHORT-TERM EXPERIMENTS

A. General Introduction

Grooming or maintenance behaviour is a common, species characteristic, movement pattern with readily definable components. In the laboratory rat, grooming behaviour may occupy as much as 25-40% of the awake time, depending on the housing conditions, with most of the behaviour seen just prior to and after the diurnal sleep period (Bolles, 1960). In terms of execution, rodents groom by licking their forepaws and then wiping them over the face. This is then followed by body licking and anogenital licking. Scratching of the head and body regions by the hindpaws occurs towards the end of this cephalocaudal sequence (Richmond and Sachs, 1980). Ethologically, grooming behaviour under high arousal states (such as novel or stressful situations) has been described as a displacement activity that may be essential in the restoration of homeostatic status (Cohen and Price, 1979). In this context, grooming has been considered as an index of behavioural adaptation to stressful conditions. Grooming behaviour is sometimes designated as "care of body surfaces" (Borchelt, 1980), maintaining the boundary between the external and internal milieu (removal of dirt, ectoparisites, and other foreign substances). Grooming then is an important activity in the behavioural repertoire of the rodent.

The most overt behavioural response elicited by centrally administered BN is grooming. In the adult rat, the grooming response is characterized by: head washing, body washing, head scratching, body scratching and anogenital licking. Sachs (1988) has pointed out that these responses (washing and scratching) may in fact be the resultant activation of two grooming "systems". In the spontaneous grooming of the developing rat, the washing movements could be observed in rats as young as 1 day (day of birth=0) and the progression of these behavioural elements of grooming followed a cephalocaudal sequence (similar to that seen in the adult rats). In contrast, scratching of the body flanks and various head regions did not appear until day 9 and did not become coordinated until much later. Indeed, they noted that scratching appeared to almost interfere with the washing style of grooming.

Although much research has been focused on the behavioural effects of BN, the bulk of studies have used adult rats. The actual development of the behavioural and physiological responses to BN has not been studied to any great degree. One short study suggests that in the 5 to 20 day old rat systemic administration of BN elicits grooming and scratching, a response only seen following central injection in the adult rat (Jackson and Kitchen, 1989a). This implies that the blood brain barrier is permeable to BN at this stage in development. In terms of the appearance of

BN and BN receptors in the CNS, Gillati and Moody (1984) showed that BN receptors reach adult-like levels by 10 days postpartum while the peptide itself reaches mature concentrations by 20-23 days postpartum. Behaviourally, grooming was demonstrated within the first week of the neonates life with quantifiable satiation effects occurring at day 15 (Jackson and Kitchen, 1989a). BN would then appear to be behaviourally active at an early stage in development.

The response profiles of other grooming inducing agents have been investigated in rats of various ages. Isaacson and colleagues showed that the grooming response to codeinone, a synthetic opioid, was significantly greater in male rats of 21 to 40 days of age as compared to older males (> 40 days of age) and similar aged females (Isaacson et al., 1987). Unfortunately, the researchers did not investigate the effects of codeine on rats younger than 20 days of age. Kirstein et al. (1990) found that 4 day old rat pups groomed in response to an ACTH peptide, ACTH1-16$_{NH2}$ though not as robustly as 21-22 day old rats. Interestingly, the form of grooming elicited in the 21-22 day old pups was different from that induced by this peptide in adult rats. A similar developmental trend has also been reported for oxytocin-induced grooming (Pedersen et al., 1988). Thus, developmental changes in peptide-induced grooming are apparent.

Physiologically, the rat undergoes profound changes in thermoregulatory control during the first 6-7 weeks of life (Adolph, 1957; Leon, 1986). The infant rat is capable of increasing metabolic rate in response to a drop in ambient temperature (Taylor, 1960) but due to its lack of insulatory capacity (e.g. fur), it loses body heat very quickly and hence is reliant on the mother and litter mates to maintain body temperature. Hypo- or hyperthermia can have significant effects on behavioural activity in the infant rat and the maintenance of a suitable environmental temperature is important for the accurate assessment of behaviour in the infant rat (Moran, 1986).

Central injection of BN to adult rats elicits changes in body temperature (Brown et al., 1977a), dependent on the ambient temperature (Tache et al., 1980). The thermoregulatory effect of BN has not been examined in the developing rat and given that alterations in body temperature affect behavioural activation, the relation of BN's behavioural effects to its effect on body temperature is of obvious import.

B. Experiment 1a: Subcutaneous Injection of BN to Developing Rats

1. Purpose

The overall objective of the first experiment was to characterize the acute effects of BN administration (s.c.) on the expression of grooming and exploratory behaviours during early ontogeny.

2. Methods

a) Animals

Rat pups were obtained from timed pregnant Sprague Dawley CD females (Charles River, St. Constant, Quebec) as well as from Sprague Dawley CD females bred in our own colony. On the day of birth (day=0), litters were standardized to 8 pups per dam with each litter containing equal numbers of males and females. Pups were kept with the mother until the day of testing (1, 5 or 10 postnatal day). The colony room environment was maintained at 20°C with a relative humidity of 60% and a 12 hr light:dark cycle (lights on 07:00 hr).

b) Experimental Procedures

On the day of testing, pups were removed from the mother, weighed and placed into clean, individual monitoring cages (15 x 13.5 x 21.5 cm for 1-10 day olds and 15 x 27 x 21.5 cm for 20 day olds). The temperature of the base of

the cage was maintained by an electric water blanket (American Medical Supplies, model K-20-C) to 33°C for 1 day olds, 30°C for 5 day olds and 28°C for 10 days). Animals were allowed 30 min to adapt to the new cage before the experimental protocol. All manipulations were performed between 10:00 and 16:30 hrs and each animal was used only once. Treatments were divided across litters in a pairwise Latin Square design such that each litter contained four treatment groups and contributed a male and female to each.

BN (Peninsula Laboratories, CA) was freshly dissolved in saline and injected subcutaneously (s.c.) in the nape of the neck over a dose range of 1 to 10 mg/kg with a saline injection serving as the control condition. BN was injected in a volume of 0.1 ml / 20 g of body weight. The injection apparatus consisted of the shaft of a 30 gauge needle connected to PE 20 plastic tubing which in turn was connected to a Hamilton syringe (100 or 250 µl). This system allowed for the slow infusion (approximately 10 µl/ s) of the peptide solution in an unrestrained pup.

The behaviour of the pups was assessed via time sampling procedure approximately 5 min after the injection. Briefly, head scratching, head washing, body scratching, body washing, sniffing, resting, and exploratory activity were scored for 2 s every 16 s over the course of 60 min following peptide administration (see table 2 for operational definitions).

Core body temperature was measured at the conclusion of the behavioural recording sessions. A vaseline lubricated thermistor probe (YSI series 700, model 702a) connected to a Digi-Sense®-JKT thermocouple thermometer was inserted approximately 3.5 cm into the rectum of the pup. The temperature was recorded, the probe withdrawn and the pup sacrificed.

c) Statistics

The frequency scores of the grooming elements as well as core body temperature were analyzed via the IBM PC statistical package SYSTAT. A 2-way analysis of variance (age x dose) design was employed. Post hoc analyses were provided by the Tukey test (n=32 per age, 8 pups per dose) with significance defined at $p<.05$. For relevant F-ratios, see table A-1 of Appendix A.

Table 2. --Behaviours Examined in Experiment 1a.

Behavioural Element	Symbol	Operational Definition
Head Washing	HW	Forepaws are brought to the face licked (perhaps) and wiped over the face and crown.
Head Scratching	HS	A hindlimb is elevated and a scratching motion directed to the ipsilateral side of the head ensues.
Body Scratching	BS	A hindlimb is elevated and a scratching motion directed to the ipsilateral body flank ensues.
Resting	REST	The animal remains stationary and/or asleep.
Exploratory Activity	EXP	The animal locomotes (including crawling) and appears to orient to a particular site in the cage.

3. Results

No differences in the responses to BN between male or female rat pups were found (data not shown) and these were then summed for the overall analysis. All doses of BN significantly increased the expression of head washing and decreased resting in the 1 day old rat pups (see table 3). The higher doses of BN (5 and 10 mg/kg) elicited significant increases in the expression of head and body scratching with the 1 and 10 mg/kg doses inducing a significant increase in exploratory locomotion (see upper panel of figure 1 for the total scratching score).

In the 5 day old pups, the 10 mg/kg dose of BN significantly increased head washing, head and body scratching and decreased resting. In the 10 day old pups, the 10 mg/kg dose of BN induced a significant increase in head washing and significantly decreased resting.

With all doses of BN (1-10 mg/kg s.c.), 1 day old pups expressed more head washing and rested less than 5 and 10 day old pups. 1 and 5 mg/kg doses of BN induced significantly more body scratching in 1 day old pups than older pups. At the highest dose of BN (10 mg/kg; s.c.), 1 day olds body scratched more than 10 day old animals. At the 5 and 10 mg/kg doses of BN s.c., 1 day old pups head scratched more than 10 day old pups. The total scratching score, obtained by adding the head and body scratch scores

Table 3. --The Behavioural Effects of Subcutaneously Administered Bombesin (BN) in Infant Rats.

AGE (DAYS)	DOSE of BN (mg/kg;s.c.)	HW	HS	BS	REST	EX
1	Saline (0.0)	15.6 ±2.6	7.5 ±2.2	16.6 ±3.2	205.9 ±2.7	5.9 ±2.1
1	1	34.4* ±2.4[d]	15.6 ±4.1	40.3 ±5.5[d]	175.3* ±5.4[d]	18.8* ±5.9[d]
1	5	73.3* ±4.1[d]	25.9* ±5.6[c]	61.3* ±7.3[d]	125.2* ±7.5[d]	13.0 ±2.8
1	10	91.9* ±8.3[d]	35.6* ±8.5[c]	72.8* ±8.0[c]	104.1* ±7.3[d]	19.5* ±4.8[d]
5	Saline (0.0)	5.6 ±0.9	2.8 ±0.8	8.1 ±1.2	212.9 ±0.9	5.6 ±1.4
5	1	10.8 ±2.0	1.9 ±0.8	10.6 ±2.8	212.4 ±2.3	5.4 ±1.1
5	5	19.8 ±3.4	8.5 ±2.3	26 ±5.9	190.1 ±8.2	5.6 ±1.1
5	10	41.9* ±7.5[c]	20.6* ±6.4	55.6* ±12.9[c]	157.4* ±14.2	4.5 ±1.1
10	Saline (0.0)	1.8 ±0.4	1.9 ±0.5	3.4 ±0.8	215.5 ±1.4	2.5 ±0.5
10	1	2.8 ±0.9	2.5 ±0.7	4.1 ±1.1	211.5 ±2.0	5.6 ±0.8
10	5	7.4 ±1.1	3.8 ±1.1	8.5 ±1.5	207.1 ±2.3	5.8 ±1.4
10	10	21.6* ±3.9	12.4 ±3.6	16.6 ±3.1	186.6* ±6.1	7.9 ±1.1

Cells contain the mean±sem. * $p.<05$ from respective age group baseline. [c] $p<.05$ from 10 day old group at that dose. [d] $p<.05$ from 5 and 10 day olds at that dose.

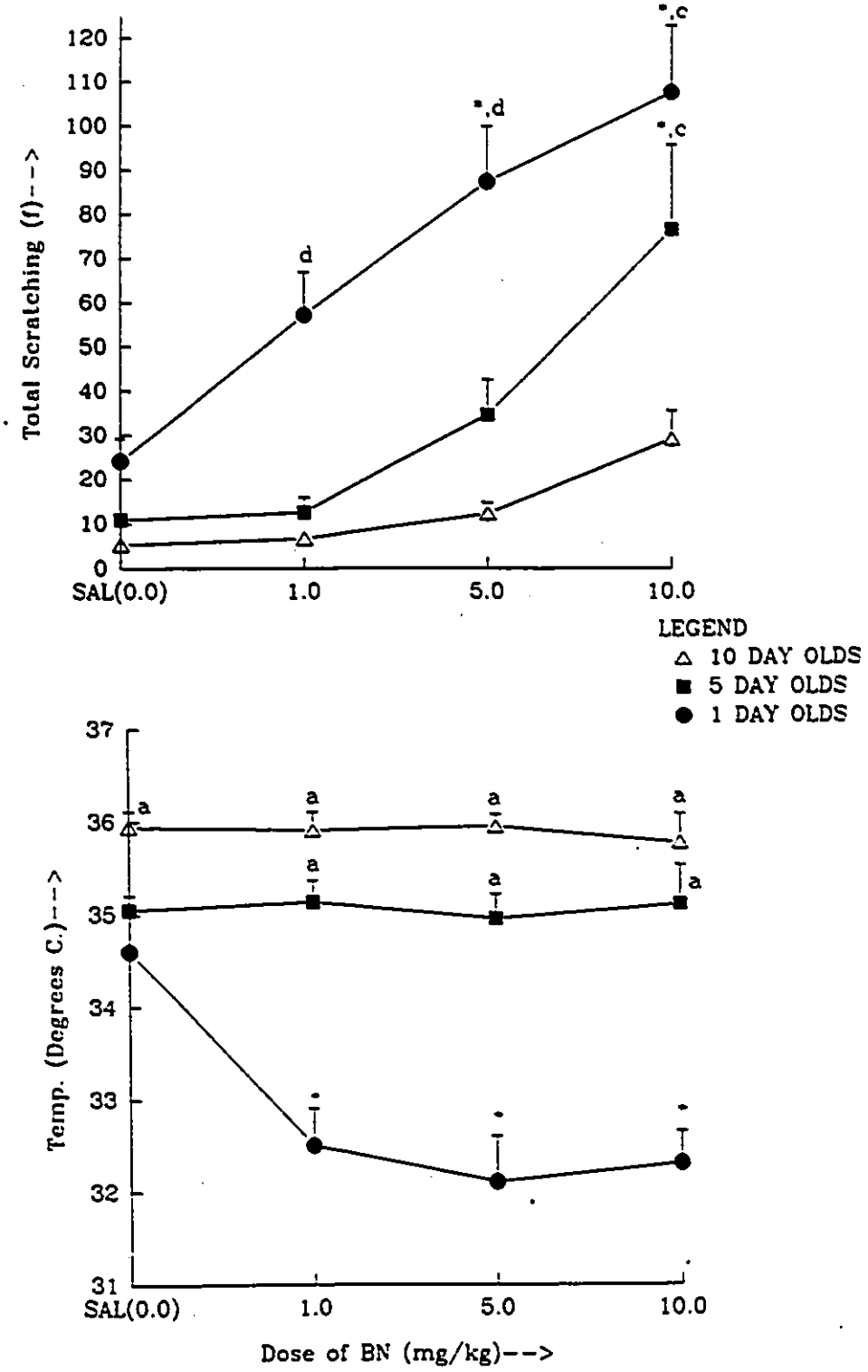

Figure 1. Top panel: BN-induced scratching in rat pups. * p<.05 from respective group baseline. Data points represent mean±sem, n=8/data point. [c] p<.05 from 1 day olds. [d] p<.05 from 5 and 10 day old pups at that dose.

Bottom panel: The effect of BN on core body temperature. * p<.05 from respective group baseline. [a] p<.05 from 1 day old pups core body temperature at that dose. Data points represent mean±sem, n=8/data point.

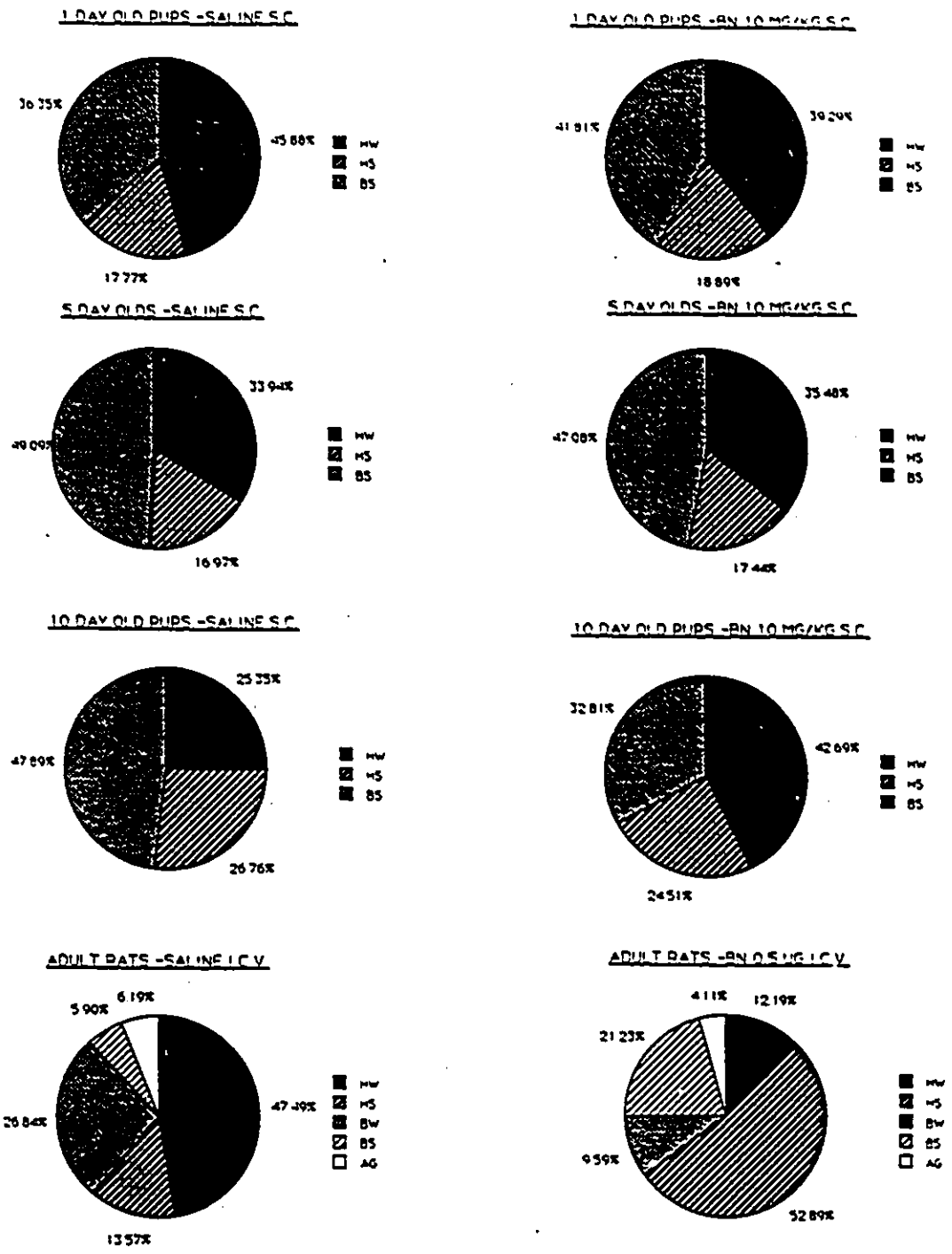

Figure 2. The composition of grooming elicited by saline or BN. Data represent the per cent contribution of grooming elements to the total grooming score.

(as shown in figure 1), illustrates this trend. One day old animals explored more frequently than 5 day old pups in response to the 1 and 10 mg/kg doses of BN (see table 3).

Figure 2 shows the differences in the composition of grooming across ages under saline and BN s.c. conditions. Comparing these data to those of a previous study indicates that in adult rats, BN i.c.v. elicits mainly head scratching while in the developing rat, BN s.c. induces predominately body scratching and head washing.

BN (1-10 mg/kg s.c.) significantly lowered core body temperature in 1 day old pups only (see figure 1; lower panel). The baseline core body temperature of 1 day old pups was significantly lower than that of 10 day old pups.

4. Discussion

BN elicited pronounced increases in washing and scratching behaviours and reduced resting in the 1 and 5 day old rat pups with less overt behavioural effects on the 10 day olds. BN induced exploratory locomotory activity in the 1 day old pups and 1 day olds were generally more active than the oldest group tested. These results are in broad agreement with those of other researchers (Jackson and Kitchen, 1989a) although some differences do exist. Principally, robust behavioural activation in 10 day old animals following BN s.c. was not evident in this study whereas Jackson and Kitchen (1989a) found that pups of this

age would scratch and wash in response to BN i.p.. Possible explanations for such differences include the route of administration (s.c. versus i.p.), different animal population and differences in behavioural assessment techniques. These authors use a time sampling interval of 5 s whereas a 2 s interval was employed in the present experiments. A consequence of this is that the behavioural scores reported by Jackson and Kitchen (1989a) were very much lower than those in this study with obvious statistical implications.

Scratching and washing behaviours were observed under baseline conditions for all ages of pups. This is in contrast to a previous study of the ontogeny of grooming behaviour (Richmond and Sachs, 1980) which reported that rat pups observed in litters performed washing movements from day 1 and scratching movements from postnatal day 6. These differences could be due to variations in the recording situation (isolation versus in litter) and stress associated with the injection procedure. In keeping with other reports (Golani and Fentress, 1985), both contact and non-contact scratching and washing movements were observed in 1 and 5 day olds though these were not directly quantified.

The results indicate a decline in behavioural responsiveness to peripherally administered BN as a function of age. Two of the possible reasons for this are that 10 day old pups are less sensitive to the behavioural effects

of BN or that maturation of the blood-brain barrier reduces the amount of systemic BN that penetrates the CNS.

In this regard, it is of interest that BN s.c. induced hypothermia in 1 day old pups only. Under baseline conditions (saline s.c.), the 1 day old animals had a core body temperature of 34.6°C which is low in the thermoneutral zone for animals of this age (Taylor, 1960). These pups could thus be mildly hypothermic despite efforts to maintain ambient temperature in the thermoneutral zone. Indeed, Takano and colleagues (1979) reported that 35°C was the threshold to induce hypothermia in young rats. The 1 day old animals appear hypothermic in response to BN s.c. and this is similar to the response of cold stressed adult rats in response to intracisternally administered BN (Brown et al., 1977a). In adult rats, this response is thought to be attributable to BN inhibiting sympathetic outflow and metabolic responses to cold exposure. The 1 day old pup is capable of increasing oxygen consumption in response to declining ambient temperature and this could be the thermoregulatory mechanism affected by BN. The hypothermic effect of BN was not observed in the 5 and 10 day old pups and this could be attributable to either s.c. BN not penetrating the CNS in large enough amounts to affect metabolic processes or the older animals were at thermoneutrality. The core body temperature of these older pups of 35.1° and 35.9°C resepectively, was well within the

thermoneutral zone for animals this age (Taylor, 1960; Fowler and Kellogg, 1975; Conklin and Heggeness, 1971) and hence BN s.c. would not be expected to affect these animals thermoregulation.

This study indicates that BN administered s.c. elicits activity in young rats that is only seen in adult rats in response to i.c.v. administered BN. When these activities are summed to yield a composite total grooming score, it is apparent that grooming seen under saline s.c. conditions and BN s.c. in young rats is different from that observed following saline i.c.v. and BN i.c.v. in adult rats. In particular, the body washing behaviour seen following saline i.c.v. in adult rats is lacking in saline s.c. and BN s.c treated developing rats. This probably reflects the inability of rats of this age group to perform this motor movement. In addition, i.c.v. BN in the adult rat elicits a scratching dominated form of grooming whereas grooming following saline i.c.v. is composed largely of washing elements. The grooming elicited by s.c. BN in the developing rat largely resembles an exaggeration of grooming observed following saline s.c. (including non-contact motor acts). Therefore, the behavioural response to BN injected s.c. would appear to mirror the development of spontaneous grooming and thus at 1 to 10 days of age does not resemble the adult response to i.c.v. BN.

In conclusion, the grooming elicited by BN s.c. appears to reflect the motor capabilities of the developing rat and therefore that of spontaneous grooming. In this regard it differs markedly from the response of adult rats to i.c.v. BN. The behavioural response declines from 1 to 10 days of age and this could reflect decreasing permeability of the blood-brain barrier to BN or a decreased sensitivity of older rats to the behavioural effects of BN. Hypothermia in response to BN s.c. was observed in 1-day old rats only. This was not a dose-dependent phenomenon and probably reflected the inability of pups of this age to thermoregulate effectively. Therefore, the BN receptors in the CNS of the developing rat are pharmacologically functional before the appearance of measurable amounts of BN-like peptides.

C. Experiment 1b: Central Injection of BN to Developing Rats

1. Introduction

The data from experiment 1a clearly demonstrate that BN can affect behavioural and possibly physiological mechanisms in the developing rat. The decrease in the grooming response to BN s.c. from days 1 to 10 indicated that either the blood-brain barrier was maturing such that permeability to BN was decreasing or that the pups sensitivity to the behavioural effects of BN was declining. The present study

sought to test the former possibility by administering BN directly into the CNS, thus circumventing the blood-brain barrier. Studies with other peptides indicate that centrally administered oxytocin (Pedersen et al., 1988) and ACTH1-16$_{NH2}$ (Kirstein et al., 1990) elicits more robust grooming as a function of age and hence implicates changes in the blood-brain barrier as the more probable reason for the diminished grooming response to systemic BN in the 10 day old rat as compared to the 1 day old.

Observations from the previous study also indicate that some self-grooming acts did not result in head or bodily contact. Golani and Fentress (1985) noted such a phenomenon in the development of grooming in mice but thus far in rats no quantitative research has been reported. It could be that non-contact elements are replaced by motor acts resulting in physical contact in the ontogeny of grooming behaviour in the rat. Richmond and Sachs (1980) indicated that spontaneous grooming in the infant rat appeared to follow a cephalocaudal sequence in terms of development and this maturational progression mirrored the known arrangement of behavioural elements in adult grooming.

2. Purpose

The objectives of experiment 1b were as follows:

1) To examine the behavioural response to centrally administered BN in the developing rat and establish whether

changes in the permeability of blood-brain barrier to BN were responsible for the decline with age in the behavioural effects of BN administered s.c..

2) To quantify the amount of non-contact "grooming" in response to BN i.c.v. and establish how it may change as a function of age.

3) To elucidate the sequential nature of the grooming response to BN and determine how this may vary as a function of age.

3. Methods

a) Animals

Rat pups were obtained from timed pregnant Sprague Dawley CD females (Charles River, St. Constant, Quebec) as well as from Sprague Dawley CD females bred in our own colony. On the day of birth (day=0), litters were standardized to 8 pups per dam with each litter counterbalanced for sex. Pups were kept with the mother until the day of testing (1, 5, 10 or 20 postnatal day). The colony room environment was maintained at 20°C with a relative humidity of 60% and a 12 hr light dark cycle (lights on 07:00 hr).

b) Experimental procedures

Pups were removed one at a time from the mother, weighed and anaesthetized with methoxy flurane (Metafane®). Anaesthetizing was accomplished by adding a minimal amount

of methoxy flurane onto cotton gauze which was located at the bottom of a centrifuge tube. The animal was then positioned head first at the mouth of the centrifuge tube until reflex could not be elicited. Animals (1-10 days of age) were then placed in a custom modified stereotaxic instrument equipped with a blocked head restraint mechanism and insulated in paper wrapping. The 20 day old animals were placed in a normal rat stereotaxic with the substitution of mouse ear bars. BN was injected into one of the lateral ventricles (coordinates from bregma: A-P -0.5 mm, D-V -3.3 mm and Lat 1.4 mm) and the animal then placed into the observation chambers described earlier. For animals of 1 to 5 days of age, skull landmarks could be located directly through the translucent skin whereas in the 10 and 20 day old animals, a small cut was made on the skin covering the skull to facilitate the identification of bregma. This cut was fused with surgical glue immediately following withdrawal of the injection cannula. All manipulations were performed between 09:00 and 16:00 hrs and each animal was used once only. Treatments were divided across litters in a pairwise Latin Square design such that each litter contained four treatment groups and contributed a male and female to each. The site of injection (right vs left lateral ventricle) was counterbalanced across sex and litter.

BN (Peninsula Laboratories, CA) was freshly dissolved in saline and injected i.c.v. over a dose range of 0.01 to 1.0 μg/ 1 μl with saline injection serving as the control injection. Following placement of the animal in the observation chamber, a video camera mounted above the cages was activated and the animal's activity taped for the next 90 min. The videotaped behavioural responses were then assessed via a custom designed analysis program (courtesy of Dr. Mathew Martin-Iversen) and the following behaviours examined: paw licking, face washing, contact body scratching, contact head scratching, non-contact body scratching, non-contact body scratching, resting, interbout resting, wall climbing, locomotory activity, sniffing, exploration, back paddling, and crawling (see table 4 for operational definitions). Post injection recovery time (behavioural recovery was defined at the point at which the animal began moving from the centre of the cage) was determined for each age group and this determined the time following placement in the cage from which the behavioural recording began. Behavioural recording was limited to 60 min following this recovery time.

Table 4. --Behaviours Measured in Experiment 1b.

Behaviour	Symbol	Definition
Interbout Pause	PAUSE	Inactivity following behavioural bouts (with a maximum duration of 300 s).
Forelimb Vibration	FL. VIB	The forelimbs contact the face (may be licked) and vibrate over the mouth and nose.
Face Wash	F.W.	The forelimbs are wiped over the face and crown regions in large, oscillatory movements.
Body Wash	B.W.	The ventral regions of the abdomen and thorax are licked vigorously.
Contact Head Scratch	H.S.	The hindpaw is brought into contact with the side of the face/head and a scratching action ensues.
Contact Body Scratch	B.S.	The hindpaw is brought into contact with the body flank and a scratching action ensues.
Non-contact Head Scratch	N.C.-H.S.	The hindpaw is directed to the head and vibrates in a scratching action but does not contact the fur.
Non-contact Body Scratch	N.C.-B.S.	The hindpaw is directed to the body flank and vibrates in a scratching action but does not contact the fur.
Rest	REST	The animal is inactive for greater than 300 s (and may be sleeping).
Crawl	CRAWL	The animal locomotes around the cage and drags the hindlimbs.
Roll on back and paddle.	BPDL	The animal rolls onto its back and proceeds to make a paddling movement with the limbs.
Locomotes	LOCO	The animal moves about the cage using all four limbs.
Sniff	SNIFF	The nose and/or head of the stationary animal vibrate.
Explore	EXP	The animal actively examines an area of the cage.
Wall Climbing	CLIMB	The animal pushes itself against the wall of the cage and thereby appears to climb the sides.

c) Statistics

Two-way analysis of variance followed by Tukey post hocs was performed on the durations of the above activities across age and dose of BN using the IBM PC statistical package, SYSTAT. Occasionally, the central injection was not successful and in order to reduce variability, those animals were excluded from the analysis to leave an n=7 for each group mean. Significance was determined at $p<.05$ (see tables A-2 and A-3 of Appendix A for relevant F-ratios).

The behavioural sequences were assessed via a variation of the chi-square test (Goodman, 1968) and the analysis of adjusted residuals (Haberman, 1973). Briefly, the transition frequencies (i.e. the number of times behavioural act "a" was followed by behavioural act "b") for the 60 min test period were entered in a 2-way contingency table and the diagonals eliminated to ensure greatest accuracy as suggested by others (Goodman, 1968; Slater, 1973; Hooff, 1982). A test of quasi-independence (Goodman, 1968) was then applied to this matrix via the BMDP 4f programme. Following a significant result ($p<.05$), standardized deviates computed by BMDP 4f were manually transformed to adjusted deviates or adjusted residuals (Haberman, 1973) to allow for the nature of the transition to be assessed (a negative adjusted residual indicates that one act inhibits the expression of the following act whilst a positive

adjusted residual indicates that one act facilitates the expression of the succeeding act) in accordance with Hooff (1982). The significance of the adjusted residual was examined via a table of normal distribution with $p<.05$ and $p<.001$.

4. Results

20 day old rat pups recovered more quickly from anaesthetic effects than did 1 day old pups (8 min vs 19 min - data not shown). BN elicited significant increases in the amount of time engaged in grooming activities across all age groups although the doses at which these behaviours were increased and the quality of the motor movement varied across age (see tables 5-8). BN, at the 1.0 μg dose, significantly increased forelimb vibration, face washing and interbout pausing (see table 5) while decreasing the amount of time spent sleeping in the 1 day old rat pups (see table 7). The 0.1 μg dose significantly lowered resting and increased the amount of time engaged in exploratory activity (see table 7).

In the 5 day old pups, BN (0.1 μg) increased the time engaged in face washing, non-contact body scratching, sniffing, interbout pausing (see table 5) and decreased the amount of time spent resting (see table 7). BN (1.0 μg) had similar effects (see table 5).

Table 5. --The Behavioural Effects of Centrally Injected Bombesin (BN) in Infant Rats.

AGE (days)	DOSE of BN (μg)	BEHAVIOURAL ELEMENTS							
		FL.VIB	F.W.	B.W.	H.S.	B.S.	PAUSE	NC-H.S.	NC-B.S.
1	0.0	0.41 ±0.41	7.6 ±4.74	0.0	0.0	0.0	481.8 ±178.7	1.5 ±1.5	2.0 ±1.7
1	0.01	3.1 ±1.7	16.8 ±7.6	0.0	0.0	0.0	730.0 ±246.1	0.0	13.1 ±10.0
1	0.1	20.4 ±9.6	123.5 ±48.6	0.0	0.0	14.1 ±9.1	1373.7 ±271.6[d]	1.1 ±1.0	40.7 ±27.2
1	1.0	134.3[*] ±77.3	424.4[*] ±174.9	0.0	0.0	11.9 ±6.7	2425.0[*] ±166.4[d]	0.0	102.5 ±6.7
5	0.0	1.2 ±1.0	1.9 ±0.7	0.0	0.0	2.5 ±2.0	8.3 ±6.1	0.0	21.4 ±19.3
5	0.01	0.8 ±0.8	3.0 ±3.0	0.0	0.0	4.8 ±4.7	215.2 ±189.5	4.3 ±4.3	36.9 ±23.1
5	0.1	47.8 ±12.2	341.2[*] ±44.7	0.0	3.2 ±2.0	74.8 ±19.8	2117.6[*] ±213.1[d]	9.8 ±4.3	243.1[*] ±46.2
5	1.0	105.7 ±13.3	845.6[*] ±97.4[a]	0.0	12.6 ±7.0	134.1 ±44.9	1842.7[*] ±138.7[d]	10.3 ±3.5	300.1[*] ±67.4[ad]

Cells contain mean±sem. [*] $p<.05$ from respective age group baseline. [a] $p<.05$ from 1 day olds at that dose. [d] $p<.05$ from 20 day olds at that dose.

Table 6. --The Behavioural Effects of Centrally Injected Bombesin (BN) in Young Rats.

AGE (days)	DOSE of BN (μg)	BEHAVIOURAL ELEMENTS							
		FL.VIB	F.W.	B.W.	H.S.	B.S.	PAUSE	NC-H.S.	NC-B.S.
10	0.0	0.0	0.0	0.0	0.0	0.0	903.9 ±281.1	0.0	0.0
10	0.01	64.8 ±34.1	28.7 ±15.5	0.0	0.7 ±0.5	42.6 ±20.3	1469.8 ±226.3[d]	6.6 ±5.1	190.4 ±85.6
10	0.1	28.1 ±6.5	122.4 ±52.7	0.0	8.6 ±2.7	198.6 ±25.0	1779.9 ±150.9[d]	65.3[*] ±18.5[a,b,d]	568.6[*] ±93.7[a,b,d]
10	1.0	88.7 ±10.7	757.2[*] ±136.8[a]	0.0	4.8 ±2.8	96.6 ±39.0	1830.1[*] ±167.7[d]	26.3 ±17.8	20.7[*] ±84.3[a,d]
20	0.0	1.9 ±1.1	67.2 ±18.5	0.0	14.9 ±6.0	16.8 ±7.0	590.3 ±170.0	0.0	0.0
20	0.01	48.4 ±12.0	433.4[*] ±88.2[a]	5.3 ±2.5	808.5[*] ±119.6[e]	1083.2[*] ±196.1[e]	698.0 ±291.5	6.5 ±5.8	80.9 ±47.0
20	0.1	53.1 ±14.1	513.0[*] ±46.3[a]	4.5 ±2.5	990.6[*] ±122.0[e]	1218.2[*] ±159.3[e]	385.5 ±214.9	2.9 ±2.9	29.4 ±11.3
20	1.0	22.9 ±7.0	715.8[*] ±75.7	2.9 ±1.0	1235.6[*] ±59.1[e]	1400.8[*] ±38.8[e]	48.1 ±3.9	0.0	0.2 ±0.2

Cells contain mean±sem. [*] $p<.05$ from respective group baseline. [a] $p<.05$ from 1 day olds at that dose. [b] $p<.05$ from 5 day olds at that dose. [c] $p<.05$ from 10 day olds at that dose. [d] $p<.05$ from 20 day olds at that dose. [e] $p<.05$ from 1, 5 and 10 day olds at that dose.

Table 7. --The Locomotor Effects of Centrally Injected Bombesin (BN) in Infant Rats.

AGE (DAYS)	DOSE of BN (μg)	BEHAVIOURAL ELEMENTS						
		REST	CRAWL	BPDL	LOCO	SNIFF	EXP	CLIMB
1	0.0	2550.7 ±461.8	2.4 ±1.6	27.2 ±7.8	0.0	10.2 ±4.4	6.3 ±3.5	0.0
1	0.01	2689.5 ±506.1[c,d]	22.2 ±16.1	41.4 ±13.5	0.0	13.2 ±7.5	31.7 ±23.0	0.0
1	0.1	1655.5* ±386.1[b,c,d]	64.5 ±23.1	57.4 ±45.0	0.0	34.7 ±17.7	80.2* ±23.9[b,c,d]	0.0
1	1.0	269.5* ±150.3	93.1 ±43.2	50.0 ±17.0	0.0	32.1 ±17.1	34.8* ±16.1	0.0
5	0.0	3486.9 ±45.0	1.3 ±0.8	54.4 ±49.2	0.0	3.4 ±1.8	0.5 ±0.5	0.0
5	0.01	3290.5 ±237.0	104.1 ±88.8	4.6 ±3.2	0.0	0.5 ±0.3	0.0	0.0
5	0.1	417.7* ±206.5	54.9 ±15.4	47.8 ±41.4	48.9 ±46.7	334.3* ±126.2[a,c,d]	17.5 ±11.4	3.4 ±3.4
5	1.0	220.1* ±98.1	8.6 ±3.6	67.9 ±28.0	4.3 ±2.6	411.9* ±138.0[a,c,d]	20.0 ±9.8	0.0

Cells values are the mean±sem. * $p<.05$ from respective age group baseline. [a] $p<.05$ from 1 day olds at that dose. [b] $p<.05$ from 5 day olds at that dose. [c] $p<.05$ from 10 day olds at that dose. [d] $p<.05$ from 20 day olds at that dose.

Table 8. --The Locomotor Effects of Centrally Injected Bombesin (BN) in Young Rats.

AGE (DAYS)	DOSE of BN (μg)	BEHAVIOURAL ELEMENTS						
		REST	CRAWL	BPDL	LOCO	SNIFF	EXP	CLIMB
10	0.0	2842.2 ±151.4	52.0 ±19.4	16.7 ±9.8	0.0	0.0	0.0	13.9 ±7.8
10	0.01	1527.0* ±387.5	56.5 ±24.0	46.9 ±21.8	0.0	10.6 ±4.5	1.0 ±0.6	27.1 ±22.9
10	0.1	520.5* ±60.4	18.7 ±6.6	79.9 ±20.2	0.0	41.6 ±12.7	0.8 ±0.8	25.6 ±25.3
10	1.0	114.7* ±85.4	18.3 ±5.5	82.3 ±23.8	4.3 ±2.8	68.7 ±29.6	21.1 ±14.5	0.0
20	0.0	2578.2 ±181.7	9.5 ±4.0	0.0	132.6 ±26.4e	64.8 ±20.9	87.2 ±17.5e	52.7 ±18.1
20	0.01	207.1* ±123.4	38.8 ±21.3	0.0	11.9* ±6.3	107.1 ±20.5	14.2* ±7.6	26.7 ±16.0
20	0.1	95.1* ±68.5	19.2 ±9.6	0.1 ±0.1	10.3* ±5.0	59.6 ±18.9	14.3* ±7.1	13.2 ±7.0
20	1.0	0.0*	21.3 ±7.6	0.0	2.8* ±1.1	23.3 ±12.1	0.2* ±0.2	5.2 ±3.7

Cells contain mean±sem. * $p<.05$ from respective age group baselines. e $p<.05$ from 1, 5 and 10 day olds at this dose.

In the 10 day old pups, BN (0.01 μg) decreased the time spent resting. The next higher dose (0.1 μg) increased non-contact body and head scratching whilst significantly decreasing resting (see table 6). The 1.0 μg dose had similar effects and significantly increased face washing.

For the 20 day old pups, all doses of BN significantly increased the time spent face washing, head scratching and body scratching (see table 6) and decreased the time spent resting and locomoting (see table 8). Body washing was also induced, albeit for very small durations.

At all doses of BN, 20 day old pups expressed significantly more contact scratching behaviours than younger pups of any age and more face washing than 1 day old pups (except the 1.0 μg dose -see tables 5 and 6). At the 0.1 and 1.0 μg doses, the 20 day old pups spent less time between behavioural bouts than younger pups of any age. 20 day old pups had higher baseline levels of locomotory behaviour than all other pups (see tables 7 and 8).

At the 0.1 μg dose of BN, 10 day old pups spent more time engaged in non-contact scratching behaviours than older or younger pups (see tables 5 and 6). 1 day old pups explored more in response to 1.0 μg of BN than all older animals (see tables 7 and 8).

In terms of sequence analysis, the response to 1.0 μg BN was examined as it was the only dose at which significant levels of grooming were reliably elicited across all ages.

Figures 3-6 show the nature of the sequences. The test of quasi-independence indicated that at all ages, the behavioural pattern elicited by BN (1.0 μg; i.c.v.) was significantly different from the pattern expected by chance ($p<.05$).

As illustrated in figure 3, at 1 day of age, the principle sequences of behaviours elicited by 1.0 μg of BN were: (1) Interbout pause→face wash→forelimb vibration→non-contact body scratching and either →contact body scratching or a conclusion of the bout (interbout pause). Here, face washing and forelimb vibration positively influenced the expression of one another to a very high degree. (2) Interbout pause→non-contact body scratching→contact body scratching. Non-contact body scratching positively influenced the expression of contact body scratching. Also of note, locomotory sequences of crawl→paddle on back→interbout pause and crawl→explore→sniff→interbout pause were elicited by BN. These locomotor sequences inhibited the expression of the grooming sequences as indicated by the significant negative connections.

In the 5 day olds, 1.0 μg BN elicited three distinct grooming sequences (as indicated in figure 4): (1) Interbout pause→face wash→forelimb vibration→face wash; (2) Interbout pause→non-contact body scratching→non-contact head scratching→non-contact body scratching and concluding with a pause; and (3) Interbout pause→non-contact body

scratching→contact body scratching→contact head scratching and either to non-contact head scratching→non-contact body scratching→interbout pause or contact body scratching→non-contact body scratching→interbout pause. The scratching sequences and washing sequences were mutually inhibitory as indicated by the significant negative connections between the constituent acts. Locomotory sequences were less prevalent at this age.

In 10 day olds, 1.0 μg BN elicited sequences that combined both scratching and washing behaviours (see figure 5): (1) Interbout pause→forelimb vibration→face wash→contact body scratch→contact head scratch→contact body scratch→face wash→roll on back and paddle→interbout pause. (2) Interbout Pause→face wash→non-contact body scratch→non-contact head scratch→non-contact body scratch→interbout pause. And (3) Interbout pause→forelimb vibration/face wash→non-contact body scratch→contact body scratch and to either →face wash or →contact head scratch→ and and the conclusion of (1). Face washing also positively influenced rolling onto the back and paddling→pause. Sequences (1), (2) and (3) indicated the establishment of contact and non-contact pathways at this age; contact body scratching negatively influenced non-contact head scratching and no statistically significant connections (either positive or negative) connected non-contact head scratching with contact head scratching.

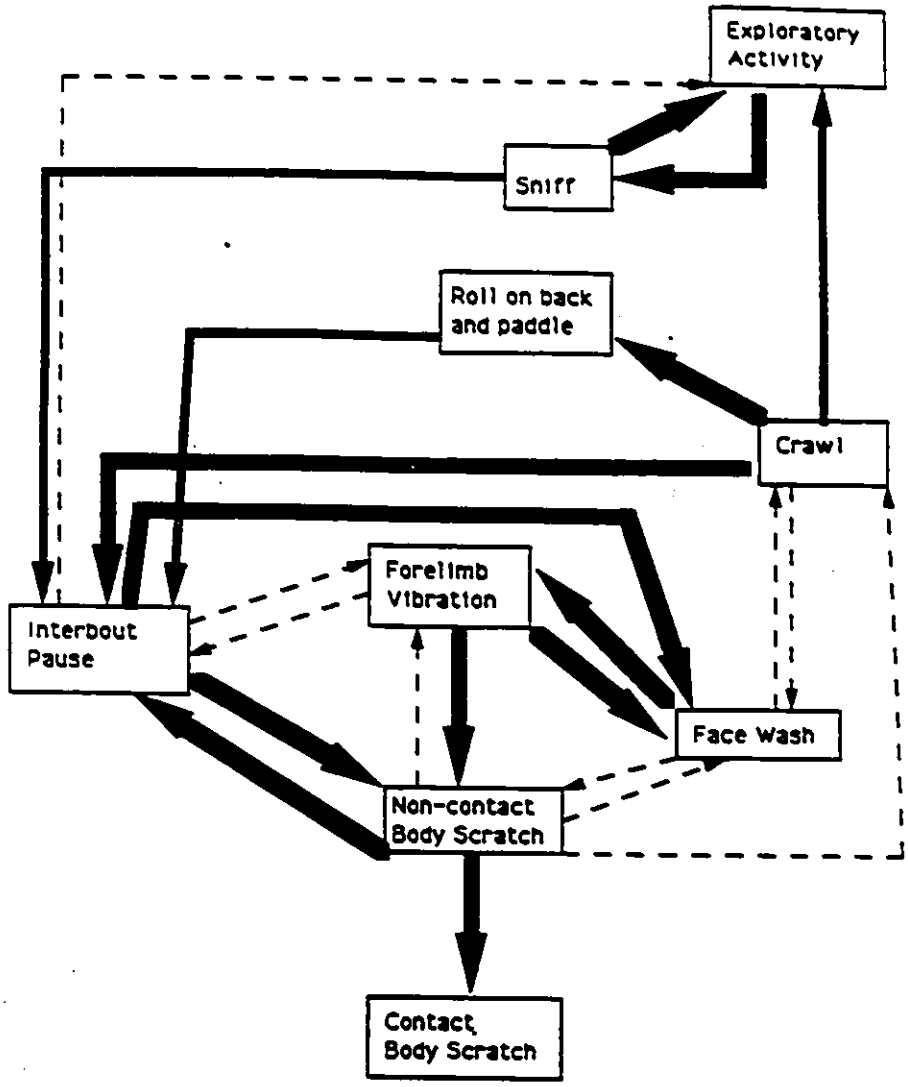

Figure 3. The sequences of behaviours elicited by BN (1.0 μg; i.c.v.) in the 1-day old rat pup. Dotted lines represent significant negative links between elements ($p<.05$). Thin solid lines represent positive links between elements ($p<.05$). Thick solid lines represent positive links between elements ($p<.001$).

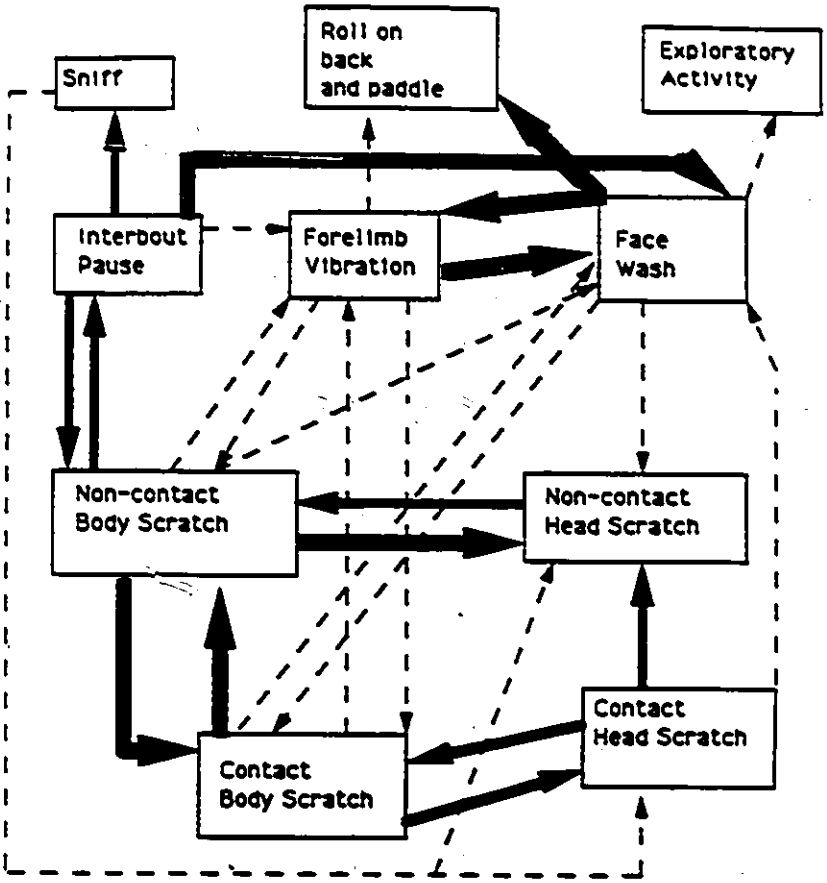

Figure 4. The sequences of behaviours elicited by BN (1.0 μg; i.c.v.) in the 5-day old rat pup. Dotted lines represent significant negative links between elements ($p<.05$). Thin solid lines represent positive links between elements ($p<.05$). Thick solid lines represent positive links between elements ($p<.001$).

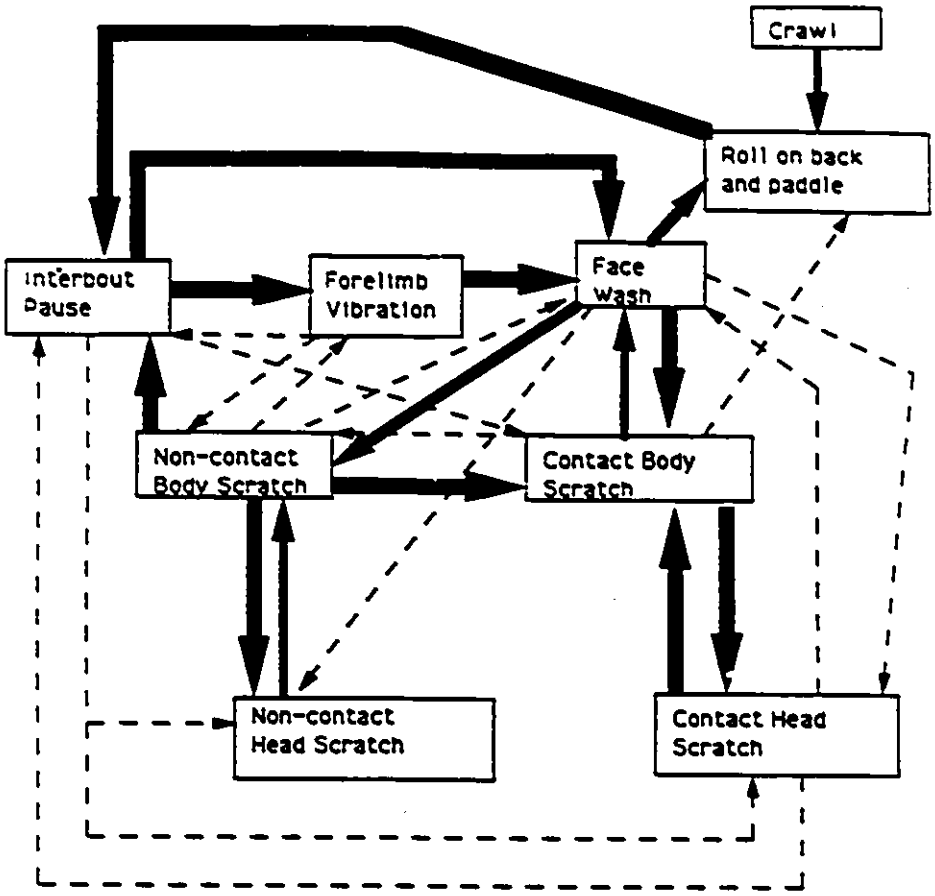

Figure 5. The sequences of behaviours elicited by BN (1.0 ug; i.c.v.) in the 10-day old rat pup. Dotted lines represent significant negative links between elements ($p<.05$). Thin solid lines represent positive links between elements ($p<.05$). Thick solid lines represent positive links between elements ($p<.001$).

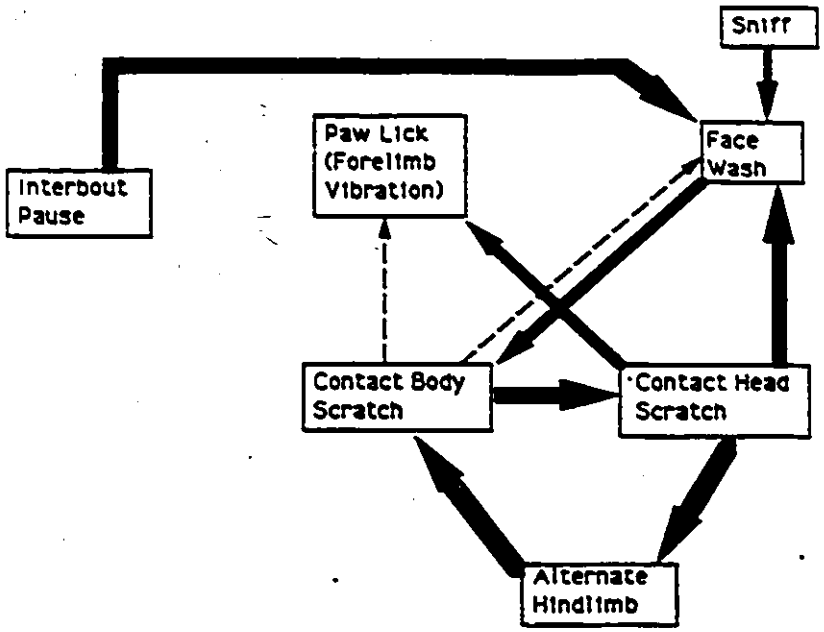

Figure 6. The sequences of behaviours elicited by BN (1.0 μg; i.c.v.) in the 20-day old rat pup. Dotted lines represent significant negative links between elements ($p<.05$). Thin solid lines represent positive links between elements ($p<.05$). Thick solid lines represent positive links between elements ($p<.001$).

At 20 days of age, 1.0 μg of BN elicited a distinct sequence: (1) Interbout pause-face wash-contact body scratch-contact head scratch-alternate hindlimb-contact body scratch-contact head scratch-face wash and from here back to interbout pause (although the connections were not statistically significant). No other sequences were apparent (see figure 6).

5. Discussion

Older rat pups recovered more quickly from the effects of anaesthetic and injection procedure than did younger pups. The 20 day old pups had a higher baseline of locomotory activity than younger pups and this locomotory activity was adult-like in appearance while that of the younger pups resembled crawling or pivoting. A similar trend in locomotory development was reported earlier and attributed to the maturation of motor and sensory systems (Almli and Fisher, 1977; Altman and Sudarshan, 1975).

An increase in behavioural sensitivity to centrally injected BN was evident as a function of age and similar findings have been reported for oxytocin (Pedersen et al., 1988) and ACTH (Kirstein et al., 1990). This indicates that the decrease in responsiveness to BN s.c. in older pups in experiment 1a was most likely due to decreasing permeability of the blood-brain barrier to the peptide and not to the

reduced sensitivity of the 10 day old pup to the grooming inducing properties of BN. The increased sensitivity of the older pups to BN i.c.v. is puzzling as the number of BN binding sites does not increase during this point in development (Gillati and Moody, 1984). It could be that endogenous BN-like peptides which are increasing in concentration during this time "prime" BN receptors, keeping them in a high affinity state and hence increasing the animal's sensitivity to BN.

More probable is the possibility that changes in the neurochemical systems and neural substrates subserving grooming and scratching are maturing during this time. Maturation of the serotonergic system(s) in the 3rd and 4th postnatal weeks have been implicated in the increased sensitivity to the grooming effects of ACTH by weanling rats (Kirstein et al., 1990). The role of serotonin in BN-induced grooming has not been examined but dopaminergic and cholinergic systems which undergo large changes from the 2nd to 4th postnatal week (Coyle and Campochiaro, 1976; Murrin and Zeng, 1986; Zeng, Hyttel and Murrin, 1988) have been implicated in the expression of BN-induced grooming in the adult rat (Merali, Kateb and Kateb, 1988; Merali and Piggins, 1990). Experimental evidence of their involvement in the infant rat is lacking and hence little is known of the neurochemical systems subserving BN's behavioural

effects. Similarly, the neural substrates for BN-induced grooming in the adult rat remain to be elucidated.

Another developmental trend was the establishment (with increasing age) of scratching as one of the primary components of BN-induced grooming. This would appear to follow the maturation of the rats' motor abilities as scratching becomes an increasingly larger constituent of the animals' activity in response to BN and shifts from the non-contact to the contact form. Scratching was observed from day 1 onwards and again differs from the findings of Richmond and Sachs (1980). Scratching was also seen in response to ACTH at day 4 (Kirstein et al., 1990). Differences in testing regimen may explain this apparent discrepancy. Richmond and Sachs (1980) tested their animals in litters whereas animals in this study (and Kirstein et al., 1990) were tested in isolation (which can have large effects on behavioural activation -Hofer and Shair, 1987). Richmond and Sachs (1980) examined spontaneous grooming whereas this study measured activity following surgical and central injection treatments and these differences in testing conditions could explain the differences in results noted. The shift in scratching from non-contact to contact form was similar to the ontogenetic changes in mouse grooming which probably reflects the maturation of motor systems (Golani and Fentress, 1985).

A third ontogenetic trend was the establishment of a scratching "subsystem". The sequence analyses indicated that scratching elements (non-contact or contact) could exist in a sequence connected to but nonetheless separate from the washing elements and that this became more robust with age. Sachs (1988) pointed out that two grooming systems, one dominated by washing elements and the other by scratching activities could exist. He suggested that activation of one system could inhibit or interfere with the other. The above data provide some support for the contention that two grooming systems exist and that at postnatal days 1 and 5 they may be mutually inhibitory. Further, as indicated by Richmond and Sachs (1980), these grooming systems may mature at different rates (e.g. washing acts are mature from day one whereas scratching elements begin in an immature, non-contact form).

At 20 days of age, the behavioural response of the preweanling rat to i.c.v. BN does not fully resemble that of the adult rat. Body washing which was seen as a component of both spontaneous and BN-induced grooming in the adult rat (see figure 2 in experiment 1a) was not observed frequently in response to BN or saline i.c.v. in the 20 day old rat. Both anogenital and body washing have been observed in rats 18-21 days of age (Almli and Fisher, 1977; Richmond and Sachs, 1980) but the testing situations employed were different from this experiment (isolation vs non-isolation

as above). A possible confound in this experiment was the anaesthesia which could have hindered the expression of washing aimed at the caudal body surfaces. Kirstein and colleagues (1990) reported body washing in response to ACTH1-16$_{NH2}$ at postnatal day 21 although no anaesthesia was used. Under the conditions used in this experiment, body washing was rarely observed and thus the behavioural response to BN i.c.v. reflected this. Locomotory activity was decreased by BN at this age which contrasts to its hyperlccomotory effects in the adult rat (Merali et al., 1983). As with grooming, this could be due to changes in neurochemical systems mediating locomotory activity, changes in the neural structures subserving motor behaviour, differences in testing regimens and differences in the locomotory ability of the 20 day old rat as compared to the adult. Further experimentation is obviously required to establish the exact reasons for this apparent difference.

In conclusion, these results extend the findings of experiment 1a and indicate that 10 and 20 day old pups respond vigorously and robustly to centrally administered BN. Scratching movements are present early in ontogeny and could form a separate grooming system from the washing form of grooming. Changes in behavicural responses to BN appear to be a function of the normal maturation of motor systems. Changes in sensitivity to BN appear to be unrelated to measurable changes in BN binding sites as these reach adult-

like levels by day 10 whereas sensitivity continues to increase.

IV. LONG-TERM EXPERIMENTS

A. General Introduction

1) Neuropeptides and Development

During the first few weeks of life, the CNS of the developing rat undergoes a large number of changes. This period, known as the brain growth spurt, is characterized by axonal growth, dendritic arborization, peak rate of synaptogenesis, gliogenesis, myelination and maturation of synaptic neurotransmission (Dobbing, 1971; Dobbing and Smart, 1973). In the rat, this spurt is entirely postnatal (Dobbing, 1974). Throughout this time period, synaptic biochemical ontogeny can be particularly susceptible to changes in the physical and/or chemical environment. Many teratogens such as toluene can have long lasting and devastating effects on the CNS and behavioural development if administered during this time period (Lorenzana-Jimenez and Salas, 1990). Similarly, physical stimulation of a rat pup (handling) or exposing it to stressors can have a profound impact on neural and behavioural ontogeny (Ader, 1970; 1975; Levine and Mullins, 1966; Meaney et al., 1988). Indeed, handling early in ontogeny can effect changes in later adult response to stress and in the levels of brain glucocorticoid receptors (Meaney et al., 1985; Meaney et

al., 1988). The rat CNS is thus highly susceptible to experiential and chemical influences during the first few postnatal weeks.

This "sensitive period" in ontogeny is also the point in development when many naturally occurring neurochemicals can effect permanent change to the CNS. The organizing effects of steroid hormones on the CNS perinatally have been well documented (see McEwen, 1988 for recent review). Specific stimulation of sex steroid receptors pre- and postnatally with exogenously administered steroid hormone can have permanent effects on steroid receptor number and density and can radically alter an organism's sexual behaviour (Arnold and Breedlove, 1985; Parsons, Rainbow and McEwen, 1984; McEwen, 1988). Similarly, endogenous dysfunction of steroid hormone production, as in adrenogenital syndrome, can have considerable behavioural effects later on in life (Money and Ehrhardt, 1973).

Over the past two decades, a considerable body of evidence has accumulated concerning the long-term effects of neuropeptide alterations early in postnatal development (see Handelmann, 1985; 1988 for reviews). Receptors for a number of neuropeptides have been found in the developing mammalian CNS both pre- and postnatally and measurable levels of many peptides can be detected in fetal and neonate nervous tissue (see Boer, Snijdewint and Swaab, 1988 for review). The dominant experimental approach at present focuses on early

manipulation of neuropeptide system(s) via administration of specific and selective receptor agonists and antagonists including neuropeptide antisera. A reasonably consistent pattern of results has emerged suggesting that early manipulation of an endogenous neuropeptide system can effect long-term changes in those behaviours and physiological mechanisms in which the neuropeptide is known to have an influence (Zadina et al., 1985; Zadina and Kastin, 1986).

Neonatal manipulation of endogenous opioids by administration of morphine or Met-enkephalin can impair motor development and reduce adult sensitivity to pain (Handelmann, 1988; Handelmann and Dow-Edwards, 1985). These effects may or may not be related to alterations in μ-receptors (Handelmann, 1983; Tempel et al., 1988). Neonatal injection of naloxone can impair body and organ development (Zagon and McLaughlin, 1989) and can affect neurogenesis (Zagon and McLaughlin, 1987). Injecting substance P into neonates reduces adult rat sensitivity to pain (Handelmann, Selsky and Helke, 1984) and increases substance P receptors in the brain (Handelmann, Shults and O'Donohue, 1987). This is reflected functionally by increased sensitivity to substance P in adulthood (Handelmann, Selsky and Helke, 1984).

Neuropeptide effects on ontogeny are not limited to sensory or physical development. Administration of ACTH to rat pups improves later adult performance on active

avoidance tasks (Nyakas et al., 1981) although a similar treatment with ACTH4-10, has a detrimental effect on adult performance in active avoidance tasks (McGivern et al., 1988). Treatment of adults with ACTH appears to improve performance on learning tasks, depending on the fragment used (Murphy and Miller, 1955; see also De Wied, 1987 for review). Neonatal exposure to vasopressin (VP) decreases VP binding sites in the kidney and is reflected functionally by decreased sensitivity to the anti-diuretic properties of VP (Handelmann et al., 1983; Handelmann and Sayson, 1984). Infant rats injected with anti-VP antisera show memory retrieval and learning impairments when tested in adulthood (Moratella et al., 1987). This mirrors the memory deficits shown in adulthood by the Brattleboro rat which is genetically deficient in VP (Van Wimersma Griedanus, 1982). Injecting infant rats with oxytocin increases novelty-induced grooming in adulthood (Noonan, Continella and Pedersen, 1989). In summary, neonatally administered neuropeptides can effect long-term changes in physical and behavioural development as well as in the sensitivity to those neuropeptides in adulthood.

In the CNS of the rat, receptors for BN can be detected prenatally before measurable immunoreactivity for the peptide itself (Gillati and Moody, 1984; Getz, Moody and Rosenstein, 1987). Receptor numbers reach adult-like levels by postnatal day 10 whereas the concentration of BN within

the CNS does not reach mature levels until approximately 21-23 days following birth. Experiments 1a and 1b demonstrated that the BN receptors were pharmacologically functional from postnatal day 1 and that a robust behavioural response could be elicited by BN s.c. for at least the first 5 days. Further, Jackson and Kitchen (1989a) found that BN s.c. could reduce food intake in rats of 15 days of age, indicating that BN's satiety inducing properties could also be elicited at an early stage in ontogeny. Stimulation and/or blockade of BN receptors during development could then have long-term effects on food and water intake, the development and expression of grooming behaviour and later adult sensitivity to BN.

2. Practical Considerations

Central to the study of the long-term outcome of neonatal manipulation of an endogenous neurochemical system are the following questions: (1) at what point in ontogeny should a manipulation be attempted, and (2) what to measure in the long-term. In the case of BN, the first question was answered by considering experiment 1a. In that study, BN s.c. elicited a quantifiable behavioural response from 1 to 10 days following birth and it was concluded from the results of experiment 1b that this activity resulted from the stimulation of BN receptors in the CNS. This response to BN s.c. declined as a function of age and if the

experiment were continued, a behavioural response at 15 days would probably have been much diminished (as a direct consequence of the maturation of the blood-brain barrier). The first 8 days following birth when BN receptors are increasing in number was thus selected as an appropriate period in which to administer BN. The use of the behaviourally active doses (5 and 10 mg/kg; s.c.), given twice daily, were selected to ensure adequate BN receptor stimulation.

With regards to the second question, it was decided to measure behavioural activation in a non-invasive manner, using mildly stressful and novel conditions throughout development. Central administration of BN was found to increase plasma corticosterone, glucose and catecholamine levels (Brown et al., 1977c; Gunion et al., 1989), all of which are also secreted in response to stress (Axelrod, 1984). Further, BN i.c.v. elicits vigorous grooming (Merali, Johnston and Zalcman, 1983) and grooming can be elicited in novel and/or stressful situations (Jolles et al., 1979; File et al., 1988). Thus the animals' behavioural responses to isolation and placement in a novel room, open field and the elevated plus maze (a measure of anxiety -Pellow and File, 1985) were measured over the course of ontogeny. It was of obvious import to assess the animals' response to BN in adulthood. On the basis of pilot work and a short study with mammalian BN-like peptides

(Piggins, personal observations; Piggins, Lafreniere and Merali, 1989), it was found that the use of a water deprivation paradigm would allow for the measurement in adulthood of the effects of BN on grooming, water and food intake. Thus, multiple behavioural measures as well as the amount of food and water consumed could be employed to detect any alterations in adulthood resulting from the exposure in infancy to exogenous BN. No attempt at receptor assay was attempted as the appropriate technique (in vitro autoradiography) was not available at the conclusion of these experiments (see General Discussion for further details).

B. Experiment 2a: The Long-term Effects of Neonatal Injections (s.c.) of BN.

1. Purpose

This experiment was undertaken in an effort to establish the possible physiological role of BN-like peptides in the mediation/modulation of stress responses and ingestive function. Thus, the primary objective of this study was to examine the long-term outcome of treating the infant rat with comparatively large doses of BN. Neonatal manipulation of endogenous neuropeptide systems can alter behavioural and physiological mechanisms in which the neuropeptide is known to have an influence. In the adult rat, BN effects changes on grooming and ingestive behaviours

and causes alterations of behavioural and physiological indices of stress. It was hypothesized that this treatment would alter the animals behavioural response to BN in adulthood as well as the animals response to a variety of novel and stressful conditions.

2. Methods

a) Animal Husbandry

Timed pregnant, female Sprague Dawley CD rats were obtained from Charles River (St. Constant, Quebec). On the day of birth (postnatal day=0), the female pups were culled and the male pups cross-fostered such that each dam had 8 male pups. Pups were randomly assigned to treatment groups and injected subcutaneously (as per experiment 1) twice a day from postnatal day 1 through 8 with one of the following: BN 5 mg/kg (group LD), BN 10 mg/kg (group HD), saline (group SAL) and non-injected but handled controls (group UNT). The treatments were distributed across mothers such that each litter contained 2 animals from each treatment condition. The injection procedure meant that litters were isolated from the mother for 60-90 min twice a day. In order to facilitate identification, pups were marked (with a non-toxic marker) immediately prior to injection. Pups were weighed every afternoon prior to injection procedure until this phase of the experiment had concluded. At this time, the ears of the saline, LD and HD

pretreated pups were clipped in a distinct pattern to facilitate later identification.

At 23 days of age, the pups were weaned from the mother and placed 4 to a cage with 1 member of each pretreatment condition represented in this weaned group. At 37 days of age, animals were housed 2 per cage with HD animals twinned with UNT animals and LD paired with SAL animals on the basis on body weight (animals closest in body weight were housed together in order to prevent one animal dominating another to a high degree). At 45 days of age, animals were isolated and housed individually. For the first 30 min of the isolation, the animal's behaviour was assessed as described below. One week later, the animals were transferred temporarily to another (unfamiliar) observation room where their grooming behaviour and locomotory activity were monitored for 60 min as described below. Following this recording session, the animals were returned to their home cage.

At 58 days of age, the animals were behaviourally assessed in the open field box and elevated plus maze, as outlined below. One week to 11 days later, the animals were stereotaxically implanted with cannulae aimed at the 3rd ventricle and following recovery, placed on a water deprivation schedule (see below for details).

b) Non-Invasive Behavioural Tests

For the following behavioural tests, a Latin Square design was employed. This was done to randomize the effects of the time of day, testing order, cage position and observer bias over litters and pretreatment groups.

(1) Novelty and Isolation-Induced Activity

In the following tests, the animal's behaviour was assessed for 2 s every 16 s for the occurrence of head washing, head scratching, body washing, body scratching, anal-genital licking, non-stereotypic sniffing, resting (interbout inactivity as well as sleeping) and exploratory activity (see table 9 for operational definitions).

Isolation-induced activity was assessed by removing the juvenile rat (aged 45 days) from the home cage (where it had been housed with another similarly aged rat) and placing it in a new cage (identical to the home cage except that the animal was now housed singly). For the succeeding 30 min the animals activities were measured according to the behavioural assessment method discussed above.

Novelty-induced activity was measured by taking the singly housed rat and transferring it to another room where it was placed in an observation cage (43 x 23 x 15 cm; identical to the rat's home cage). These cages were equipped with strategically placed infra-red beams which assessed the animals locomotory activity (distance

Table 9. --Behaviours Measured Under Isolation and Novelty Conditions.

Behavioural Measure	Symbol	Definition
Head Washing	HW	The forepaws (licked or unlicked) are wiped over the face and crown.
Head Scratching	HS	The hindpaws are brought into contact with the side of the head and a scratching motion ensues.
Body Washing	BW	The ventral surface of the abdomen and thorax are licked.
Body Scratching	BS	The body flanks are contacted by the hindpaws and a scratching motion ensues.
Anogenital Licking	AG	The anal or genital regions are contacted by the tongue.
Sniffing	SNIFF	The animal remains stationary and sniffs the immediate environment.
Explore	EXP	The animal locomotes around the cage and sniffs and/or orients to a particular feature of the cage.
Resting	REST	The animal remains stationary or is asleep.

traversed) in cm. The experimenter, sitting behind a one-way mirror, assessed the animals' behaviour (as per above) for the next 60 min whilst the motor activity was measured by the automated system as described previously.

(2) Open Field and Elevated Plus Maze Measurements

Open field activity was examined by placing the animal in a box (47 x 47 x 25.5 cm) having sides equipped with strategically placed infrared beams. Every interruption of these beams incremented the activity score by 1. The total number of beam interruptions over a 10 min test period was recorded.

Activity in the elevated plus maze was examined using a variation of the methodology of Pellow and File (1985). Following the 10 min test in the open field, the animal was placed in the centre of the maze (the arm faced was randomized over the litter). For the succeeding 10 min, the frequency of entry and the time spent on the open arms as well as the amount of time spent in the closed arms and the centre of the maze were measured.

c) Invasive Procedures

(1) Surgery

Animals were anaesthetized with sodium pentobarbital (65 mg/kg; Somnital®) and placed in the stereotaxic instrument. A 30 gauge cannula (Plastic One, VA) aimed at the 3rd ventricle (coordinates from Bregma: A-P -4.3 mm, Lat

0.0 mm and D-V - 4.3 mm) (Paxinos and Watson, 1982) was implanted.

(2) Water Deprivation Schedule

Following surgical recovery (approximately 5 days), animals were placed on a 20 hr water deprivation schedule (Purina rat chow was available ad libitum). Under this regimen, animals were given water for 240 min each day together with access to a wet mash of 40% water and 60% milled Purina rat pellets for the first 30 min. Wet mash contained in a porcelain bowl was placed in a plexiglass holder which was kept in the cage at all times.

(3) Peptide Administration

Animals were allowed 7-9 days to stabilize on the above schedule before the experiments commenced. BN was administered in a pairwise Latin Square design. At least one 24 hr period elapsed between each treatment. BN was dissolved in saline and administered over the following doses (0.0(sal), 0.01, 0.1 and 1.0 μg/3 ul; i.c.v.). Behavioural as well as ingestive activity was assessed as described below for the following 30 min.

In the next experiment, BN was dissolved in saline and administered systemically over the following doses (0.0 (sal), 2, 4 and 8 μg/kg; i.p. in a volume of 0.1 ml/ 100 g body weight).

Table 10. --Behaviours Measured Under the Water Deprivation Schedule.

Behavioural Measure	Symbol	Definition
Head Washing	HW	The forepaws (licked or unlicked) are wiped over the face and crown.
Head Scratching	HS	The hindpaws are brought into contact with the side of the head and a scratching motion ensues.
Body Washing	BW	The ventral surface of the abdomen and thorax are licked.
Body Scratching	BS	The body flanks are contacted by the hindpaws and a scratching motion ensues.
Eating	EF	The animal orients to the feeding bowl such that its mouth is in or near the bowl and is chewing.
Drinking	DF	The animal orients to the water spout and licks it.
Explore	EXP	The animal locomotes around the cage and sniffs and/or orients to a particular feature of the cage.
Resting	REST	The animal remains stationary or is asleep.

The above behaviours were chosen to be monitored as they are relatively easy to characterize and quantify. An examination of interobserver reliability performed under similar conditions to the above indicated an overall α reliability ratio of $r=0.97$ (2 observers, 8 animals rated) and Kendall coefficients of concordance for the individual behaviours ranged from 0.87 for HW to 0.98 for BW.

(4) Behavioural Testing

Following BN administration, the wet mash and water were presented simultaneously. The following behaviours were then scored via time sampling procedure (each animal was monitored for 2 s every 16 s for 30 min): Grooming (head washing, body washing, body scratching and head scratching), feeding frequency, drinking frequency, resting and exploratory activity (see table 10 for operational definitions and note on interobserver reliabilty).

d) Histology

At the conclusion of the experiment, each animal was anaesthetized with a near lethal injection of sodium pentobarbital (Somnotol®) and injected i.c.v. over 70 s with 6 μl of filtered cresyl violet solution. The animal was decapitated and the brain removed. The brain was set on an ice-cooled cutting surface and sectioned with a tissue cutter. The presence of the dye in the 3rd and/or 4th ventricles was recorded.

e) Statistics

All frequency and duration measures of behaviour in the non-invasive tests were examined statistically by a one-way analysis of variance design with the Tukey post hoc test ($p<.05$) using the IBM PC statistical package, SYSTAT. For the invasive tests, frequency counts of behaviour as well as

the amount of food and water consumed were analyzed in a 2-way analysis of variance with repeated measures design (Winer, 1971) using the SAS programme (see tables A-4 and A-5 of Appendix A for relevant F-ratios). Post hoc analyses were performed by using the Tukey test with significance set at $p<.05$. Body weight data underwent logarithmic transformation and were analyzed as above with significance set at $p<.05$. Those animals that failed to receive the full injection volume (as judged by flow rate), were discarded to leave n=7 per pretreatment group. It is noteworthy that the behavioural responses of these animals to the high dose of BN was also much diminished.

3. Results

No group differences were found on the day of fur covering or eye opening (data not shown). The HD group had consistently lower body weights than the other groups (up 65 days of age) but this never reached statistical significance (see table 11). Isolation elicited exploratory activity but little grooming in all pretreatment groups and no group differences were apparent (see table 12). Animals in all pretreatment groups groomed and locomoted in response to transfer to a novel environment but no group differences were found (see table 13). Animals were consistently active in the open field activity box but few animals in any group

Table 11.--Body Weight Gain in Rats Neonatally Pretreated with BN.

AGE (days)	BODY WEIGHT (g)			
	UNT GROUP	SAL GROUP	LD GROUP	HD GROUP
1	6.2 ±0.25	6.4 ±0.87	6.6 ±0.6	6.7 ±0.2
8	17.0 ±0.73	17.5 ±2.5	17.3 ±1.9	15.4 ±0.6
10	22.3 ±1.0	22.5 ±2.8	23.0 ±2.7	20.7 ±0.6
21	50.2 ±1.8	51.8 ±6.3	52.7 ±5.7	47.3 ±1.2
30	89.8 ±4.0	90.3 ±10.4	95.2 ±10.8	86.4 ±2.7
37	138.5 ±5.6	145.4 ±16.7	144.6 ±16.6	135.7 ±2.9
55	289.9 ±9.2	305.5 ±32.2	307.9 ±33.3	281.4 ±6.7
62	346.9 ±12.9	368.3 ±37.9	372.5 ±41.2	341.4 ±9.8
71	354.9 ±10.4	353.5 ±35.7	345.8 ±37.8	373.9 ±16.1
78	383.1 ±12.1	406.1 ±38.4	407.9 ±42.2	380.4 ±9.7
85	372.9 ±12.7	391.8 ±37.1	394.8 ±37.6	370.1 ±10.8
99	403.9 ±13.6	424.3 ±31.6	425.5 ±40.5	409.5 ±8.7

Cells contain mean±sem, n=7-8/group.

Table 12. --The Effects of Neonatal Pretreatment with BN on Isolation-Induced Activity in Juvenile Rats (45 days of age).

GROUP	BEHAVIOURAL ELEMENTS							
	HW	HS	BW	BS	AG	REST	SNIFF	EX
UNT	7.0 ±1.3	1.9 ±0.4	9.6 ±1.9	2.3 ±0.5	0.6 ±0.4	22.1 ±6.3	6.8 ±1.3	74.4 ±6.4
SAL	6.0 ±0.5	2.0 ±0.9	7.1 ±2.0	2.0 ±0.7	1.0 ±0.4	21.1 ±5.2	6.8 ±0.8	80.3 ±6.0
LD	6.3 ±0.9	3.1 ±1.1	11.1 ±1.9	3.1 ±1.0	1.5 ±0.3	17.1 ±4.4	7.9 ±1.6	78.3 ±5.5
HD	6.0 ±1.0	1.9 ±0.9	10.1 ±2.0	1.8 ±0.4	1.6 ±0.6	18.6 ±4.6	6.6 ±1.2	85.4 ±5.4

Cells contain mean±sem, n=8/group.

Table 13. --The Effects of Neonatal Pretreatment with BN on Novelty-Induced Activity in Young Adult Rats (52 days of age).

GROUP	BEHAVIOURAL ELEMENTS								
	HW	HS	BW	BS	AG	REST	SNIFF	EX	LOCO (cm)
UNT	14.4 ±1.4	3.1 ±1.0	20.6 ±2.5	2.4 ±0.9	1.5 ±0.4	135.4 ±6.8	47.5 ±6.3	65.4 ±7.0	918.3 ±121.8
SAL	18.8 ±2.6	4.1 ±1.2	18.9 ±3.3	3.1 ±0.7	2.0 ±0.6	117.8 ±9.7	44.6 ±3.8	81.6 ±7.6	1303.4 ±300.2
LD	14.1 ±2.1	5.1 ±1.2	17.0 ±2.3	2.4 ±0.5	1.6 ±0.5	120.3 ±9.1	40.6 ±6.3	85.8 ±11.5	1450.8 ±181.6
HD	13.8 ±1.5	2.3 ±0.8	16.9 ±2.9	1.8 ±0.8	1.1 ±0.4	136.3 ±11.0	42.4 ±5.2	93.8 ±17.5	1259.6 ±298.0

Cells contain mean±sem, n=8/group.

Table 14. --The Effects of Neonatal Pretreatment with BN on the Activity of Rats (58 days of age) in the Open Field and Elevated Plus Maze.

GROUP	BEHAVIOURAL MEASURE				
	OPEN FIELD ACTIVITY (F)	OPEN ARM ENTRY (F)	TIME IN OPEN ARM (S)	TIME IN CENTRE (S)	TIME IN CLOSED ARM (S)
UNT	1017.8 ±119.7	0.5 ±0.4	7.3 ±5.5	306.2 ±35.1	331.4 ±30.5
SAL	1156.3 ±113.1	1.1 ±0.4	12.0 ±5.1	301.5 ±15.2	286.5 ±16.8
LD	1192.1 ±128.7	0.4 ±0.3	3.3 ±2.5	274.6 ±14.1	314.9 ±13.4
HD	1231.6 ±85.4	0.9 ±0.3	8.5 ±3.0	331.2 ±12.3	260.5 ±13.7

Cells contain mean±sem, n=8/group.

ventured onto the open arms in the elevated plus maze test. Once again, no group differences were evident on these measures (see table 14).

One animal in each pretreatment group showed impaired injection rate and these were discarded from data analyses. Histological examination indicated that the cannulae of the remaining 28 rats were in the 3rd ventricle.

Under the water deprivation schedule, all pretreatment groups showed similar responses in terms of grooming behaviour (HW, HS, BW, BS, and the total scratching score), eating and drinking behaviours, exploratory activity, and resting under control (saline; i.c.v.) conditions. In addition, all animals consumed similar amounts of wet mash and water over the test period under control conditions. In all pretreatment groups, BN i.c.v. dose-dependently increased the expression of grooming elements (HW, HS, BW, BS, and the total scratching score), lowered the frequency of drinking and eating behaviours and reduced the amount of water and wet mash consumed in the test period (see table 15 and figures 7-9). Generally, in terms of the BN dose-response curve, a ceiling effect was evident at the highest dose (1.0 μg) of this peptide. The HD and SAL groups were found to be consistently more sensitive to the effects of BN i.c.v. than the UNT or LD groups (as indicated by the intragroup dose-response data). Specifically, at the 0.1 μg dose, BN elicited significant increases in the frequency of

expression of all grooming elements (HW, HS, BW, BS, and the total scratching score) and decreased significantly the frequency of eating behaviour in the HD and SAL animals (see table 15 and figure 7). In addition, BN (0.1 μg) significantly reduced wet mash and water intake in the HD and SAL groups (see figures 8 and 9). In contrast, significant increases in grooming elements (HW, HS, BW, BS, and the total scratching score) occurred only at the highest dose of BN (1.0 μg) for the UNT group. Similarly, eating frequency and the amount of wet mash and water consumed were significantly decreased at the 1.0 μg dose of BN for this group of animals (see table 15 and figures 7-9). The LD animals showed a moderate level of sensitivity with HW significantly increased at the 0.1 μg dose of BN.

Significant group differences were found between the pretreatment groups in terms of behavioural and ingestive measures over the course of the BN i.c.v. dose-response curve. Specifically, significant differences were found between pretreatment groups at the 0.1 μg dose of BN. At this dose, the HD and SAL groups were found to scratch (and in particular body scratch) and body wash more frequently and engage in eating behaviour less frequently than LD and UNT groups (see table 15 and figures 7-9). In addition, the HD animals consumed less wet mash than the UNT or LD animals (although no pretreatment effect and only a weak interaction effect was detected on this measure -see table A-4 of

Table 15. --The Behavioural Effects of Centrally Injected BN in Adult Rats (>75 days old) Neonatally Pretreated with BN.

GRP	DOSE BN (μg)	BEHAVIOURAL ELEMENTS							
		HW	HS	BW	BS	EF	DF	RE	EX
UNT	0.0	4.4 ±0.7	2.3 ±0.6	1.3 ±0.5	2.7 ±0.6	80.6 ±2.7	17.0 ±1.5	2.0 ±0.6	14.6 ±2.3
UNT	0.01	4.0 ±1.3	1.9 ±0.3	2.6 ±1.0	2.6 ±0.8	84.7 ±4.6	16.7 ±1.7	1.1 ±0.7	11.0 ±2.6
UNT	0.1	6.1 ±0.6	3.9 ±1.2	2.7 ±1.0	6.1 ±1.9	72.4 ±2.0	19.0 ±2.0	4.1 ±1.5	13.9 ±2.1
UNT	1.0	15.9* ±2.1	39.3* ±5.2	9.3* ±1.4	34.3* ±3.6	25.3* ±3.0	10.7 ±1.2	10.3 ±3.8	19.0 ±1.8
SAL	0.0	3.7 ±1.0	1.9 ±0.7	1.2 ±0.5	0.9 ±0.5	70.7 ±3.8	22.3 ±2.9	2.3 ±1.1	19.4 ±3.3
SAL	0.01	5.4 ±1.2	1.1 ±0.6	2.3 ±0.6	2.0 ±1.1	75.3 ±2.7	21.4 ±1.9	2.1 ±0.6	14.9 ±2.6
SAL	0.1	16.3* ± 2.3^{U}	24.7* ± 4.2^{U}	11.1* ± 2.1^{UL}	28.4* ± 2.3^{UL}	37.3* ± 6.6^{UL}	17.1 ± 1.7	4.3 ± 1.0	15.6 ± 2.6
SAL	1.0	18.4* ±3.3	32.7* ±5.7	10.6* ±2.3	34.1* ±4.6	23.6* ±3.1	13.1 ±3.8	10.6 ±3.1	20.4 ±4.4
LD	0.0	4.1 ±0.9	2.0 ±0.8	2.1 ±0.9	1.7 ±0.7	78.0 ±3.0	19.7 ±1.8	3.4 ±1.6	11.7 ±2.6
LD	0.01	5.6 ±1.0	2.7 ±1.1	1.9 ±0.3	2.4 ±0.7	76.3 ±4.7	18.3 ±1.6	3.9 ±1.8	14.9 ±3.4
LD	0.1	12.3* ±1.6	12.6 ±4.6	4.0 ±1.4	13.0 ±4.3	61.9 ±8.4	19.0 ±2.4	3.4 ±1.1	15.6 ±2.7
LD	1.0	17.1* ±2.5	41.9* ±4.3	10.7* ±1.7	38.6* ±3.2	24.3* ±4.4	12.7 ±3.4	3.1 ±1.0	20.4 ±3.0
HD	0.0	4.0 ±0.8	2.6 ±1.4	2.1 ±0.4	1.4 ±0.6	78.3 ±5.7	19.4 ±1.4	1.4 ±1.3	16.1 ±4.3
HD	0.01	3.3 ±0.7	3.4 ±2.5	1.6 ±0.4	3.9 ±2.5	71.0 ±5.0	23.6 ±2.4	1.3 ±0.5	19.9 ±5.1
HD	0.1	16.3* ± 1.5^{U}	38.0* ± 4.9^{UL}	10.0* ± 1.4^{UL}	38.0* ± 3.7^{UL}	28.0* ± 2.5^{UL}	17.3 ± 2.0	7.3 ± 2.2	16.0 ± 3.5
HD	1.0	20.7* ±2.9	43.7* ±7.2	7.7* ±1.7	41.3* ±5.7	25.1* ±3.5	10.4 ±3.3	9.9 ±4.8	15.3 ±4.4

Cells contain mean±sem. * $p<.05$ from respective group baselines. ^{U} $p<.05$ from UNT group at that dose. ^{L} $p<.05$ from LD group at that dose. GRP abbreviates pretreatment group.

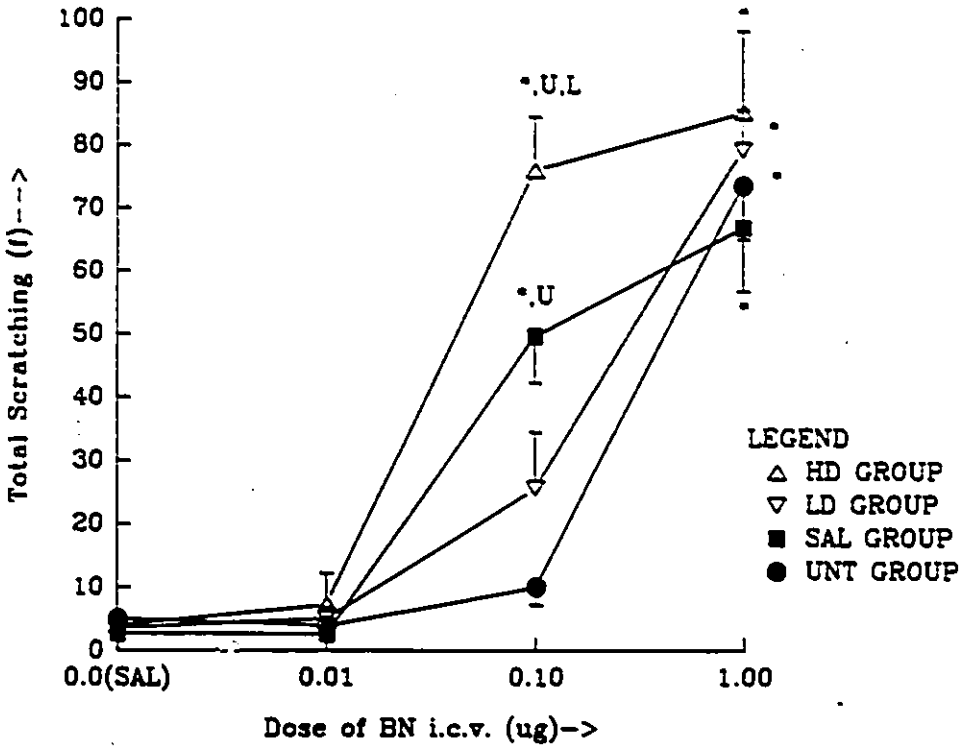

Figure 7. Total scratching elicited by BN (i.c.v.) in adult rats neonatally pretreated with BN. Data points represent mean±sem, n=7/group.
* p<.05 from respective group control condition (saline; i.c.v.).
U p<.05 from UNT group at that dose.
L p<.05 from LD group at that dose.

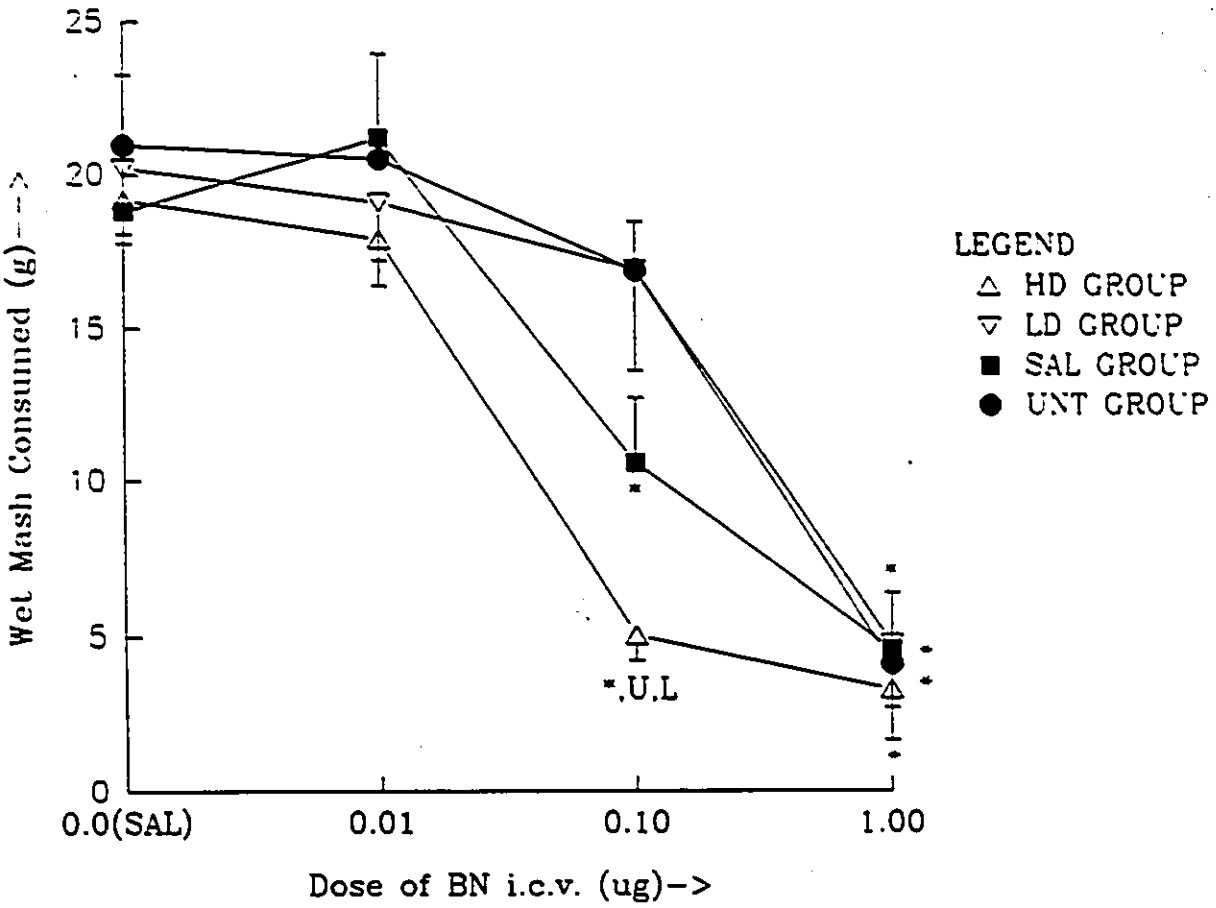

Figure 8. The effect of BN (i.c.v.) on wet mash consumption in adult rats neonatally pretreated with BN. Data points represent mean±sem, n=7/group.
* p<.05 from respective group control condition (saline; i.c.v.).
U p<.05 from UNT group at that dose.
L p<.05 from LD group at that dose.

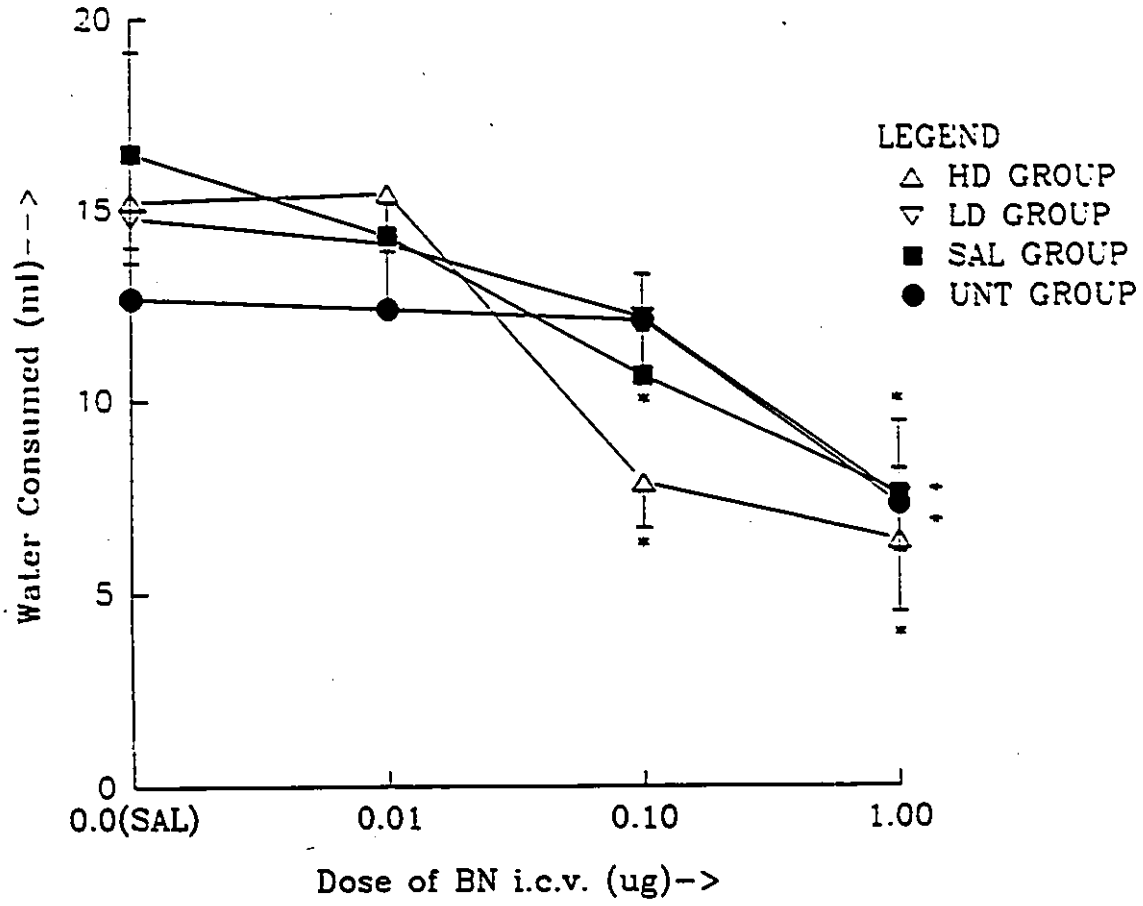

Figure 9. The effect of BN (i.c.v.) on water consumption in adult rats neonatally pretreated with BN. Data points represent mean±sem, n=7/group.
* p<.05 from respective group control condition (saline; i.c.v.).
u p<.05 from UNT group at that dose.

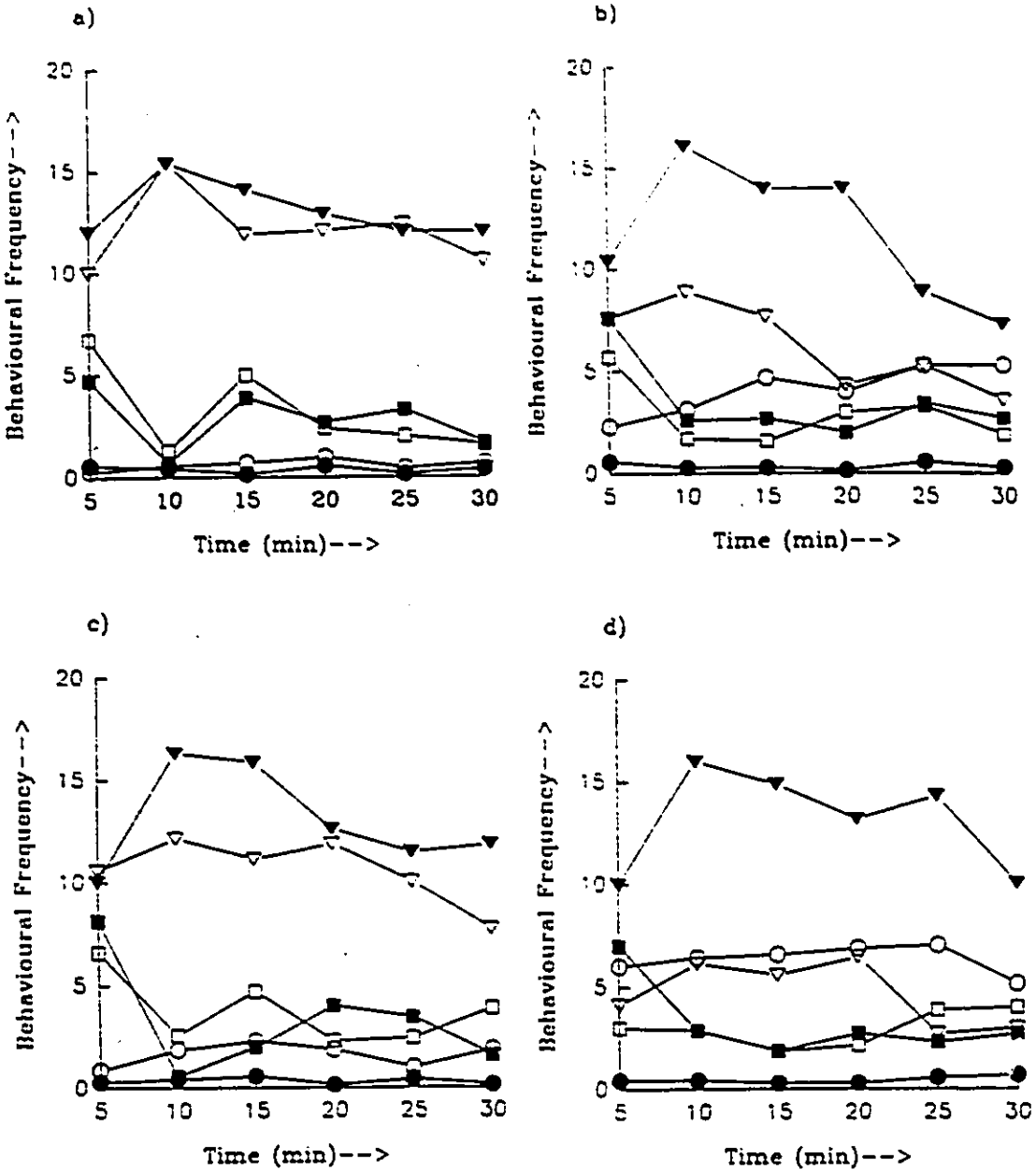

Figure 10. The effect of BN (0.1 μg; i.c.v.) on the frequency of expression of eating, drinking and head scratching. Data points represent mean, n=7/group. Panel a) shows the UNT group data, panel b) shows the SAL group data, panel c) shows the LD group data and panel d) shows the HD group data.
▼ represents eating frequency under saline i.c.v. condition.
▽ represents eating frequency under BN condition.
■ represents drinking frequency under saline i.c.v. condition.
□ represents drinking frequency under BN condition.
● represents head scratching under saline i.c.v. condition.
o represents head scratching under BN condition.

Table 16. --The Behavioural Effects of Systemically Administered BN in Adult Rats (>75 days old) Neonatally Pretreated with BN.

GROUP	DOSE BN (μg)	BEHAVIOURAL MEASURE		
		EF	REST	EX
UNT	0.0	81.1 ±5.0	17.6[M] ±2.2	11.1 ±2.6
UNT	2.0	76.3 ±1.9	6.9 ±2.1	14.4 ±2.5
UNT	4.0	68.6 ±4.7	9.9 ±4.8	17.0 ±1.9
UNT	8.0	33.7* ±8.1	51.4* ±8.8	19.4 ±2.8
SAL	0.0	75.0 ±4.1	7.7 ±2.4	15.6 ±2.6
SAL	2.0	63.0 ±1.7	10.6 ±5.2	26.6 ±2.0
SAL	4.0	55.6 ±8.9	18.9 ±9.5	22.1 ±3.5
SAL	8.0	37.9* ±9.5	34.4* ±9.5	18.6 ±3.9
LD	0.0	77.9 ±4.0	5.7 ±1.1	15.6 ±3.1
LD	2.0	73.0 ±6.1	10.7 ±2.7	14.3 ±4.1
LD	4.0	61.1 ±8.2	13.9 ±7.7	22.6 ±5.4
LD	8.0	37.9* ±6.8	46.0* ±11.5	16.1 ±3.1
HD	0.0	75.3 ±2.5	2.9 ±0.7	15.0 ±2.7
HD	2.0	72.7 ±3.1	8.4 ±1.7	19.6 ±1.9
HD	4.0	59.9 ±8.9	17.6 ±6.4	17.6 ±2.0
HD	8.0	47.1* ±6.0	26.0* ±10.7	25.3 ±3.0

Cells contain mean±sem. * $p<.05$ from respective group baseline. [M] $p<.05$ from HD group at that dose.

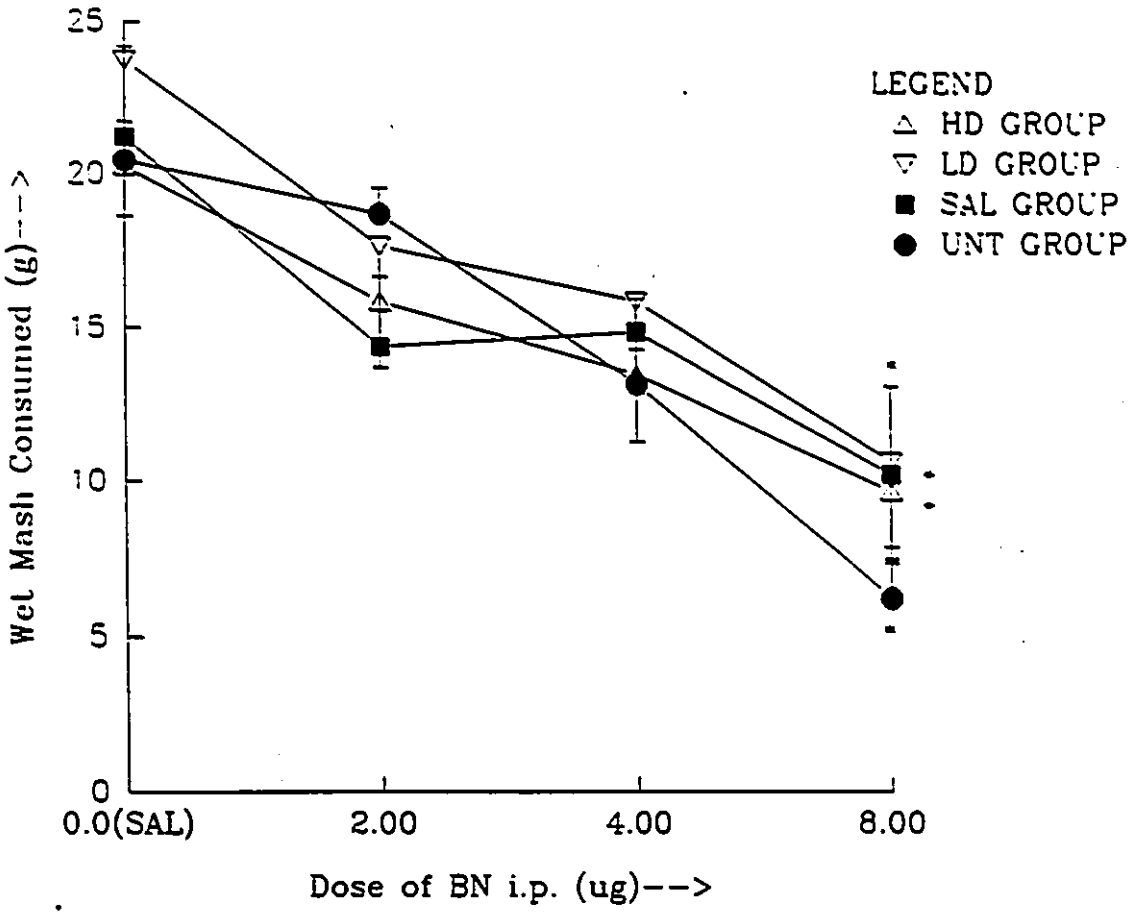

Figure 11. The effect of BN (i.p.) on wet mash consumption in adult rats neonatally pretreated with BN. Data points represent mean±sem, n=7/group.
*** p<.05 from respective group control condition (saline; i.p.).**

Appendix A). Also at this dose, the HD and SAL groups head washed more than the UNT group (although no pretreatment or interaction effect was detected on this measure -see table A-4 of Appendix A). Further, at 0.1 μg BN, the HD group head scratched more frequently than the UNT and LD groups whilst the SAL group head scratched more often than the UNT group (figure 7 illustrates this effect on the total scratching score). These group differences were not significant at the 1.0 μg dose of BN.

Figure 10 shows the pattern of eating and drinking frequencies as well as that of head scratching in 5 min bins across the 30 min test period. The pattern for all groups in the control condition (saline i.c.v.) and at the 0.1 μg dose of BN are illustrated. For each group under the control condition, eating frequency increased rapidly and maintained a high level over the first 15-20 min of the test period whilst drinking frequency declined and showed an inconsistent pattern.

Head scratching was expressed at low frequencies across all time bins. In those groups significantly affected by BN at the 0.1 μg dose (SAL and HD, see panels b) and d) respectively of figure 10), eating frequency did not rise steeply after the initial 5 min but maintained a moderate level and then gradually declined. Head scratching increased gradually over this time period. However, increases in head scratching did not necessarily mirror the

changes in eating frequency. No such trend was noted in the UNT group (panel a); figure 10) and the LD group demonstrated an intermediate pattern (panel c); figure 10).

Under the water deprivation schedule, systemic injection of BN reduced the consumption of wet mash and decreased feeding behaviour in all pretreatment groups in a dose-related manner. Grooming elements, drinking behaviour and water intake were unaffected by BN i.p. in all groups (data not shown). No intergroup differences were found in terms of either feeding (see table 16) or wet mash intake (see figure 11). The UNT group rested more in response to saline i.p. than the HD animals. BN (8 μg/kg; i.p.) inhibited food intake in all groups and the frequency of eating behaviour was decreased for all groups at this dose. Resting increased in all animals at the 8.0 μg/kg dose of BN.

4. Discussion

During the non-invasive behavioural tests, all rats appeared fairly active when assessed under isolation, open field and novel conditions. However, in the elevated plus maze, few animals entered the open arms of the maze. Grooming and locomotory activity were prevalent in the novel environment whilst isolation elicited mainly exploratory activity. No intergroup differences were apparent and thus

pretreatment appeared to have no effect on these behavioural measures.

Animals in all groups adapted to the water deprivation schedule, consuming consistent amounts of water and wet mash within 7-10 days thus corroborating earlier observations (Piggins, Lafreniere and Merali, 1989). BN i.c.v. elicited grooming activity characterized by scratching, reduced ingestive behaviours and decreased the amount of wet mash and water consumed over the 30 min test period. BN i.p. reduced wet mash consumption and eating behaviour but had no effect on water consumption or drinking behaviour. Resting was generally found to increase with the high dose of the peptide (8 μg/kg; i.p.). The results are consistent with previous data from this testing regimen (Piggins, personal observations; Piggins, Lafreniere and Merali, 1989) and are in agreement with the known behavioural and satiety-inducing effects of BN (De Caro et al., 1985; Gibbs et al., 1979; Kulkosky, Gibbs and Smith, 1982; Negri, 1986).

Several intergroup differences were apparent under the water deprivation schedule to centrally but not peripherally administered BN. These suggest that subchronic neonatal treatment with BN appears to alter in adulthood, the sensitivity of the endogenous BN-like system in the CNS. LD pretreated animals were less sensitive than the HD pretreated animals in terms of the effect of BN (0.1 μg; i.c.v.) on body washing, scratching, eating and amount of

food consumed (although the sensitivity effect as measured on wet mash consumption was not nearly as pronounced as the differences noted on the scratching frequency scores due to the lack of a pretreatment effect or strong interaction effect -see table A-4 of Appendix A). Also at this dose, the LD group body scratched and body washed less than the SAL group. The SAL group showed greater sensitivity to BN (0.1 μg; i.c.v.) on many behavioural and ingestive measures than the UNT group, indicating an apparent injection effect (although differences on measures of consumption of wet mash were not as pronounced -see table A-4 of Appendix A). In short, it appears that in comparison to the SAL group, LD animals were subsensitive and the HD animals borderline hypersensitive to the effects of BN i.c.v.. These data also indicate an injection effect over and above the effects of neonatal handling.

This trend was also apparent when the data were broken down into 5 min bins. Under the 0.1 μg dose of BN, the eating frequency of both the SAL and HD groups failed to show the steep rise after the first 5 min (as it had under the control condition). The LD group showed only a moderate change in this pattern and the UNT group little if any change. The frequency of head scratching in the SAL and HD groups did not rise that much over the test period when feeding behaviour declined. This supports the contention that the suppression of food intake following BN i.c.v. is

not necessarily due to activation of grooming/scratching mechanisms.

A number of possible mechanisms can be implicated to account for the pretreatment effects. Subchronic exposure in infancy or adulthood to peptides can increase or decrease the number of receptors for that peptide in the CNS (Handelmann, 1988). This can be manifested behaviourally by altered sensitivity to the peptide (Handelmann, Selsky and Helke, 1984; Handelmann, Shults and O'Donohue, 1987).. Dose-dependent changes in BN receptor number following neonatal treatment may account for the differences between the LD and HD groups. However, an injection effect was apparent (as shown by the SAL group) and little BN-like immunoreactivity is present in the developing rat CNS during the period of injection (Gillati and Moody, 1984). It seems likely that other endogenous neurotransmitter systems were activated by the injection procedure. Neurotransmitters can act as morphogens in the developing CNS (Lauder, 1988) and it could be that the development and synaptogenesis of neurons containing BN-like peptides was altered indirectly by the other neurochemical system(s).

The SAL condition represents the concomitant effects of handling and the stress of the injection on the development of BN and/or neurochemical systems. Neonatal stress can have long-term effects on the development of behavioural and physiological adaptation to stress in adulthood (e.g.

Gonzalez et al., 1990) and the effects of handling on adult stress responses are well known. Beta-endorphin is released in response to stress from postnatal days 4 to 12 (Iny et al., 1987). It could be that beta-endorphin release was induced by saline injections and beta-endorphin indirectly altered the development of the endogenous BN-like peptide system.

Both handling and physical stimulation lower brain and body temperature (Sullivan, Shokrai and Leon, 1988; Sullivan, Wilson and Leon, 1988) in the infant rat and this could have long lasting effects on behaviour (Hutchings, 1968; McIver, 1968; Schaeffer, 1968). Further, dams treat cold exposed pups differently and contact them more than warm pups (Jans and Leon, 1983; Leon et al., 1978). Of all groups, the UNT pups were removed from the litter for the shortest period of time. The UNT pups were weighed and marked twice a day whereas the other pups were weighed, marked and then injected (injection volumes had to be measured which also increased time away from the litter). It remains a possibility that the treated pups may have been affected as a consequence of hypothermia. However, as neither the temperature of the pups post-injection nor the treatment of the pups by the mothers was examined, no conclusion can be drawn.

Considerable evidence exists to suggest that the ontogeny of central glucocorticoid receptors (GCs) and the

behavioural response of developing rat pups to exogenously administered ACTH1-10_{NH2} are dependent, to some degree, upon the ontogeny of the serotonergic system (Kirstein et al., 1990; Mitchell, Iny and Meaney, 1990). It could be that the development of BN-related system(s) in the CNS is also dependent on the ontogeny of other neurotransmitter systems. The above treatments could have altered the development of BN systems directly (LD and HD groups) and/or indirectly via effects on other neurotransmitter systems (SAL). The role of serotonin in BN-induced activity in the adult rat is unknown but evidence exists to suggest that dopamine could partially modulate/mediate the scratching effects of BN (Merali, Johnston and Zalcman, 1983; Merali and Piggins, 1990; Piggins and Merali, 1989). Direct assessment of dopaminergic involvement in the behavioural effects of BN in the developing rat has yet to be performed and as such it is premature to make further inferences.

The above results indicate that injection of saline or BN (5 or 10 mg/kg; s.c.) twice a day for the first 8 postnatal days had no effect on unconditioned behavioural responses, including novelty or isolation-induced activity, and activity elicited in the open field or elevated plus maze. Injecting animals subcutaneously with saline or BN (10 mg/kg s.c.) does increase adult sensitivity to BN (0.1 μg; i.c.v.) as compared to handled, non-injected controls. Neonatal pretreatment with BN (5 mg/kg; s.c.) appears to

reduce adult sensitivity to some behavioural effects of BN (0.1 μg; i.c.v.) as compared to animals pretreated neonatally with saline or BN (10 mg/kg) s.c.. Neonatal pretreatments had no effect on later adult sensitivity to systemically administered BN. These effects could be due to long-term changes in central BN system(s), changes in maternal care of treated pups or to changes in other neurochemical systems mediating/modulating the effects of i.c.v. BN.

C. Experiment 2b: The Long-term Effects of Neonatal Injections of a BN Receptor Antagonist.

1. Introduction

Recent studies suggest that long-term behavioural changes occur following neonatal treatment with neuropeptide antagonists (Handelmann, 1988). Neonatal treatment with vasopressin antagonists decreases later adult performance on passive avoidance tasks (Snijdewint and Boer, 1988). Similarly, neonatal treatment with vasopressin antiserum, which sequesters endogenous vasopressin, results in decreased adult performance on both active and passive avoidance tasks (Moratella et al., 1987). Neonatal treatment with naloxone has profound effects on somatic and CNS development (Najam and Panksepp, 1989; Zagon and McLaughlin, 1987). Hence, blockade of neuropeptide effects

in the CNS during infancy can effect long-term changes in neural and behavioural development.

The results of experiment 2a implied that neonatal treatment with a neuropeptide agonist (i.e. BN) altered later adult response to that neuropeptide. Blockade of neuropeptide receptors would also be expected to affect behaviours in which the neuropeptide is involved. The next study was then undertaken to investigate the effects of neonatal treatment with a BN-receptor antagonist.

[D-Phe^{6},ΨLeu^{13}-Cpa^{14}]BN(6-14) is a recently synthesized BN analogue which was found to have specific BN receptor antagonist properties in rat pancreatic acini membranes (Von Schrenk et al., 1990). Preliminary tests indicated that pretreatment with 5 or 10 mg/kg s.c. of this antagonist reduced grooming elicited by BN (10 mg/kg; s.c.) in 4 day old rat pups by 20 and 45%, respectively. No overt behavioural effects of the antagonist were observed. It was thus decided to use the methodology of experiment 2a to examine the long-term effects of neonatal exposure to 5 and 10 mg/kg doses of the antagonist.

2. Purpose

The purpose of this experiment was to establish the long-term consequences of neonatal exposure to the recently synthesized BN receptor antagonist, [D-Phe^{6},ΨLeu^{13}-Cpa^{14}]BN(6-14). In particular, the goal was to diminish or block the

effects of endogenous BN-like peptides by blocking BN receptors from postnatal day 1 through to postnatal day 8.

3. Methods

The methods and experimental protocol used in this experiment were the same as in experiment 2a with the following exceptions: (1) 5 mg/kg s.c. antagonist pretreatment (LDA group) and 10 mg/kg s.c. antagonist (HDA group) were substituted for the BN pretreatments and (2) females (of Charles River stock) were bred in the departmental animal care facilities as timed-pregnant females were unavailable from the commercial source at the commencement of this study.

4. Results

No group differences were found on measures of body development (day of eye opening, day of appearance of bodily fur or body weight gain) over the first 3 weeks of life (data not shown). One animal (HDA group) was crushed by the mother on day 8 and a similar aged pup from another litter was substituted until isolation. No group differences were found in body weight changes over the succeeding 42 days (data not shown). Isolation elicited mainly exploratory activity and little grooming (see table 17).

Table 17. --The Effects of Neonatal Pretreatment with [D-Phe^{6}, ψLeu^{13}-Cpa^{14}]BN(6-14) on Isolation-Induced Activity in Juvenile Rats (45 days of age).

GROUP	BEHAVIOURAL ELEMENTS							
	HW	HS	BW	BS	AG	REST	SNIFF	EX
UNTA	4.25 ±0.9	4.1 ±0.8	6.6 ±1.8	3.8 ±1.0	1.0 ±0.4	13.1 ±3.1	6.8 ±1.3	89.0 ±4.6
SALA	5.5 ±1.5	3.3 ±1.1	8.8 ±2.8	4.0 ±0.8	1.1 ±0.7	13.3 ±3.5	6.8 ±0.8	86.6 ±5.1
LDA	4.8 ±1.4	2.6 ±0.8	5.1 ±1.5	2.6 ±1.1	0.9 ±0.5	12.9 ±4.0	7.9 ±1.6	90.9 ±4.5
HDA	3.6 ±0.9	5.3 ±1.9	7.4 ±1.9	4.9 ±1.3	0.5 ±0.4	15.4 ±4.4	6.6 ±1.2	78.1 ±8.9

Cells contain mean±sem, n=7/group.

Table 18. --The Effects of Neonatal Pretreatment with [D-Phe^{6}, ΨLeu^{13}-Cpa^{14}]BN(6-14) on Novelty-Induced Activity in Young Adult Rats (52 days old).

GROUP	BEHAVIOURAL ELEMENTS								
	HW	HS	BW	BS	AG	REST	SNIFF	EX	LOCO (cm)
UNTA	13.9 ±1.8	3.3 ±0.8	16.5 ±1.7	2.6 ±0.9	0.5 ±0.4	106.9 ±4.6	24.4 ±4.4	105.8 ±7.1	1014.8 ±180.6
SALA	9.0 ±2.6	4.1 ±1.0	17.0 ±3.4	4.2 ±1.2	0.4 ±0.2	114.5 ±13.2	23.5 ±5.6	100.6 ±11.8	1371.8 ±269.6
LDA	10.9 ±1.7	1.8 ±0.5	12.9 ±2.8	3.4 ±1.1	0.6 ±0.4	113.9 ±12.6	24.8 ±4.3	97.6 ±9.6	1221.4 ±201.9
HDA	11.0 ±2.4	3.9 ±1.8	16.0 ±2.7	4.4 ±2.3	3.4 ±2.3	116.3 ±10.9	32.3 ±4.0	98.5 ±7.0	1330.0 ±262.0

Cells contain mean±sem, n=7/group.

Table 19. --The Effects of Neonatal Pretreatment with [D-Phe^{6},ΨLeu^{13}-Cpa^{14}]BN(6-14) on the Activity of Rats (58 days of age) in the Open Field and Elevated Plus Maze.

GROUP	BEHAVIOURAL MEASURE				
	OPEN FIELD ACTIVITY (F)	OPEN ARM ENTRY (F)	TIME IN OPEN ARM (S)	TIME IN CENTRE (S)	TIME IN CLOSED ARM (S)
UNTA	1178.5 ±93.6	1.5^{H} ±0.9	14.5^{H} ±8.3	329.7 ±18.5	270.2 ±18.5
SALA	1353.4 ±92.6	1.3^{H} ±0.4	14.1^{H} ±5.5	327.2 ±17.3	272.8 ±17.3
LDA	1016.0 ±94.6	0.6^{H} ±0.4	6.5^{H} ±4.3	315.1 ±19.1	285.0 ±19.1
HDA	1197.6 ±108.1	5.4 ±1.3	60.6 ±17.0	376.0 ±18.9	224.1 ±18.9

Cells contain mean±sem, n=7/group. ^{H} $p<.05$ from the HDA group.

Transfer into a novel environment induced grooming, locomotory activity and subsequently resting (see table 18). No intergroup differences were found on either of these tests . All animals showed much activity in the open field test but no intergroup differences were apparent (see table 19). In the elevated plus maze, animals from the UNT, SAL, and LDA groups remained largely in the centre and closed arm portions of the apparatus. However, HDA animals entered and spent significantly more time on the open-arms of the plus maze than any other group (see table 19). No other intergroup differences were found using this apparatus.

One animal (from the LDA group) died during surgery but the remaining animals recovered quickly and adapted to the water deprivation schedule 7-9 days postoperation. In order to complete statistical analyses, the animals that responded the most inconsistently to BN i.c.v. were removed from analyses (1 from the SALA group and 1 from the UNTA group).

Under the water deprivation schedule, animals of all groups showed similar levels of grooming behaviours (HW, HS, BW, BS as well as the total scratching score), feeding and drinking behaviours, and intake of water and wet mash over the test period in response to control injection (saline; i.c.v.). Central administration of BN dose-dependently elicited increases in grooming elements (HW, HS, BW, BS, and total scratching) and decreased eating behaviour in all

pretreatment groups (see table 19 and figure 12) (see tables A-6 and A-7 of Appendix A for relevant F-ratios). In addition, BN i.c.v. dose-dependently reduced the consumption of wet mash (see figure 13) and water (see figure 14) in all pretreatment groups (except water intake for the UNTA group) although drinking behaviour was only affected in the LDA animals (see table 20). Generally, the dose-response curves of the behavioural responses to BN i.c.v. demonstrated a ceiling effect at the highest dose of the peptide (1.0 μg) (see figures 12-14 and table 20). At this dose, the water consumption of the UNTA animals was unaffected by BN and they consumed significantly less water than all other animals. No intergroup differences were apparent on any other behavioural or consumption measure over the BN i.c.v. dose-response curve.

Under the water deprivation schedule, systemically administered BN dose-dependently reduced wet mash consumption and decreased feeding behaviour in all pretreatment groups. Neither water intake nor grooming behaviours (HW, HS, BW, BS and the total scratching score) were affected by BN i.p. (data not shown) in any pretreatment group. No intergroup differences in behavioural or intake measures to saline i.p. were found (see table 21 and figure 15). BN (8 μg/kg; i.p.) reduced wet mash consumption in all animals. Eating frequency was

Table 20. --The Behavioural Effects of Centrally Injected BN in Adult Rats (>75 days old) Neonatally Pretreated with [D-Phe^{6}, ΨLeu^{13}-Cpa^{14}]BN(6-14).

GRP	DOSE BN (μg)	BEHAVIOURAL ELEMENTS							
		HW	HS	BW	BS	EF	DF	RE	EX
UNTA	0.0	4.62 ±0.9	2.1 ±0.8	1.9 ±0.9	2.7 ±0.9	79.3 ±3.7	20.3 ±1.9	1.6 ±0.9	12.6 ±3.5
UNTA	0.01	6.4 ±1.0	7.9 ±1.8	4.3 ±1.4	7.0 ±1.7	68.3 ±5.0	25.4 ±2.8	2.0 ±0.8	14.9 ±2.4
UNTA	0.1	9.7 ±0.9	33.3* ±4.7	7.1* ±1.3	28.9* ±4.2	41.1* ±4.6	25.0 ±2.7	4.4 ±1.8	14.6 ±7.4
UNTA	1.0	18.1* ±2.9	45.1* ±4.5	7.3* ±1.2	35.4* ±3.9	36.7* ±7.3	20.4 ±3.1	7.4 ±2.5	7.4 ±2.5
SALA	0.0	3.6 ±0.8	3.1 ±1.3	1.4 ±0.3	1.9 ±0.5	81.3 ±5.0	18.7 ±2.3	3.9 ±1.1	9.4 ±2.7
SALA	0.01	4.1 ±0.8	6.4 ±2.7	2.6 ±0.6	7.7 ±3.3	73.3 ±4.9	21.6 ±2.8	1.1 ±0.7	11.9 ±2.6
SALA	0.1	9.4* ±1.3	21.1* ±3.6	5.1 ±1.7	19.3* ±3.4	58.0 ±3.4	22.4 ±2.9	4.0 ±1.1	12.1 ±2.7
SALA	1.0	21.0* ±2.9	47.1* ±3.9	7.0 ±1.4	39.9* ±4.3	33.7* ±4.5	13.1 ±2.9	6.6 ±1.8	13.6 ±1.9
LDA	0.0	6.3 ±1.2	4.3 ±0.9	2.3 ±0.9	3.9 ±0.9	76.7 ±5.4	21.1 ±1.5	2.7 ±1.1	12.6 ±2.2
LDA	0.01	3.6 ±0.8	8.1 ±3.5	2.4 ±0.4	9.3 ±3.4	77.9 ±4.7	16.3 ±1.6	2.4 ±0.5	9.9 ±1.9
LDA	0.1	10.1 ±1.4	21.6* ±5.8	6.6 ±2.6	19.3 ±4.9	50.7* ±7.2	21.7 ±4.1	1.7 ±0.2	14.0 ±2.0
LDA	1.0	15.6* ±1.9	42.3* ±3.9	6.0 ±2.0	37.3* ±4.7	31.1* ±3.9	10.9 ±2.0	14.4* ±4.0	13.7 ±1.1
HDA	0.0	2.6 ±0.6	3.4 ±0.9	1.3 ±0.5	3.6 ±1.2	83.1 ±3.3	20.0 ±2.1	1.6 ±0.6	11.6 ±3.0
HDA	0.01	5.9 ±1.0	14.7 ±3.8	3.9 ±1.1	13.1 ±2.7	66.1 ±6.3	23.4 ±2.4	2.0 ±0.9	13.0 ±1.8
HDA	0.1	9.1* ±1.1	20.7* ±6.0	5.9 ±1.4	21.6* ±5.9	57.3* ±7.2	19.6 ±1.1	2.6 ±0.8	15.7 ±3.0
HDA	1.0	21.6* ±1.9	47.0* ±2.1	6.1 ±2.4	38.1* ±2.1	27.3* ±2.6	13.4 ±3.5	5.4 ±1.8	13.4 ±2.4

Cells contain mean±sem, n=7/group. * $p<.05$ from respective group baseline. GRP abbreviates pretreatment group.

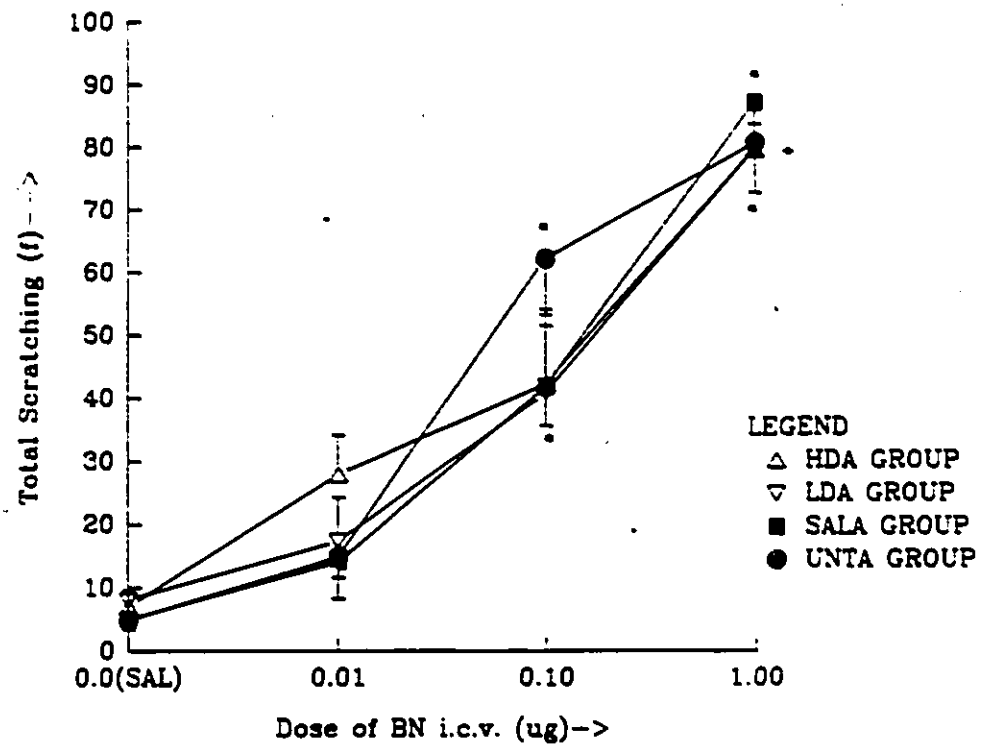

Figure 12. Total scratching elicited by BN (i.c.v.) in adult rats neonatally pretreated with [D-Phe^{6}, $\bar{\text{T}}$Leu^{13}-Cpa^{14}]BN(6-14). Data points represent mean±sem, n=7/group.
* $p<.05$ from respective group control condition (saline; i.c.v.).

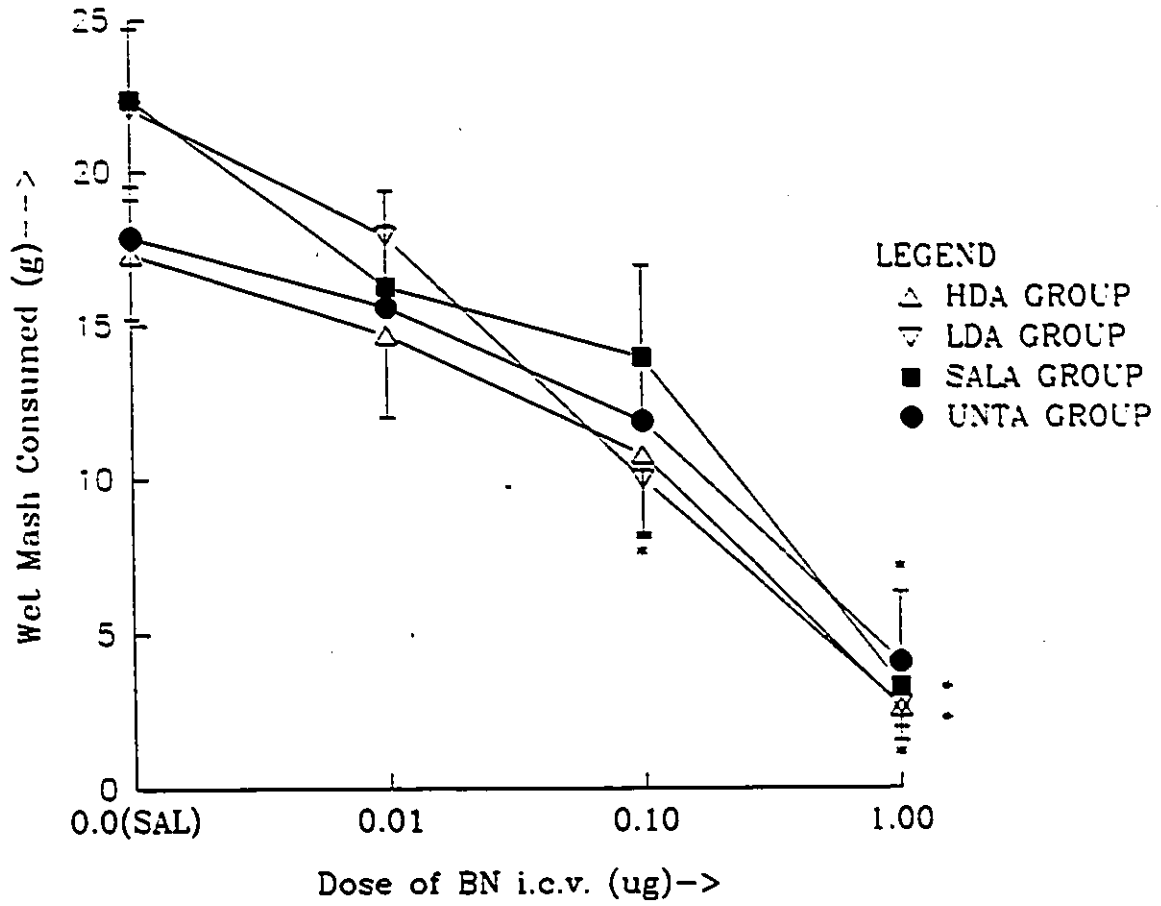

Figure 13. The effect of BN (i.c.v.) on wet mash consumption in adult rats neonatally pretreated with [D-Phe^{6}, Leu^{13}-Cpa^{14}]BN(6-14). Data points represent mean±sem, n=7/group.
* $p<.05$ from respective group control condition (saline; i.c.v.).

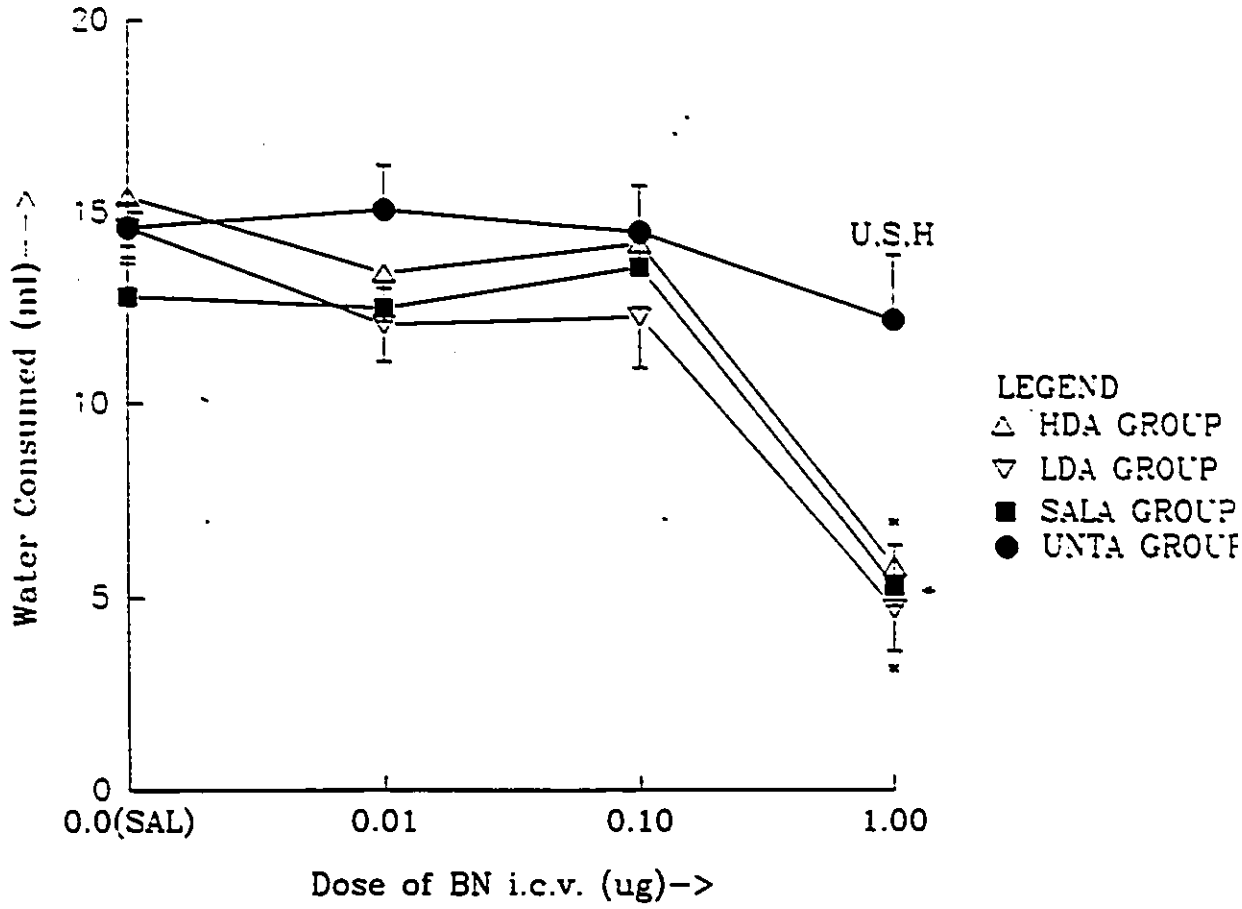

Figure 14. The effect of BN (i.c.v.) on water consumption in adult rats neonatally pretreated with [D-Phe^{6}, $^{\Psi}$Leu^{13}-Cpa^{14}]BN(6-14). Data points represent meantsem, n=7/group.
* p<.05 from respective group control condition (saline; i.c.v.).

Table 21. --The Behavioural Effects of Systemically Administered BN in Adult Rats (>75 days old) Neonatally Pretreated with [D-Phe^{6}, ΨLeu^{13}-Cpa^{14}]BN(6-14).

GROUP	DOSE BN (μg)	BEHAVIOURAL ELEMENTS		
		EF	REST	EX
UNTA	0.0	77.1 ±4.6	7.0 ±4.4	10.0 ±3.4
UNTA	2.0	78.9 ±4.0	4.0 ±1.1	7.3 ±2.9
UNTA	4.0	67.6 ±6.7	13.4 ±3.7	13.4 ±3.9
UNTA	8.0	47.7* ±4.1	35.6* ±7.6	19.9 ±4.5
SALA	0.0	76.1 ±4.6	8.0 ±2.7	12.1 ±2.4
SALA	2.0	80.1 ±4.5	4.9 ±2.0	7.9 ±2.1
SALA	4.0	76.6 ±5.3	6.3 ±1.3	11.1 ±3.1
SALA	8.0	57.0 ±8.4	28.9 ±8.3	8.7 ±1.6
LDA	0.0	77.1 ±3.3	5.1 ±2.4	11.4 ±2.1
LDA	2.0	68.3 ±6.0	8.0 ±1.8	13.7 ±3.4
LDA	4.0	56.6 ±9.1	18.6 ±6.7	14.3 ±5.3
LDA	8.0	53.4 ±8.4	18.0 ±6.3	20.0 ±3.9
HDA	0.0	87.3 ±4.9	2.6 ±1.0	9.0 ±4.0
HDA	2.0	79.9 ±3.3	5.7 ±1.0	10.9 ±3.2
HDA	4.0	66.9 ±8.0	18.0 ±6.5	11.0 ±2.0
HDA	8.0	43.1* ±7.9	49.7* ±10.2	5.7^{UL} ±1.5

Cells contain mean±sem, n=7/group.
* $p<.05$ from respective group baseline.
^{UL} $p<.05$ from UNTA and LDA group at that dose.

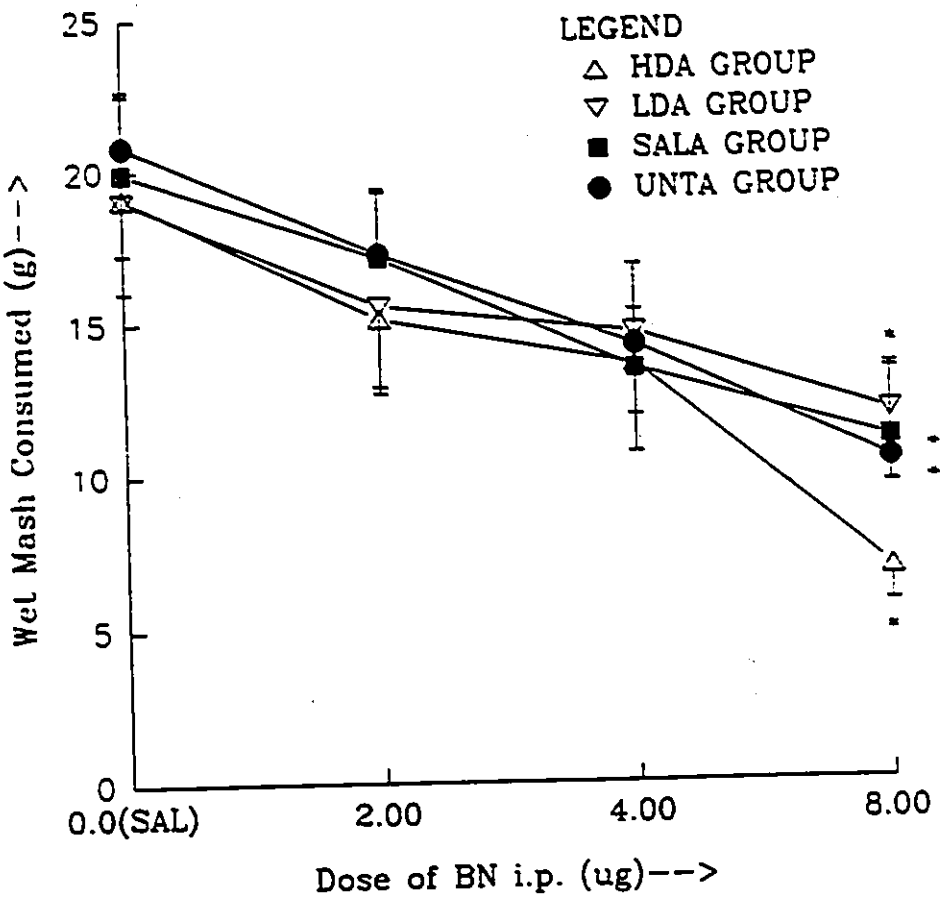

Figure 15. The effect of BN (i.p.) on wet mash consumption in adult rats neonatally pretreated with [D-Phe^{6}, ΨLeu^{13}-Cpa^{14}]BN(6-14). Data points represent mean±sem, n=7/group.
* p<.05 from respective group control condition (saline; i.p).

generally reduced at this dose except in the SALA and LDA groups where it was borderline non-significant. Resting was increased in SALA and UNTA animals at the 8 µg/kg dose of BN i.p. but not the LDA group (no intergroup differences were found). This treatment had no effect on exploratory activity but the HDA group showed less exploratory activity than the UNTA or LDA groups (see table 21).

No other changes to BN i.c.v. or i.p. were found though the LDA and HDA animals showed greater variability on most behavioural and consumption indices.

5. Discussion

Neonatal treatment with the BN receptor antagonist [D-Phe^{6},ΨLeu^{13}-Cpa^{14}]BN(6-14) failed to influence a variety of measures of brain and physical development. Body growth, as reflected by body weight gain was unaffected (data not shown) by these neonatal treatments and the mean group weights were remarkably similar over a number of weeks. Nursing and subsequent ingestive behaviour would thus appear to have been unaffected by pretreatment with the antagonist.

The data from the novelty and isolation tests indicate that exploratory/locomotory activity was elicited under both conditions whereas increased grooming was observed in the novel environment only. No intergroup differences were observed within the context of these tests indicating that antagonist pretreatment had no effect on novelty or

isolation-induced behavioural arousal. These results are in agreement with those of the previous study (experiment 2a) and indicate that the isolation test may be too short to elicit large increases in grooming. Neither test situation appears to differentiate the neonatal pretreatment conditions and hence it would seem that either BN based mechanisms have little if any involvement in the mediation/modulation of behaviour under these conditions or that recovery (due to plasticity) is complete at this point.

Placement of rats in the open field box elicited much activity but no intergroup differences were noted. HDA pretreated animals entered the open arm of the elevated plus maze more frequently and for longer than animals of any other group. Such activity within this apparatus has been interpreted as indicating an anxiolytic action of drug treatment in acute studies (Pellow and File, 1985). Agents which decrease anxiety, such as benzodiazepines, increase the entries and time spent on the open arms of this apparatus (Pellow et al., 1985). In terms of neuropeptides, NPY (Heilig et al., 1989) and a CCK receptor subtype-B antagonist (Hughes et al., 1990) have anxiolytic actions as measured on this instrument. BN has been shown to block the orexigenic action of NPY (Morley et al., 1987) and NPY i.c.v. reduces grooming activity (Corp et al., 1990). However, it is unknown whether the BN-receptor antagonist

employed in this experiment has actions on the above neurochemical systems.

The behaviour of the animals under the water deprivation schedule was essentially similar to that of the animals in experiment 2a and of animals in previous studies. As the dose of BN i.c.v increased, scratching and washing behaviours increased while ingestive behaviours and consumption of wet mash and water decreased. The only apparent intergroup difference was at the 1.0 μg dose of BN where the UNTA animals consumed less water than all other groups. The reasons for this are not apparent but given that no other changes in consumption measures were found, then this was probably an anomalous finding. Overall, neonatal pretreatment with [D-Phe^{6},ΨLeu^{13}-Cpa^{14}]BN(6-14) at either dose failed to alter the response to BN i.c.v in adulthood as compared to saline pretreated or untreated, but handled controls. Permanent changes in BN receptor based mechanisms do not appear to have occurred in this experiment.

Likewise, the responses of the experimental population to BN i.p. were similar to that of the previous experiment and no consistent intergroup differences were noted. Wet mash consumption decreased as a function of dose of BN and the frequency of ingestive behaviour followed a similar trend. Water intake and drinking behaviour were unaffected by any dose of BN i.p.. The only significant intergroup

difference to be noted was that the HDA animals explored less than the UNTA or LDA animals following BN (8 μg/kg; i.p.). This group of animals had the highest level of resting and consumed the least amount of wet mash following BN i.p. (8 μg/kg). This could indicate increased sensitivity to BN i.p. but the evidence is inconclusive.

The dose-response curves to BN (i.c.v. or i.p.) compare favourably to those of experiment 2a, indicating that this group of animals was not on the whole different from that of the previous experiment. However, the behavioural response of the UNTA group to BN i.c.v. was considerably different from that of the UNT group in experiment 2a. In the present study, the UNTA group displayed a greater sensitivity to BN i.c.v. than the UNT group. Reasons for this are not immediately apparent. The experiments were carried out at different times of the year (autumn vs winter) but home colony room conditions were similar. The only apparent difference was that the litters for the present study were obtained from females bred within the department whereas those of experiment 2a were derived from mothers bred at Charles River. Timed-pregnant females from Charles River were unavailable at the beginning of the present experiment and hence females acquired from Charles River were bred in the colony room. This could have played a role in this experiment as prenatal stress (as might occur through transport of timed-pregnant rats from Charles River to the

university) can affect the behaviour and neural development of infant pups (Fride et al., 1986; Fride, Soreq and Weinstock, 1986; Fride and Weinstock, 1988). Hence, the prenatal environment may have influenced the outcome of the present study.

In the adult rat, long-term administration of a receptor antagonist can result in behavioural hypersensitivity to receptor agonists and may involve increased numbers of the receptor(see Fleming and Westfall, 1988; Johnson and Fleming, 1989; Wolfe and Molinoff, 1988 for recent reviews of this and related phenomena). This phenomenon has been demonstrated with chronic injection regimes of a variety of dopamine receptor antagonists including haloperidol (Muller and Seeman, 1978) and SCH 23390 (Creese and Chen, 1985). This phenomenon has also been demonstrated with dopamine antagonists in the infant rat (Saleh and Kostrzewa, 1988). However, a similar effect of [D-Phe^{6},ΨLeu^{13}-Cpa^{14}]BN(6-14) on BN receptors does not seem to have occurred in the present experiment.

Outside of prenatal stress, there are a number of other factors that could contribute to the negative finding reported here. First, the period during which the antagonist was administered is a stage when BN development is characterized by increasing numbers of CNS receptors but no quantifiable BN-immunoreactivity (Gillati and Moody, 1984). Although BN immunoreactive neurons have been

described in the developing rat CNS (Panula et al., 1988) these appear rather limited in number. The relevance of stimulation of BN receptors by endogenous BN-like peptides at this stage of ontogeny appears minimal. Thus blockade of BN receptors may have had little or no functional consequences.

Second, the BN i.c.v. dose-response curve covers a broad range, with large gaps between doses. Possible changes in behavioural sensitivity at intermediate doses (e.g. 0.3 or 0.5 μg) were not assessed and thus the sensitivity of this curve for phenomena occurring over intermediate doses was lacking. Similarly, if alterations in behavioural responsiveness had occurred at lower doses (0.05 or 0.075 μg) they too would have been missed. Thus it can only be concluded that under the conditions employed in this study, no changes in central BN functioning were apparent.

Third, the lack of long-term effects by subchronic treatment of neonatal pups with neuropeptide receptor antagonists is not without precedence. Snijdewint and Boer (1986, 1988) reported that a number of vasopressin receptor antagonists had no effect on physiological or behavioural parameters on body and brain development measures. They hypothesized that the doses of antagonist used may have been too small or that the receptor blockade effects were compensated by increased rates of vasopressin synthesis.

The antagonist doses used in this experiment did not, in pilot studies, reverse entirely the behavioural effects of exogenous BN and so may not have been fully efficacious. The second suggestion could be a factor although BN-like immunoreactivity was immeasurable in the developing rat brain during the period in which the antagonist was administered. It also remains possible that functional recovery due to endemic plasticity may have been complete enough to mask underlying alterations. The development of more potent antagonists as well as research on possible neuropeptide synthesis effects of BN-receptor blockade could address these issues.

Fourth, data concerning the in vivo kinetics of the binding of [D-Phe^{6},ΨLeu^{13}-Cpa^{14}]BN(6-14) to the receptor are lacking and thus the length of time that the antagonist remains active is unknown. Zagon and McLaughlin (1987) have reported that long-term effects on endogenous opioids only occurs if a prenatally administered opioid receptor antagonist occupies the receptor site for upwards of 12 hrs. [D-Phe^{6},ΨLeu^{13}-Cpa^{14}]BN(6-14) may be metabolized too quickly to have lasting effects.

The results of this study indicate that under the dose regimen and testing conditions employed, subchronic treatment of infant rats with [D-Phe^{6},ΨLeu^{13}-Cpa^{14}]BN(6-14) does not appear to have long-term consequences in terms of adult sensitivity to BN i.c.v or i.p. . However, the 10

mg/kg s.c. treatment did appear to alter anxiety on the elevated plus maze through as yet unknown mechanism(s).

V. GENERAL DISCUSSION

The aim of the experiments reported here were multifold: (1) to characterize the acute behavioural effects of subcutaneously administered BN to developing rat pups; (2) to examine the effects of BN administered i.c.v. to the developing rat and elucidate the sequential nature of the behavioural responses over ontogeny; (3) to examine how subchronic neonatal treatment with BN and a BN-antagonist would affect physical and behavioural development and later sensitivity to BN. Experiments 1a and 1b were designed to determine whether maturation of endogenous BN system(s) played a role in the development and/or expression of spontaneous grooming. Experiments 2a and 2b attempted to elucidate a physiological role for endogenous BN-like peptides in the development of grooming and ingestive behaviours and behavioural adaptation to stress. The data from these experiments when considered individually and collectively reveal a number of interesting trends.

A. Effects on Grooming

Subcutaneous administration of BN to rat pups 1 to 10 days of age elicits grooming with no apparent sex differences. This corroborates earlier work with BN i.p. (Kitchen and Jackson, 1989a) and other neuropeptides known to induce grooming in the neonatal rat (Kirstein et al., 1990; Pedersen et al., 1988). The form of grooming activity elicited by s.c. administration of BN to rat pups 1 to 10 days of age does not resemble that induced by i.c.v. administration of BN to adult rats. BN s.c. induced grooming contains the same elements as observed under saline s.c. conditions but with the accentuation of the expression of these elements. Three principle elements characterized grooming in the 1-10 day old rat pup: head washing, head scratching and body scratching. These data contrast to a degree with the findings of Richmond and Sachs (1980) but differences could be attributable to different testing situations. Other studies, using similar procedures to the present experiment, reported scratching to be present from days 4-5 onwards (Jackson and Kitchen, 1989a,b; Kirstein et al., 1990). No body washing was observed and this agreed with previous reports suggesting that body washing is not seen until the beginning of the 3rd postnatal week (Bolles and Woods, 1964: Richmond and Sachs, 1980). In contrast, adult grooming in response to BN i.c.v. is characterized by

scratching although the expression of washing activities is also elevated. This same pattern was noted in experiments 2a and 2b (under water deprivation conditions) when the animal was motivated to eat and drink and points to the robustness of the response.

The behavioural response to BN s.c. in the developing rats declined as a function of age. This could be attributable to the decreasing permeability of the blood-brain barrier to BN rather than a decrease in sensitivity to the behavioural effects of BN in the older pups. Central administration of BN to pups of this age invoked a vigorous grooming response. Indeed, 10 and 20 day old pups groomed more in response to central BN than younger pups, indicating an increased sensitivity to the neuropeptide. Such increased sensitivity to some neuroactive substances in ontogeny had been previously demonstrated (e.g. Campbell, Baldessarini and Teicher, 1988) and could reflect the state of neurochemical flux within the developing rat CNS .

A developmental gradient of change in the sequential organization of the behavioural response to BN i.c.v. was also apparent. The behavioural response to BN at 1 and 5 days of age indicated that some of the motor acts were approximations of the adult form. In particular, scratching motions failed to contact the body at this age. This substantiates previous reports indicating non-contact grooming pathways in the ontogeny of grooming in the mouse

(Golani and Fentress, 1986) and the rat (Bolles and Woods, 1964). Further, in contrast to earlier reports (Richmond and Sachs, 1980), these data indicated that infant rats as young as 1 day could make scratching movements under both saline and BN (i.c.v. or s.c.) conditions. A subsystem of scratching activity was apparent within BN induced grooming activity at these ages. At 10 and 20 days of age, these non-contact scratching activities were replaced by the mature contact form and the scratching subsystem was clearly established within the grooming activity elicited by BN i.c.v. .

The above data provide evidence for the suggestion that scratching is part of a different grooming system from washing (Sachs, 1988). Likewise, the behaviour elicited by BN i.c.v. in adult rats illustrates that in comparison to baseline levels, changes in scratching (body and head) frequencies were considerably greater than the changes in washing activities. Further, the HD animals of experiment 2a which were borderline hypersensitive to BN showed larger increases in scratching activities than washing activities relative to the other pretreatment groups. Conversely, the LD group data indicate that this group's subsensitivity to BN i.c.v. was primarily due to a lower degree of scratching.

The results of the novelty-induced behavioural tests in both chronic studies substantiate this suggestion. Grooming elicited by placement of rats in a novel environment was

characterized by washing behaviours. In the context of the neonatal subchronic agonist (BN s.c.; 2x daily) treatments, evidence was presented indicating long-term alterations in sensitivity to BN i.c.v. as shown by changes in scratching activities. Grooming under novel conditions was unaffected by these pretreatments. These data indicate that although the activity elicited by BN s.c. early in ontogeny contains the elements present in spontaneous grooming, pretreatment with BN does not affect the development and/or expression of washing dominated grooming. Thus, BN would not appear to play a major physiological role in the ontogeny of this form of grooming.

This result was not entirely unexpected. Oxytocin injected i.c.v. to adult rats induces a form of grooming characterized by washing activities. Neonatal i.c.v. treatment with oxytocin increases the expression in adulthood of novelty-induced grooming. Neonatal oxytocin then influences the expression of behaviour in which it is involved. This principle was established for the long-term effects of other neuropeptides such as substance P (Handelmann, 1988) and could well be the case here. As BN elicits a form of grooming qualitatively different from that elicited by novelty, it would not be expected to enhance its expression or ontogeny.

One way to test this would be to repeat the pretreatments of experiment 2a and then test with i.c.v.

substance P or beta-endorphin, two neuropeptides known to elicit scratching activity when injected centrally to adult rats. It is of interest that a substance P receptor antagonist has been shown to inhibit the expression of BN-induced scratching although it in itself has neurotoxic effects (Cowan et al., 1985). Both substance P (Qurion and Dam, 1986) and opiate receptors (Coyle and Pert, 1976) are present within the CNS of the neonatal rat and if subchronic BN alters the ontogeny of scratching behaviour, it may also have some effects on the development of these neuropeptide systems. Alternatively, the experiment could be reversed whereby neonates would be exposed to substance P or beta-endorphin and the behavioural response to i.c.v. BN investigated in adulthood.

In this context, it is of interest to note that O'Donohue and colleagues (1984) suggested that BN may play a role in sensory input modulation. If this were the case, then such a role appears functional early in ontogeny. Much of the scratching activity elicited by BN s.c. or i.c.v. in the infant rat was non-contact, suggesting central mediation. In the adult mouse, application of lidocaine to body flanks and neck does not diminish BN-induced scratching, implying that sensory feedback appears negligible for the continued expression of BN-induced behaviour (Wheeler et al., 1988). The present study fails to shed further light on the role of BN in sensory

processing but illustrates that altered BN sensitivity could be manifested by changes in scratching.

The mechanism by which the long-term effects of neonatal treatment with BN are manifested are unknown. Down-regulation of peripheral BN receptors by BN has been reported in vitro (Swope and Schonbrunn, 1990) and apparent behavioural sensitization occurred when BN was administered centrally twice within 12 hours (Gmerek and Cowan, 1983). In terms of scratching, if the receptor changes discussed earlier occurred, then increased numbers would be expected in the NTS or other nuclei within contact of the 4th ventricle. At these sites, the greatest sensitivity to the scratching and feeding effects of BN has been demonstrated (Flynn, 1989; Johnston and Merali, 1988b). In addition, increased sensitivity to some secretagog functions would also be expected (Gunion et al., 1989). One way to investigate this would be to repeat the pretreatments of experiment 2a and implant cannulae aimed at these particular nuclei. Feeding and grooming responses could then be examined in response to BN intraNTS as could blood levels of fatty acids, epinephrine, corticosterone and glucose.

An examination of BN receptors was not performed in the present experiment for a number of reasons. First, an appropriate assay was not readily available at the conclusion of the chronic investigations. The most appropriate procedure would have been in vitro

autoradiography. This would quantify receptor changes in specific nuclei. A whole brain homogenate binding assay could have been attempted with "grind and bind" techniques but the results may not have been readily interpretable. Receptor numbers could be differentially affected in various regions of the brain such that an overall assay would not have detected such changes and may have failed to indicate any change at all. The second reason why an assay was not attempted concerned the number of treatments to which the animals were exposed. At the conclusion of experiments 2a and 2b, the animals had received 4 i.c.v. procedures and 4 i.p. injections. Further, they had been implanted with indwelling cannulae and maintained under an intake schedule for 42 days, procedures which in and of themselves could have affected central neurochemical systems and hence interpretation of receptor assays would have been difficult. In order to characterize possible changes in central BN receptors, the experiment would have to be repeated twice; once to repeat the above findings and second to sacrifice the animals at 65 days of age to measure central BN receptor changes via in vitro autoradiography.

Subchronic neonatal BN exposure could have altered the development of another endogenous neurochemical system(s). Previous discussions indicated that central serotonin could play an important role in the modulation of the behavioural effects of neonatal ACTH i.c.v. (Kirstein et al., 1990) and

the ontogeny of GC receptors (Mitchell et al., 1990). In this context, it is of particular interest that the 5-HT_{1B} agonist, 1-(3-trifluoromethylphenyl)piperazine (TFMPP), elicited grooming and scratching in the 4 day old but not 20 day old pup (Kirstein et al., 1990). Neonatal administration of the 5-$HT_{1B/1C}$ agonist, 1-(3-chlorophenyl)piperazine (mCPP), also induces grooming in the 5 day but not the 10 day old pup (Jackson and Kitchen, 1989b). In the adult mouse, the 5-HT_2 agonist, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), induces ear scratching and this is inhibited by the 5-HT_{1B} agonists TFMPP and 5-Methoxy-3-(1,2,3,5-tetrahydropyridyl)indole succinate (RU 24969) as well as the selective 5-HT_2 antagonist, ketanserin (Darmani et al., 1990; Deegan and Cook, 1958). It would not be inconceivable that long-term changes in endogenous serotonergic system(s) would affect the expression of grooming/scratching elicited by i.c.v. BN.

The role of serotonin in the mediation/modulation of BN-induced activity has not, to date, been investigated in depth. The dopamine D_1 receptor antagonist (R)(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol HCl (SCH 23390) inhibits the expression of BN-induced scratching (Piggins and Merali, 1989; Merali and Piggins, 1990). This agent also binds to serotonergic receptors (McQuade et al., 1988) but the importance of this action is unknown. Central serotonergic lesions facilitate the

expression of grooming elicited by the dopamine D_1 receptor agonist 2,3,4,5-tetrahydro-1,8-dihydroxy-1-phenyl-1H-3-benzazepine-7,8-diol HCl (SKF 38393) (Lucki and Kucharik, 1988) although grooming induced by SKF 38393 is characterized by washing elements (Merali and Piggins, 1990). Given the reported alterations in serotonergic functioning in ontogeny (Mabry and Campbell, 1974; Jackson and Kitchen, 1989b) it would be of great interest to investigate the possible role of this neurotransmitter in the mediation/modulation of the behavioural effects of BN throughout development.

The above studies indicate that central BN receptors are pharmacologically functional from an early stage in ontogeny despite the absence of measurable amounts of the peptide. Furthermore, the behavioural response to BN i.c.v. shows measurable changes, both quantitatively and qualitatively, over development.

B. Effects on Thermoregulation

BN s.c. elicited hypothermia in the 1 day old rat pup in a manner unrelated to dose. These animals appeared very slightly hypothermic as their body temperature under control conditions was in the lower thermoneutral range for animals of this age (Taylor, 1960). Animals of this age are more susceptible to drops in ambient temperature than adults

owing to a lack of heat conservation capability. Thermoregulation at this age is achieved through filial and maternal contact (Leon, 1986). However, 1 day old pups are capable of increasing metabolic rate (as measured by O_2 consumption- Taylor, 1960) and the beta-adrenergic:brown adipose tissue complex is functional at this stage in development (Svoboda et al., 1984). In cold-exposed adult rats, i.c.v. BN elicits hypothermia which is caused by inhibition of regulatory heat production through reduced sympathetic outflow to brown adipose tissue (Brown et al., 1987; Shido et al., 1987). This could be the same mechanism through which BN elicits body temperature changes in the infant rat.

In order to verify, the experiment could be repeated using modern equivalents of the equipment (metabolic chambers) described by Taylor (1960). The ambient temperature could be raised to keep the animals closer to the thermoneutral norm and this could prevent BN's effect on body temperature. A higher ambient temperature was not employed here as BN has been reported to raise body temperature in heat exposed adult animals. Such an action in the present experiment could have lead to overheating and consequently seizures as well as dehydration of the pups. Body core temperature was not measured following BN i.c.v. as the equipment was unavailable at the beginning of these experiments. However, such a measure would be confounded by

the fact that the anaesthetic employed, methoxy flurane, also decreases metabolism (Animal Care Services information sheet) and thus the animals would have been hypothermic under control conditions. Hypothermia in the neonate can lead to behavioural depression and this may explain why 1 and 5 day old pups showed little grooming following saline i.c.v.. If BN i.c.v. also lowered body temperature under these conditions, then this could also contribute to the lower behavioural levels observed following BN i.c.v. in the younger pups as compared to 20 day olds. The older rats, being capable of greater heat conservation would be largely unaffected by BN as indeed the BN s.c. data indicate (outwith the declining permeability of the blood-brain barrier).

In terms of the subchronic studies, core body temperature was not taken as this procedure can damage internal tissue in the developing rat, particularly when taken daily. The injection procedure of removing the mother from the home cage, weighing the pups, injecting the pups and then marking them resulted in pups that felt cold to the touch at the conclusion of these twice daily protocols. The long-term importance seems implicit. A single exposure in infancy to cold can alter the development of endogenous serotonergic system(s) (Giulian et al., 1974). Subchronic exposure to low or high Ta during rearing can have permanent effects on adaptation to alterations in temperature

(Ferguson et al., 1981; Hahn, 1956; Young and Dawson, 1988). The effects of handling have, to some degree, been attributable to early alterations in body temperature (Schaeffer, 1968).

Meaney and colleagues (1987) indicated that endogenous thyroid hormone could modulate the long-term GC receptor changes associated with the handling phenomenon. In short, the increase in hippocampal GC binding was reversed by neonatal pretreatment with thyroid hormone synthesis inhibitor. This is of particular interest in the present context as thyrotrophin-releasing factor reverses and blocks i.c.v. BN induced hypothermia in cold-exposed adult rats (Brown, Rivier and Vale, 1977b). And further, thyrotrophin release is suppressed by i.c.v. BN in cold-exposed rats (Brown and Vale, 1980 as quoted by Brown et al. ,1988). It has been hypothesized that increased circulating levels of thyroid hormone may mediate the effects of handling on adrenocortical response to stress (Meaney et al., 1987). If BN reduced or suppressed thyrotrophin-releasing factor in cold exposed rat pups for the first week on life, then it could be that the handling effects on later stress responses were diminished.

On the other hand, if the BN treated rat pups were detectably hypothermic or more hypothermic than other pups in the litter, then these pups may be treated differently by the dam (Leon, 1986). A number of studies have indicated

that differential treatment of pups by the mother can have important long-term changes in the behaviour and physiology of the offspring (Ottinger et al., 1962; Levine, 1967). Such pups could be better fed by the mother and nutritional status during early ontogeny has marked effects on later adult behaviour (Walker and Aubert, 1988; Rudy and Castro, 1990). The possible relations between early ambient temperature, maternal behaviour and nutritional status, and the long-term significance remain to be fully investigated.

C. Effects on Anxiety

In adult rats, central injection of BN elicits behaviours observed under stressful conditions as well as influencing a number of physiological indices of stress. However, the behaviour, a scratching form of grooming, as discussed earlier is qualitatively different from grooming observed when the animal is placed in a novel (and hence stressful) environment. Behaviourally then, BN may not have a role in the development of behaviours evoked by stress. Indeed, manipulation of endogenous BN in ontogeny did not alter the animals behaviour under isolation or novel environment conditions. The isolation test did not evoke much grooming (as compared to exploratory behaviour) and this may indicate that the test period was too short. It has been suggested that grooming may occur at the end of a

stressful event and could serve as a form of behavioural habituation (Jolles et al., 1979). Grooming was not frequently observed under the conditions of the present test and in future tests it would probably be more accurate to assess behaviour over 60 min. A similar recommendation could be applied to the test of open field activity.

Neonatal treatment with BN had no anxiolytic or anxiogenic effect as measured on the elevated plus maze (as compared to appropriate controls). However, the animals neonatally pretreated with 10 mg/kg dose of [D-Phe^{6},ΨLeu^{13}-Cpa^{14}]BN(6-14) showed an apparent anxiolytic effect (as shown by the number of entries and time spent in the open arms of the maze). This result indicates that the antagonist may have an as yet unknown action on other endogenous anxiety-related neurochemical systems. Subchronic neonatal treatment with the benzodiazepines, diazepam or lorazepam, increased social interaction in adolescence, reflecting an anxiolytic action (File, 1986) and benzodiazepines generally have anxiolytic action on the elevated plus maze (Pellow et al., 1986). Similarly, drugs which decrease central serotonergic activity generally have anxiolytic effect on the elevated plus maze whilst drugs that increase central serotonergic activity have largely anxiogenic or no action at all (Chopin and Briley, 1987). Neonatal depletion of central serotonin with the neurotoxin 5,7-dihydroxytryptamine has an anxiolytic action in adult

animals on this maze (Briley, Chopin and Moret, 1990). Hence, if [D-Phe^{6},ΨLeu^{13}-Cpa^{14}]BN(6-14) had an effect on the development of other endogenous neurochemical systems, then it could well have been on GABAergic or serotonergic mechanisms.

Other tests of anxiolytic or anxiogenic activities could have been used and would have been useful to ascertain the consistency of the above effect. However, the number of tests employed was deliberately limited as only a relatively short period of time was available before the invasive procedures commenced. Most of the data from pilot studies and from the literature deal with animals at the 300 to 400 g weight range, leaving little time post weaning for tests. Also, the added variable of the effects of repeatedly handling in adolescence was kept to a minimum by the non-invasive procedures.

D. Effects on Food and Water Intake

The ingestive measures of the chronic studies used wet mash and water intake following a water deprivation schedule for a number of reasons. Early pilot work indicated that under such a schedule, rats appeared to regulate food (dry standard Purina rat chow pellets) intake to coincide with the presentation of the test water bottle. Attempts to quantify the amount consumed, either in pellet or milled

form, were unsuccessful. It was found that presenting a mixture of milled Purina rat chow with water (40% by weight), allowed for easier and more accurate measurement of food intake. Further, the animals stabilized on this regime and consumed most of their daily water (from the test bottle) within the initial 30 min.

It was found on subsequent trials that the behavioural responses to BN i.c.v. could be assessed as could effects on water and food consumption. Preliminary data indicated that even at moderately high doses of BN, the animals would initially orient to and eat the wet mash. Thus, scratching elicited by BN did not initially interfere with the ingestive behaviours. Similarly, the effects of BN i.p. on food intake were reproduced. The effects of centrally administered BN-like peptides were assessed under the above conditions and the schedule differentiated the peptides anorectic and scratch-inducing potencies in manner similar to their known activities on pharmacological preparations. Thus this water deprivation schedule formed an important endpoint for the chronic studies.

In both studies, BN at 0.1 μg elicited scratching behaviour in most animals (save the UNT group of experiment 2a). This was also the dose at which most animals reduced wet mash consumption. Examination of the 5 min bin data of experiment 2a indicated that the animals were capable of orienting to and consuming the wet mash even when induced to

head scratch by BN (0.1 μg). Eating frequency (an accurate measure of wet mash consumption) failed to increase steeply after 5 min (as it had under control conditions) and this would account for the reduced level of food intake. However, as indicated by figure 10, the frequency of head scratching, a robust behavioural indicator of central BN receptor stimulation, did not increase at a rate sharp enough to account for the dimunition of feeding behaviour. This could indicate that BN, acting within the CNS, reduces food intake independently of its effects on head scratching.

Water intake tended to be inhibited only at the higher doses employed. As shown in figure 10, i.c.v. BN (0.1 μg) had no overt effect on the pattern of drinking over the 30 min test period (although the volume of water consumed over this period was significantly reduced in the HD and SAL animals). Flynn (1989) and others (Calisher and Avery, 1982) reported that water intake was only affected by i.c.v. BN in the presence of food. Their data imply that effects on water intake could be secondary to effects on food intake. However, water intake was not significantly affected by peripherally administered BN, even when wet mash consumption was reduced considerably. These data indicate that it remains a possibility that i.c.v. BN, acting through as yet unknown CNS mechanism(s), could mediate/modulate water intake.

Under the peripheral BN dose-response curve, wet mash intake was inhibited at the high dose of BN (8 μg/kg; i.p.). These decreases were largely matched by similar reductions in the eating frequency. Manipulation of BN-related systems neonatally had no overt effects on BN response under these conditions, indicating that any changes noted above were probably centrally rather than peripherally mediated.

The mechanisms of these effects are unknown and without the assessment of BN receptors both peripheral and central, the attribution of the above to changes in the numbers of BN receptors remains speculative. Receptor changes in the NTS as well as the PVN would be expected given the sensitivity of these regions to BN's effects on feeding. Changes peripherally, if any, appear to have been too small for detection with the present method. BN has been shown to down-regulate its own peripheral receptor (Swope and Schonbrunn, 1990) but no changes in functional sensitivity could be detected in the present experimental protocol.

Alternatively, BN could have altered the development of another neurotransmitter system(s) as discussed in previous sections. Serotonergic mechanisms have been implicated in the control of food intake (Blundell, 1977) and large numbers of 5-HT_3 receptors have been found in the NTS and limbic system of the rat (see Pratt et al., 1990 for review). The serotonergic agonist d-fenfluramine has recently been demonstrated to reduce feeding via a CCK-

dependent mechanism (Cooper et al., 1990). Thus long-term changes in central serotonergic functioning could have a role in peptidergic regulation of food intake.

VI. CONCLUSIONS

The data from the above experiments when considered as a whole yield the following:

1) Peripheral injection of BN to developing rats elicits grooming activity. The form of grooming was qualitatively different from that seen following central administration of the peptide to adult rats. These differences appear to reflect the development of motor capabilities of the rat and therefore that of spontaneous grooming.

2) Peripheral injection of BN to developing rats induces a further drop in core body temperature of mildly hypothermic neonates.

3) The behavioural response to peripherally administered BN declines as a function of age and this probably reflects the maturation of the blood-brain barrier.

4) Central injection of BN to rats of 1 to 20 days of age elicits grooming behaviour which is characterized by immature (non-contact) behavioural elements. An increase in sensitivity to BN through development was apparent and at 20 days of age, the grooming response to BN was characterized largely by mature (contact) scratching behaviours. Sequentially, there was an elimination of other behaviours elicited by BN through ontogeny such that at 20 days, a scratching subsystem of grooming was distinguishable.

5) Findings 1 to 4 indicate that BN receptors in the developing rat CNS and the neural mechanisms underlying BN-induced behaviour are functional from day 1 onwards in ontogeny. Thus BN, when administered centrally, may serve as a useful pharmacological tool in studying the ontogeny of grooming/scratching behaviour(s).

6) Neonatal injections with BN or saline did not alter behavioural responses to a novel environment, isolation or anxiety eliciting situations. These data indicate that BN probably does not play a significant role in the development and/or expression of behaviour under the above circumstances.

7) Neonatal injections with BN or saline alters later adult sensitivity to BN i.c.v. as compared to non-injected

but handled controls. No change in sensitivity to peripherally injected BN was apparent.

8) Neonatal injections with [D-Phe^{6},ψLeu^{13}-Cpa^{14}]BN(6,14) had no effect on late tests of stress or novelty-induced activity. However, neonatal pretreatment with the high dose of the BN receptor antagonist had an apparent anxiolytic effect (as shown by the number of entries and time spent in the open arms of the elevated plus maze). Adult sensitivity to i.c.v. administered BN was unaffected by these neonatal pretreatments with the BN-receptor antagonist. The mechanism(s) by which the BN receptor antagonist elicited the apparent anxiolytic effect is unknown.

9) These effects (6-8), could be due to altered development of systems utilizing BN-like peptides as neuromodulators and/or neurotransmitters. These effects could include changes in receptor numbers as well as changes in synaptic contacts with other neuronal systems.

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Table A-1. --Summary Statistics for Experiment 1a

Behavioural Element	F-Ratio		
	Age Effect	Dose Effect	Interaction
Head Washing	F(2,84)=143.31 p<.000001	F(3,84)=73.73 p<.000001	F(6,84)=124.98 p<.000001
Head Scratching	F(2,84)=18.30 p<.000001	F(3,84)=13.40 p<.000001	F(6,84)=1.23 not sig.
Body Scratching	F(2,84)=49.56 p<.000002	F(3,84)=27.21 p<.000001	F(6,84)=4.08 p<.0012
Total Scratching	F(2,84)=38.1 p<.000008	F(3,84)=22.63 p<.000001	F(6,84)=2.87 p<.013
Resting	F(2,84)=77.35 p<.000001	F(3,84)=58.41 p<.000001	F(5,84)=8.97 p<.000001
Exploratory Activity	F(2,84)=16.05 p<.000001	F(3,84)=3.21 p<.027	F(6,84)=1.80 not sig.
Temperature	F(2,84)=102.11 p<.000003	F(3,84)=5.07 p<.003	F(6,84)=4.49 p<.00054

Table A-2. --Summary Statistics for Experiment 1b (first part). 181

Behavioural Element	F-Ratio		
	Age Effect	Dose Effect	Interaction
Forelimb Vibration	F(3,96)=0.253 not sig.	F(3,96)=10.374 p<.000006	F(9,96)=2.23 p<.026
Face Wash	F(3,96)=11.67 p<.000001	F(3,96)=66.92 p<.000001	F(9,96)=3.48 p<.00092
Body Wash	F(3,96)=11.89 p<.000001	F(3,96)=1.25 not sig.	F(9,96)=1.29 not sig.
Contact Head Scratch	F(3,96)=281.40 p<.000001	F(3,96)=34.97 p<.000001	F(9,96)=33.69 p<.000001
Contact Body Scratch	F(3,96)=177.18 p<.000001	F(3,96)=30.73 p<.000001	F(9,96)=19.92 p<.000001
Interbout Pause	F(3,96)=21.37 p<.000001	F(3,96)=24.83 p<.000001	F(9,96)=10.27 p<.000001
Non-contact Head Scratch	F(3,96)=9.92 p<.000001	F(3,96)=5.77 p<.0011	F(9,96)=4.22 p<.00013
Non-contact Body Scratch	F(3,96)=23.37 p<.000001	F(3,96)=17.29 p<.000001	F(9,96)=6.52 p<.000001

Table A-3. -- Summary Statistics of Experiment 1b (second part).

Behavioural Element	F-Ratio		
	Age Effect	Dose Effect	Interaction
Rest	F(3,96)=21.78 p<.000001	F(3,96)=116.57 p<.000001	F(9,96)=9.01 p<.000001
Crawl	no stats performed	no stats performed	no stats performed
Back Paddle	no stats performed	no stats performed	no stats performed
Locomotory Activity	F(3,96)=6.92 p<.00028	F(3,96)=4.72 p<.0041	F(9,96)=6.40 p<.000001
Sniff	F(3,96)=9.83 p<.00001	F(3,96)=5.68 p<.00127	F(9,96)=5.00 p<.000016
Exploratory Activity	F(3,96)=9.59 p<.000013	F(3,96)=1.97 not sig.	F(9,96)=6.27 p<.000001
Climb	no stats performed	no stats performed	no stats performed

Table A-4. --Summary Statistics for Experiment 2a (i.c.v. BN data).

Behavioural Element	F-ratio		
	Pretreatment Effect	Dose Effect	Interaction Effect
Head Washing	F(3,24)=2.75 not sig.	F(3,72)=62.75 p<.0001	F(9,72)=1.85 not sig.
Head Scratching	F(3,24)=6.80 p<.0018	F(3,72)=93.20 p<.0001	F(9,72)=4.25 p<.0002
Body Washing	F(3,24)=2.03 not sig.	F(3,72)=41.27 p<.0001	F(9,72)=3.79 p<.0006
Body Scratching	F(3,24)=8.19 p<.0006	F(3,72)=142.71 p<.0001	F(9,72)=6.44 p<.0001
Total Scratching	F(3,24)=7.25 p<.0013	F(3,72)=118.18 p<.0001	F(9,72)=5.08 p<.0001
Eating Frequency	F(3,24)=8.78 p<.0004	F(3,72)=138.93 p<.0001	F(9,72)=5.05 p<.0001
Drinking Frequency	F(3,24)=0.82 not sig.	F(3,72)=12.07 p<.0001	F(9,72)=0.97 not sig.
Resting	F(3,24)=0.41 not sig.	F(3,72)=8.48 p<.0001	F(9,72)=1.29 not sig.
Exploring	no stats performed	no stats performed	no stats performed
Food Intake (g)	F(3,24)=2.45 not sig.	F(3,72)=75.26 p<.0001	F(9,72)=2.244 p<.0175
Water Intake (ml)	F(3,24)=0.35 not sig.	F(3,72)=27.06 p<.0001	F(9,72)=1.31 not sig.

Table A-5. --Summary Statistics for Experiment 2a (i.p. BN data).

Behavioural Element	F-Ratio		
	Pretreatment Effect	Dose Effect	Interaction
Food Intake (g)	F(3,24)=0.71 not sig.	F(3,72)=23.22 p<.0001	F(9,72)=0.55 not sig.
Eating Frequency	F(3,24)=1.09 not sig.	F(3,72)=29.17 p<.0001	F(9,72)=0.56 not sig.
Rest	F(3,24)=1.33 not sig.	F(3,72)=3.82 p<.0134	F(9,72)=1.86 not sig.
Explore	F(3,24)=1.99 not sig.	F(3,72)=17.05 p<.0001	F(9,72)=1.00 not sig.

Table A-6. --Summary Statistics for Experiment 2b (i.c.v. BN data).

Behavioural Element	F-ratio		
	Pretreatment Effect	Dose Effect	Interaction Effect
Head Washing	F(3,24)=0.28 not sig.	F(3,72)=82.39 p<.0001	F(9,72)=1.71 not sig.
Head Scratching	F(3,24)=0.60 not sig.	F(3,72)=119.28 p<.0001	F(9,72)=1.35 not sig.
Body Washing	F(3,24)=0.36 not sig.	F(3,72)=11.99 p<.0001	F(9,72)=0.23 not sig.
Body Scratching	F(3,24)=0.26 not sig.	F(3,72)=84.81 p<.0001	F(9,72)=0.87 not sig.
Total Scratching	F(3,24)=0.38 not sig.	F(3,72)=107.40 p<.0001	F(9,72)=1.08 not sig.
Eating Frequency	F(3,24)=0.59 not sig.	F(3,72)=74.13 p<.0001	F(9,72)=1.27 not sig.
Drinking Frequency	F(3,24)=1.62 not sig.	F(3,72)=10.43 p<.0001	F(9,72)=1.27 not sig.
Resting	F(3,24)=1.68 not sig.	F(3,72)=13.96 p<.001	F(9,72)=1.91 not sig.
Exploring	no stats performed	no stats performed	no stats performed
Food Intake (g)	F(3,24)=0.63 not sig.	F(3,72)=51.46 p<.0001	F(9,72)=0.63 not sig.
Water Intake (ml)	F(3,24)=4.64 p<.01	F(3,72)=51.62 p<.0001	F(9,72)=2.84 p<.0064

Table A-7. --Summary Statistics for Experiment 2b (i.p. BN data).

Behavioural Element	F-Ratio		
	Pretreatment Effect	Dose Effect	Interaction
Food Intake (g)	$F(3,24)=0.21$ not sig.	$F(3,72)=25.72$ $p<.0001$	$F(9,72)=0.51$ not sig.
Eating Frequency	$F(3,24)=1.08$ not sig.	$F(3,72)=20.30$ $p<.0001$	$F(9,72)=1.11$ not sig.
Rest	$F(3,24)=1.91$ not sig.	$F(3,72)=24.43$ $p<.0001$	$F(9,72)=2.15$ $p<.036$
Explore	$F(3,24)=1.27$ not sig.	$F(3,72)=1.57$ not sig.	$F(9,72)=1.97$ not sig.