OPIOID REGULATION OF INGESTIVE BEHAVIOR IN THE DOMESTIC FOWL

by

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(ABSTRACT)

Six studies were conducted to examine the role of endogenous opioid peptides in the regulation of ingestive behavior in the domestic fowl. In the first study, the dose-response relationships of two opioid antagonists, naloxone and naltrexone, were evaluated in Rock-Cornish (RC) and Single-Comb White Leghorn (SCWL) cockerels. Naloxone and naltrexone decreased food and water intake in both stocks. In a separate experiment, the effect of naloxone on water intake was evaluated independent of food intake. Naloxone depressed water intake in normally hydrated and saline-loaded chicks. These results indicate endogenous opioid peptides influence food and water consumption independently in the domestic fowl.

The sensitivities of RC and SCWL stocks to naloxone were compared in a second investigation. There was no difference in the efficacy of naloxone in attenuating ingestive behavior when the stocks were compared at either the same age or similar body weight. Therefore, genetic selection for meat or egg production has not significantly altered naloxone-sensitive opioid mechanisms regulating food and water intake in the domestic fowl.

A third study extended the investigation of opioid regulation of ingestive behavior to Japanese quail (*Coturnix coturnix japonica*). Administration of naloxone attenuated feeding, but not drinking, suggesting that water in Japanese quail is not influenced by endogenous opioids.

The fourth study was performed to determine whether opioid regulation of ingestive behavior in the domestic fowl is mediated at sites within the central nervous system or peripheral tissues. An initial experiment examined the effects of two opioid antagonists with differing ability to traverse the blood-brain barrier (bbb). Food and water intake were attenuated by both antagonists. However, at equally potent doses the antagonist which does not readily traverse the bbb (quaternary naloxone) was more effective than its congener which crosses the bbb (tertiary

naloxone). Central administration of tertiary naloxone attenuated water consumption, but not feeding. No alterations in ingestive behavior were observed when these levels of tertiary naloxone were injected im. Therefore, these results suggest that opioid regulation of food intake occurs at sites outside the bbb, whereas water intake is at least, in part, centrally mediated.

The remaining studies were conducted to identify the specific opioid receptor subtypes which mediate ingestive behavior in the domestic fowl. In the fifth study, ingestive responses to central (intracerebroventricular; ICV) and peripheral (im) administration of the mu agonist, morphiceptin, and the delta agonist, [Met^5]-enkephalin, were studied. The mu and delta opioid receptors are the receptors for which naloxone has the highest and lowest affinity, respectively. ICV administration of morphiceptin stimulated drinking, whereas im administration stimulated feeding. [Met^5]-enkephalin stimulated food intake by both routes of administration. These results indicate that the mu opioid receptor mediates water intake in the central nervous system and food intake peripherally, while the delta opioid receptor mediates food intake centrally and peripherally. Failure to detect central opioid mediation of food intake in previous studies was likely due to the low affinity naloxone exhibits for the delta receptor.

The final study examined the feeding, drinking and temperature responses to ICV administration of β-endorphin. β-endorphin has equal affinity for the mu, delta and epsilon opioid receptors. ICV administration of β-endorphin induced increases in feeding, drinking and colonic temperature in RC and SCWL.

These studies indicate that endogenous opioid peptides influence food and water intake in the domestic fowl. Opioids appear to influence food and water consumption at sites within and outside the bbb, and via different receptor subtypes. Furthermore, there seems to be no difference in naloxone-sensitive opioid systems influencing ingestive behavior in meat and egg stocks of chickens.

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Introduction

The success of any species depends largely on an ability to achieve a balanced energy and nutrient exchange with its environment. Mild deviations from this equilibrium produce dramatic changes in the physiochemical makeup of the animal. When energy intake is not adequate to offset expenditure, the animal must either draw on body stores to compensate for the deficit or, if possible, reduce expenditures. When energy intake exceeds expenditure the excess dietary constituents are stored within the body, predominately as fat, producing an increase in body weight. Therefore, the regulation of food intake is one of the primary mechanisms used by animals to maintain energy homeostasis.

A number of theories have been developed to explain how organisms regulate food intake. The glucostatic theory (Mayer, 1953) is based on blood glucose levels being monitored by hypothalamic glucoreceptors which, in turn, control ingestive behavior. In general, when blood glucose levels are low, feeding is enhanced and when blood glucose levels increase, food intake is decreased. The lipostatic theory of food intake regulation, proposed by Kennedy (1953), is based on the brain being able to sense the state of fat depots and adjust food intake to maintain constant fat stores. Increases or decreases in fat depots result in compensatory changes in food intake. The aminostatic theory (Rogers and Leung, 1973) relates food intake to changes in the amino acid composition of the diet. The change in intake is dependent upon the nature of the dietary amino acid alteration, excess, deficiency or imbalance, and upon the particular amino acid(s) involved.

Although there is evidence to support each individual theory, it is probable that the systems act in concert, integrated by the central nervous system to regulate ingestive behavior and maintain energy homeostasis. Therefore, a great deal of investigation has been directed toward an understanding of the neuroanatomical and neurochemical relationships which control food intake. It has become increasingly apparent that neuropeptides play an integral role in the complex process of food and water intake regulation. Several gastrointestinal peptides have been shown to mediate satiety signals in both the periphery and central nervous system (CNS) (Hoebel, 1985). In addition,

monoamines and peptides found within the CNS have appetite suppressing effects (Hoebel, 1984). However, relatively few neuroactive substances have been shown to enhance appetite. For this reason attention has focused on the endogenous opioid peptides, which appear to be major stimulators of ingestive behavior (Morley and Levine, 1982).

The modern era of opiate research began with the discovery of stereospecific opiate receptors within the CNS of animals and humans. Van Pragg and Simon (1966) were among the first to report detection of opiate receptors in brain homogenates. It was relatively easy to demonstrate binding of opiate ligands, but more difficult to discern whether the binding was specific. In an effort to precisely determine the amount of specific receptor binding Goldstein *et al.* (1971) used stereospecificity as a criterion. Evidence for the existence of stereospecific opiate binding in the brain was published independently by Pert and Snyder (1973), Simon *et al.* (1973), and Terenius (1973). These investigators, utilizing similar modifications of the Goldstein *et al.* (1971) procedure, found that 50 to 90% of opiate binding was stereospecific, i.e., replaceable by unlabeled opiate but not by its inactive enantiomer.

Subsequent investigations were devoted to the characterization of the stereospecific binding sites and a compilation of evidence regarding their relationship with opiate receptors. The evidence gathered by a number of laboratories regarding the distribution of binding sites within the nervous system, their *in vivo* pharmacological potency, and their *in vitro* binding affinity gave credence to the theory that these sites were indeed pharmacological receptors (Simon, 1982). The discovery of stereospecific opiate receptors and the finding that they existed in a wide variety of vertebrates (Pert *et al.*, 1974), including humans (Kuhar *et al.*, 1973), stimulated investigation into the existence of endogenous opioid ligands.

In 1975, two independent laboratories were successful in isolating endogenous compounds from brain tissue which behaved like morphine in pharmacological assays (Hughes, 1975; Hughes *et al.*, 1975a) and in receptor binding assays (Pasternak *et al.*, 1975). Prior to determining the structure of the endogenous ligand, Hughes (1975) and Hughes *et al.* (1975a) proposed the name enkephalin for the active compound isolated by their laboratory. Pasternak *et al.* (1975) named their active substance morphine-like factor (MLF). At about the same time, Teschemacher *et al.*

(1975) isolated a larger and chemically different peptide from bovine pituitary gland which they named pituitary opioid peptide (POP).

By the end of 1975, Hughes *et al.* (1975b) had sequenced the active substance previously named enkephalin and determined that it was actually two different pentapeptides. The peptides had either a methionine or a leucine residue at the carboxy terminal end and were named [Met^5]-enkephalin and [Leu^5]-enkephalin, respectively. The authors also suggested that the amino acid sequence of [Met^5]-enkephalin was contained within the structure β-lipotropin (β-LPH), a peptide of unknown function first isolated from pituitary glands more than a decade earlier (Li *et al.*, 1965).

Bradbury *et al.* (1976) discovered that the C-terminal fragment (β-LPH 61-91) of β-LPH, found in the pituitary, had potent opioid activity. The peptide was named β-endorphin, β derived from β-LPH. Ling *et al.* (1976) determined that two other fragments of β-LPH, β-LPH 61-76 and β-LPH 61-77, also had opioid activity and were, therefore, named α- and γ-endorphin, respectively.

The original terminology for describing opioid peptides was agreed upon at the International Narcotics Research Conference (INRC) in 1975. Dr. Eric Simon suggested the term endorphine, endo from endogenous and orphine from the suffix of morphine, to describe any endogenous opioid peptide. The "e" was later dropped to conform with the conventional nomenclature for peptides and biogenic amines. As a result, the term enkephalin was used to specifically refer to the two pentapeptides discovered by Hughes *et al.* (1975b) and the term endorphin was used as a generic term for all endogenous opioid peptides.

During the late 1970's and early 1980's research on endogenous opioid peptides rapidly expanded into four principle areas including: (1) the discovery of new peptides, (2) the discovery of precursor molecules, (3) the classification of receptors, and (4) the characterization of the physiological effects of opioid peptides. Martin *et al.* (1976), investigated the concept of heterogeneous opioid receptors by contrasting the neurophysiological and behavioral effects of opiate drugs, reported three separate classes of receptors. Those which were acted upon by morphine were designated μ (mu), those acted upon by ketocyclazocine were designated κ (kappa), and those acted upon by the compound SKF 10,047 (N-allynormetazocine) were designated σ (sigma) receptors. A fourth receptor class, delta (δ), for which enkephalins have particularly high affinity, was char-

acterized by Lord *et al.* (1976). More recently, 2 additional classes of opioid receptors have been characterized including epsilon (ε, Wuster *et al.*, 1979) and lambda (λ, Grevel and Sadee, 1983) which display high affinity for β-endorphin and morphine, respectively.

The search for precursor molecules continued in the late 1970's and early 1980's with the discovery of a precursor molecule isolated from the pituitary of mice by Mains *et al.* (1977). The molecule was the precursor of both adrenocorticotrophic hormone (ACTH) and β-endorphin and was accordingly named pro-opiocortin. The sequence of pro-opiocortin was elucidated by Nakanishi *et al.* (1979) using DNA cloning techniques. Following sequence determination, it was apparent that the molecule also coded for the melanocyte stimulating hormones, α- and β-melanocyte stimulating hormone (MSH) (Chretien *et al.*, 1979). The name of the molecule was, therefore, expanded to pro-opiomelanocortin. There has also been speculation as to the existence of a pre-proopiomelanocortin. The m-RNA nucleotide sequence for pro-opiomelanocortin indicates the presence of a signal peptide at the beginning of the pro-opiomelanocortin molecule which may be cleaved as the molecule crosses the membrane of the endoplasmic reticulum (Kitchen, 1984).

The original theory that enkephalins were synthesized as part of the pro-opiomelanocortin molecule (Hughes *et al.*, 1975b) was later shown to be false (Hughes, 1979). Therefore, the nature of the enkephalin precursor(s) was unknown through the late 1970's. A 13 amino acid extended [Leu^5]-enkephalin peptide with potent opioid activity was isolated and sequenced from the pituitary of pigs by Goldstein *et al.* (1979). The peptide was designated dynorphin (1-13), (dyn from the Greek dynamis) because of its extraordinary opioid activity. Further classification of the dynorphin structure established that the opioid was actually a heptadecapeptide (dynorphin 1-17) with its biological activity accounted for by the first 13 residues (Goldstein *et al.*, 1981). An octapeptide, dynorphin (1-8), structurally identical to the N-terminal sequence of dynorphin (1-13), was extracted from porcine hypothalamus by Minamino *et al.* (1980). A second extended [Leu^5]-enkephalin, was also isolated from porcine hypothalamus by Kangawa and Matsuo (1979) and designated α-neo-endorphin. Subsequent investigation revealed yet another extended [Leu^5]-

enkephalin which was extracted as a side fraction from the previous isolation of α-neo-endorphin and dynorphin (1-8). The new peptide was named β-neo-endorphin (Minamino *et al.*, 1981).

The first extended [Met^5]-enkephalins were found in porcine hypothalamus ([Met^5]-enkephalin-Arg^6) by Huang *et al.* (1979) and in the striatum and adrenal medulla ([Met^5]-enkephalin-Arg^6-Phe^7) by Stern *et al.* (1979). Kimura *et al.* (1980) isolated three large enkephalin containing peptides (ECP) from bovine adrenal medulla (BAM). The peptides ranged in size from 3 to 5 kilodaltons and were designated Peptide I, F, and B. Peptide I was found to contain three extended [Met^5]-enkephalins, BAM-12P (Mizuno *et al.*, 1980a), BAM-20P and BAM-22P (Mizuno *et al.*, 1980b). Two additional peptides with opioid activity were found in extracts of bovine adrenal medulla. The first, an octapeptide ([Met^5]-enkephalin-Arg^6-Gly^7-Leu^8) isolated by Kilpatrick *et al.* (1981a) and the second a fraction of Peptide I (designated Peptide E) (Kilpatrick *et al.*, 1981b). All of the peptides isolated from bovine adrenal medulla were thought to be biosynthetic intermediates with one common precursor. The structure of the precursor was finally elucidated by cloning DNA sequences complimentary to the bovine mRNA coding for the protein. Pre-proenkephalin was sequenced by Noda *et al.*, (1982) and Gubler *et al.*, (1982). Subsequently, pre-proenkephalin was found to contain at least four enkephalins, [Met^5]-enkephalin, [Met^5]-enkephalin-Arg^6-Phe^7, [Met^5]-enkephalin-Arg^6-GLy^7-Leu^8, and [Leu^5]-enkephalin. The enkephalin sequences are each bounded by paired basic amino acid residues which act as cleavage signals for processing enzymes (Noda *et al.*, 1982). Pre-proenkephalin and pro-enkephalin differ only in the presence of a putative signal peptide at the beginning of the protein molecule.

The precursor of [Leu^5]-enkephalin and the extended [Leu^5]-enkephalins was also determined by DNA cloning techniques. Kakidani *et al.* (1982) published the primary structure of the precursor protein which was found to contain the sequences of α-neo-endorphin, β-neo-endorphin, and dynorphin. They proposed that this pre-proenkephalin be termed pre-proenkephalin B and the precursor of the [Met^5]-enkephalins pre-proenkephalin A. Rossier (1982) suggested that a more appropriate designation for pre-proenkephalin B may be pre-prodynorphin. Both terms are currently being utilized in the literature. The current relationship between precursor molecules and opioid peptides is summarized in Table 1.

In addition to the opioid peptides discovered while searching for precursors, several other peptides with opiate activity have been discovered. In 1978, the INRC classified a new class of substances, the exorphins, which were derived from food proteins. Henschen *et al.* (1980) obtained an opioid heptapeptide by chloroform-methanol extraction of β-casein designated β-casomorphin. Exorphins have also been isolated from pepsin hydrolysis of wheat gluten and α-casein (Zioudron and Klee, 1978). Takagi *et al.* (1979) extracted a dipeptide (Tyr-Arg), with opioid activity, from bovine brain. The dipeptide was named kyotorphin after the city of Kyoto, Japan. Same *et al.* (1980) isolated an opioid from the brain, blood, and cerebrospinal fluid (CSF) of rats and humans which they named humoral endorphin (H-endorphin).

The purpose of this investigation was to elucidate the role of endogenous opioid peptides in the regulation of food and water intake in the domestic fowl. This dissertation will be presented as six chapters, each of which has been or will be submitted for publication as a research paper or note. The first chapter examines the effects of opioid antagonists on ingestive behavior in the domestic fowl. The second chapter directly compares the sensitivity of rapidly growing meat stocks and slow growing egg stocks to opioid antagonists. The third chapter explores opioid regulation of ingestive behaviors in Japanese quail. Chapter 4 is a study conducted to determine whether opioids influence ingestive behavior in the domestic fowl at sites within the central and/or peripheral nervous system. Chapters 5 and 6 are studies investigating the effects of opioid agonists on ingestive behavior in the domestic fowl. In Chapter 5, the effects of mu and delta agonists were evaluated following intracerebroventricular and intramuscular administration. In Chapter 6, the feeding, drinking and temperature responses to intracerebroventricular β-endorphin were examined.

Table 1. Opioid Peptide Families[1]

Pro-opiomelanocortin	Proenkephalin (Human)	Prodynorphin (Porcine)
β-endorphin:	**[Met^5]-enkephalin:**	**α-neo-endorphin:**
(Human) Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu	Tyr-Gly-Gly-Phe-Met	Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-Lys
	[Leu^5]-enkephalin:	**β-neo-endorphin:**
	Tyr-Gly-Gly-Phe-Leu	Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro
	[Met^5]-enkephalin-7:	**Dynorphin (1-8):**
(Ostrich) Tyr-Gly-Gly-Phe-Met-Ser-Ser-Glu-Arg-Gly-Arg-Ala-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Val-Lys-Ser-Ala-Tyr-Lys-Lys-Gly-Gln	Tyr-Gly-Gly-Phe-Met-Arg-Phe	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile
	[Met^5]-enkephalin-8:	**Dynorphin (1-13):**
	Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr
		Dynorphin (1-17): Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln

[1]Adapted from Akil *et al.*, 1984

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CHAPTER 1

THE EFFECTS OF OPIOID ANTAGONISTS ON INGESTIVE BEHAVIOR IN THE DOMESTIC FOWL

INTRODUCTION

Since the discovery of brain opioid receptors in 1973 and the isolation of their endogenous ligands later in the decade (for review see, Simon, 1982; Akil *et al.*, 1984; Kitchen, 1984) there has been considerable investigation into the role of these compounds in physiological and behavioral processes. Endogenous opioid peptides have been implicated as regulators or modulators of a myriad of processes, including thermoregulation, cardiovascular and pulmonary function, learning, and ingestive, social and sexual behavior (Olson *et al.*, 1984).

Since the initial studies of Grandison and Guidotti (1977) who demonstrated that injection of β-endorphin into the ventromedial hypothalamus (VMH) facilatated feeding in the rat, much attention has focused on ingestive behavior. Subsequent investigations, using either opioid agonists or antagonists, have provided corroborative evidence for the role of opioid peptides in the regulation of food and water intake (Levine *et al.*, 1985).

Opioid antagonists are generally believed to act physiologically, altering ingestive behavior by the blockade of opioid receptors with a resultant attenuation of the actions of endogenous opioid peptides (Olson *et al.*, 1984; Levine *et al.*, 1985). Opioid antagonists, such as naloxone and naltrexone, demonstrate high affinity for opioid receptors (Snyder, 1975) and have been shown to decrease the ingestive stimulation induced by central administration of dynorphin-(1-13) (Morley and Levine, 1981) and β-endorphin (Leibowitz and Hor, 1982).

Holtzman (1974) first demonstrated an attenuation of food intake when the opioid antagonist naloxone was administered to rats. Additional investigations using mammalian species showed that antagonists were capable of suppressing food intake under a variety of conditions, e.g., satiated (King *et al.*, 1980), deprived (Brown and Holtzman, 1979; Frenk and Rogers, 1979; Thornhill *et al.*, 1982), 2-deoxy-D-glucose (Ostrowski *et al.*, 1981), stress-induced (Morley and

Levine, 1980), and insulin-induced hypoglycemia (Ostrowski *et al.*, 1981). While both food and water consumption were affected, water intake appeared to be particularly sensitive to the effects of antagonists and is possibly the primary behavior affected (Brown and Holtzman, 1979). Opioid antagonists have been effective in attenuating water intake induced by deprivation (Holtzman, 1975; Brown and Holtzman, 1979; Woods and Leibowitz, 1985), angiotensin II (Ostrowski *et al.*, 1981), hypertonic saline (Brown *et al.*, 1980), and hypervolemia (Ostrowski *et al.*, 1981).

Endogenous opioid peptides whose effects may be blocked by antagonists have been isolated from *Aves*. Peptides isolated from the turkey (*Meleagris gallopavo*) (Chang *et al.*, 1980) and the ostrich (*Struthio camelus*) (Naude *et al.*, 1980) have been sequenced (Chang *et al.*, 1980; Naude *et al.*, 1981) and demonstrate high affinity for opioid receptors in binding assays (Yamashiro *et al.*, 1980; Yamashiro *et al.*, 1982). There have been relatively few studies examining the affects of opioid antagonists in *Aves*. Cooper and Turkish (1981) and Deviche and Schepers (1984a) observed a decrease in food intake when naloxone was administered to pigeons. In contrast to findings with mammals, there was no effect on water intake, indicating that the regulation of water intake in pigeons is independent of opioid regulation.

The present study was conducted to evaluate the effect of endogenous opioids on ingestive behavior in the domestic fowl. Opioid antagonists were used to determine the existence and characterize the nature of opioid regulation in Leghorn and commercial broiler-type chickens.

MATERIALS AND METHODS

Animals. Commercial broiler and Single-Comb White Leghorns (SCWL) cockerels were reared in heated batteries until three and six weeks of age, respectively, and then transferred to individual cages. The chicks were provided starter mash [20 % protein, 2864 kcal/kg metabolizable energy (ME)] and water *ad libitum*, and were exposed to continuous light.

Experiments 1 and 2. These experiments were conducted to determine the effects of peripheral (intramuscular, im) injections of naloxone HCl on food and water intake in 6-week old broilers (Experiment 1) and 8-week old SCWL (Experiment 2). A replicated completely randomized design was used with 20 chicks (10/replicate) assigned at random to four treatment groups

consisting of 0, 2.5, 5, or 10 mg/kg body weight naloxone HCl (Endo Laboratories, Inc. Garden City, N.Y.) injected im in a volume of 1 ml. Physiological saline (0.9 % NaCl) served as a control solution. Immediately prior to initiating the experiment, feeders and waterers were removed and the chicks were weighed to the nearest 1 g. Injections were administered in random order into the pectoral muscle 15 min prior to the return of food and water. Food and water intake was measured to the nearest 1 g and 5 ml, respectively, with measurements being made at 30 min intervals for 300 min and again at 24 hr.

Experiments 3 and 4. These experiments were similar to Experiments 1 and 2, except naltrexone HCl (Endo Laboratories, Inc. Garden City, N.Y.) was substituted for naloxone HCl and Experiment 3 consisted of a single replicate (10 birds). Naltrexone is a congener of naloxone which exhibits greater potency and a longer period of efficacy (Blumberg and Dayton, 1973). The purpose of these experiments was to determine whether alterations in ingestive behavior induced by opioid receptor blockade are unique to naloxone or if they may be elicited by related antagonists.

Experiment 5. This experiment was conducted to determine if naloxone HCl was capable of specifically attenuating water intake independent of effects on food intake. The experiment was conducted as a replicated completely randomized design with ten 6-week old commercial broiler chicks (5 birds/replicate) assigned at random to one of four treatment groups. Treatments were as described in Table 1; the first and second injections were separated by a 45 minute interval. Immediately prior to the first injection feed and water were withdrawn and the chicks weighed to the nearest 1 g. Chicks were then administered their respective treatments in random order. Water was returned 15 minutes after the second injection and measurements made at 30 min intervals for 300 min. Food was withheld for the 300 min measurement period.

Statistical Analysis. In Experiments 1 through 4, cumulative food and water intake at each time period were analyzed using analysis of variance. Dose-response relationships within observation periods were evaluated using linear and quadratic contrasts (Steel and Torrie, 1980). In Experiment 5, data were transformed to $(\sqrt{Y + .5})$ to achieve homogeneity of variances and normality. Data were analyzed using analysis of variance and orthogonal contrasts were used within

observation periods to evaluate treatment differences (Steel and Torrie, 1980). Reported values are the untransformed data. Significance implies $P \leq .05$.

RESULTS

Experiments 1 and 2. Administration of naloxone caused a significant dose-dependent attenuation of food and water intake in broilers (Figure 1). The dose relationship for the reduction in food intake within observation periods was quadratic through 180 min and linear from 210 through 300 min. The minimally effective dose tested was 2.5 mg/kg body weight while 5.0 mg/kg body weight produced the maximum response. Water consumption was attenuated in a quadratic manner through 300 min. The relative efficacy of the doses was similar to that observed for food intake with 5.0 mg/kg body weight being most efficacious. Naloxone had no effect on either food or water intake at 24 hr.

As in broilers, the administration of naloxone to SCWL also resulted in a significant dose-dependent reduction in food and water intake (Figure 2). Food intake was decreased linearly through 180 min and again at 240 min. Water intake was attenuated in a linear manner at 30 min, quadratically from 60 through 90 min and linearly from 120 through 300 min. No treatment effect was noted on either food or water consumption at 24 hr.

Experiments 3 and 4. The effects of intramuscular injection of naltrexone HCl on food and water intake in broilers and SCWL are shown in Figure 3 and 4, respectively. Administration of naltrexone to broilers resulted in a significant dose-dependent attenuation of food and water intake. Food intake was decreased in a linear manner through 60 min and quadratically from 90 through 300 min. Water intake was decreased in a quadratic manner from 60 through 300 min. The minimally effective dose tested was 2.5 mg/kg body weight for both food and water consumption while the maximally effective dose was 5 mg/kg body weight for food intake and 10 mg/kg body weight for water intake. Naltrexone had no effect on either food or water consumption at 24 hr.

Naltrexone also produced a significant dose-dependent suppression in food and water consumption in SCWL. Food intake was attenuated in a quadratic manner from 60 through 300 min, with 2.5 mg/kg body weight being the most effective dose prior to 180 min and 10 mg/kg body

weight the most effective at subsequent time periods. A quadratic dose relationship was observed for the depression in water intake through 240 min and a linear relationship from 270 through 300 min. A similar suppression in water intake was observed with the 2.5 mg/kg body weight and 5 mg/kg body weight doses, while the 10 mg/kg body weight dose attenuated water intake to the greatest degree. No effect of treatment was noted on either food or water consumption at 24 hr.

Experiment 5. The results of Experiment 5 are summarized in Table 2. Intracellular dehydration induced by a 5 mM saline load resulted in a significant increase in water consumption relative to the isotonic saline injected treatments from 90 through 300 min. The response to saline load was significantly decreased for 150 min by the administration of naloxone. An attenuation in water consumption, relative to the isotonic saline control, was also observed from 60 to 150 min when naloxone was administered without saline load. Beyond 150 min the depressant effects of naloxone began to dissipate leading to rapid compensatory increases in water intake in naloxone-treated birds.

DISCUSSION

The results of this study demonstrate that the peripheral (im) administration of opioid antagonists to broiler and SCWL chickens produced a dose-dependent attenuation of food and water consumption. The suppression of ingestive behaviors occured at doses from 2.5 to 10 mg/kg body weight with maximum attenuation usually occurring at a dose of 5 mg/kg body weight.

Broiler and SCWL chickens were utilized in order to evaluate the efficacy of opioid antagonists in altering ingestive behavior in the domestic fowl and to determine if the genetic selection for low and high body weight in egg-producing and meat producing stocks, respectively, altered sensitivity to these compounds. Previous studies comparing meat and egg-laying stocks have shown that these stocks have different responses to putative regulators of ingestive behavior. Denbow *et al.* (1981, 1983) have shown that broilers and SCWL respond differently to the central administration of biogenic amines. Epinephrine was effective in increasing food intake in broilers while having no effect in SCWL. On the other hand, the central administration of 5-hydroxytryptamine (5-HT) increased water intake of sated SCWL and decreased water intake of food-deprived SCWL

(Denbow *et al.*, 1983) but produced no alteration in water intake in broilers (Denbow *et al.*, 1982). Recently, Lacy *et al.* (1986) found that intrahepatic infusions of lipids decreased feeding in SCWL but did not alter food intake in broilers. The attenuation of feeding and drinking observed in the present study demonstrates that opioid antagonists are effective in reducing ingestive behaviors in both stocks of chickens and that the genetic selection for egg-production and low body weight has not abolished the sensitivity to opioid antagonists. The possibility remains that the stocks differ in the degree of sensitivity to these substances.

Reductions in food intake have been observed in rats (King *et al.*, 1980) and pigeons (Cooper and Turkish, 1981; Deviche and Schepers, 1984a) in response to opioid antagonists. However, while opioid antagonists have consistently produced attenuation of water intake in mammals (Holtzman, 1975; Brown and Holtzman, 1980; Sanger, 1981; Baldwin and Parrott, 1985) an effect on drinking behavior has not heretofore been observed in *Aves* (Cooper and Turkish, 1981; Deviche and Schepers, 1984a)

Food and water consumption have been shown to be proportional in commercial stocks of chickens (Medway and Kare, 1959; Marks, 1981; Pesti *et al.*, 1985) which is suggestive of a common regulatory mechanism. Therefore, although naloxone and naltrexone reliably decreased water consumption in both broiler and SCWL chickens, the similarity in time course between the food intake and water intake responses and the incongruity with studies using pigeons necessitated further study to confirm that the attenuation of water intake was not simply an artifact of the effect on food intake.

Experiment 5 was conducted to evaluate the effect of naloxone on water intake independent from it's effects on food intake. The results obtained demonstrated that naloxone attenuated drinking in normally hydrated chickens and in chickens induced to drink by hypertonic saline-induced intracellular dehydration. Furthermore, the decrease in water consumption occurred independently of alterations in food consumption. Food and water intake, therefore, appear to be regulated by similar but independent opioid antagonist-sensitive regulatory systems. The genetic basis for such independent systems has been described by Marks (1980). It is plausable that a dis-

similarity in the naloxone-sensitivity of water consumption exists between pigeons and commercial stocks of chickens.

The mechanisms by which opioid antagonists induce alterations in ingestive behavior still remain a matter of speculation. Two possible modes of action have been theorized. One mechanism is based on the central reward theory proposed by Belluzzi and Stein (1977) and supported by the work of Carr and Simon (1984). The central reward theory proposes that endogenous opioid peptides mediate drive-reduction reward and, therefore, enhance the reward value of ingestive acts. Accordingly, opioid antagonists would interrupt normal ingestive behaviors by interfering with this reward system rendering food and water consumption less satisfying.

A second mechanism, first proposed by Cooper (1980), attributes antagonist-induced alterations in ingestive behavior to changes in the threshold of satiety. The satiety theory originated from the observation that naloxone served to further depress feeding behavior in animals already exhibiting reduced intake due to osmotic challenge. Therefore, opioid antagonists appeared to interrupt an opioid mediated mechanism which inhibited satiety. The satiety theory received support in subsequent studies by Siviy *et al.* (1982) and Kirkam and Blundell (1984) which have shown that naloxone hastens the termination of feeding and drinking by interfering with processes that serve to sustain consumption.

The possiblity exists that opioid antagonists may act in a pharmacological manner by altering motor ability, or by inducing a non-specific malaise. These reasons are not supported in the present study because no differences in motor activity, appearence of discomfort or overt behaviors were observed between control and experimental treatments. These observations are substantiated by other studies in which similar dosages were used and in which no malaise was observed (Brown and Holtzman, 1980; Holtzman, 1975; King *et al.*, 1980, Ostrowski *et al.*, 1981). Also, Segal *et al.* (1979) found no alterations in the locomotor activity of rats at doses of naloxone as high as 20 mg/kg body weight.

Further evidence against antagonists acting by means of a non-specifc malaise is that in both rats (Brown and Holtzman, 1980) and pigeons (Deviche and Schepers, 1984b) the actions of opioid antagonists have been shown to be stereospecific. Also, opioid antagonists exhibit behav-

ioral specificity for ingestive behaviors as has been demonstrated by the absence of any effect of naloxone on water intake in pigeons (Cooper and Turkish, 1981; Deviche and Schepers, 1984a) and the dissociation of prey killing and prey eating in mouse killing rats (Walsh *et al.*, 1984).

High doses of naloxone have been found to produce varying degrees of conditioned taste aversion (CTA) in rats (Frenk and Rogers, 1979), but no reliable correlation has been found between the magnitude of the CTA and the suppression of water consumption (Wu *et al.*, 1979; Ostrowski *et al.*, 1980). In addition, Frenk and Rogers (1979) were unable to suppress drinking with lithium chloride despite it's ability to produce an extensive CTA. Therefore, there appears to be little evidence that naloxone exerts it's effects by inducing a non-specific malaise.

Based on the results of this study it is possible to conclude that opioid antagonists produce an attenuation of food and water intake in meat and egg-laying stocks of chickens. The induction of this effect is most likely a manifestation of the interaction of these compounds with opioid receptors, resulting in the inhibition of the actions of endogenous opioid peptides. Therefore, it appears that endogenous opioid peptides play a significant role in the regulation of food and water intake in the domestic fowl.

SUMMARY

The effects of opioid antagonists on food and water intake in commercial stocks of chickens were investigated. Four experiments were conducted to examine the effects of naloxone (N-allylnoroxymorphone) and naltrexone (N-cyclopropylnoroxymorphone) in broiler and Single-Comb White Leghorn cockerels. Birds were injected intramuscularly with either naloxone HCl or naltrexone HCl at doses from 2.5 to 10 mg/kg body weight. Food and water were offered *ad libitum* 15 min post-injection.

In broilers, naloxone dose-dependently attenuated food and water consumption for 300 min, while in Leghorns naloxone attenuated food and water intake for 240 and 300 min, respectively. Naltrexone dose-dependently reduced food and water consumption for 300 min in both broilers and Leghorns. Neither naloxone nor naltrexone significantly altered food or water intake at 24 hr.

A fifth experiment was conducted to verify the specificity of opioid antagonism for water intake. Broiler cockerels received an intraperitoneal injection of either isotonic saline (.15 M NaCl) or hypertonic saline (2.5 M NaCl) followed by an intramuscular injection of either isotonic saline or naloxone HCl (5 mg/kg body weight). Food was withheld for the entire experiment while water was offered *ad libitum* 15 min following the second injection. Naloxone significantly attenuated drinking in normally hydrated and osmotically challenged birds for 150 min. The results suggest a role for endogenous opioid peptides in the regulation of food and water intake in meat and egg-laying stocks of chickens.

Chapter 1 has been published in Pharmacology, Biochemistry and Behavior 27:25-33.

TABLE 1. Experimental procedure for Experiment 5

Treatment	First injection [1]	Second injection [2]
A	isotonic saline	isotonic saline
B	isotonic saline	naloxone HCl
C	hypertonic saline	isotonic saline
D	hypertonic saline	naloxone HCl

[1] Intraperitoneal injections of isotonic saline (.15 M NaCl) and hypertonic saline (2.5 M NaCl) were delivered in a volume of 2 ml/kg body weight, resulting in a 5mM NaCl load for animals receiving hypertonic saline.

[2] Intramuscular injections of isotonic saline and naloxone HCl (5 mg/kg body weight) were administered into the pectoral muscle in a volume of 1 ml. This injection was given 45 min after the first injection.

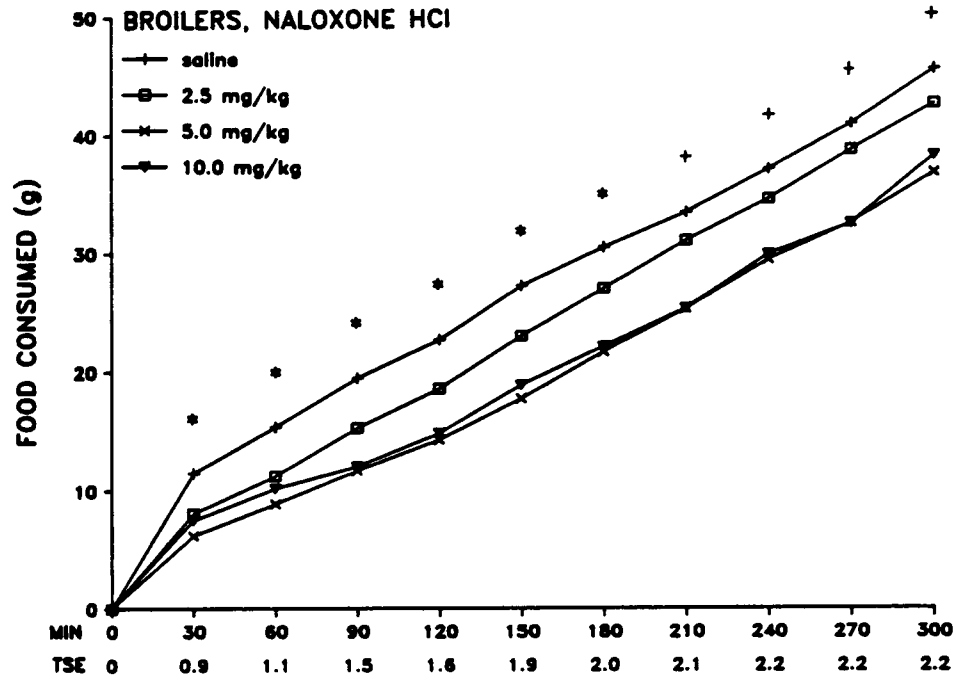
TABLE 2. The effect of intramuscular injections of naloxone hydrochloride on mean cumulative water intake (ml) of broiler cockerels with and without saline load pretreatment (Exp. 5)

	Time (min)									
Treatment	30	60	90	120	150	180	210	240	270	300
A) isotonic saline + isotonic saline [1]	10.5	15.0	19.0	19.5	23.0	25.0	26.5	27.5	28.5	29.5
B) isotonic saline + naloxone HCl	3.5	4.0	4.0	4.0	5.0	5.5	14.0	22.0	24.0	26.0
C) hypertonic saline + isotonic saline	18.0	32.0	48.0	49.0	50.0	52.5	52.5	57.0	59.5	61.0
D) hypertonic saline + naloxone HCl	0	1.5	4.5	6.0	32.0	58.0	70.0	82.5	89.5	102.5
Standard error of the treatment mean	1.8	2.1	2.1	2.3	5.4	8.5	8.8	9.2	9.2	9.1
F value of contrasts [2]										
A *vs* B	1.80	4.33*	9.24*	10.09*	4.53*	3.53	2.11	1.08	0.73	0.68
C *vs* D	15.50*	30.31*	58.90*	55.00*	9.88*	2.36	0.67	0.06	0.01	0.53
AB *vs* CD	.02	1.47	6.35*	6.04*	4.94*	6.37*	6.04*	7.44*	7.62*	12.20*

[1] The birds received two injections 45 min apart. The first injection was either isotonic saline (.15M NaCl) or hypertonic saline (2.5M NaCl), administered ip in a volume of 2 ml/kg body weight. The second injection was either isotonic saline or 5.0 mg/kg body weight naloxone HCl administered im.

[2] F values reported are those obtained from analyses of transformed ($\sqrt{Y + .5}$) data.

* Critical F (1, 35) = 4.10, P ≤ .05; therefore, these contrasts are significant.



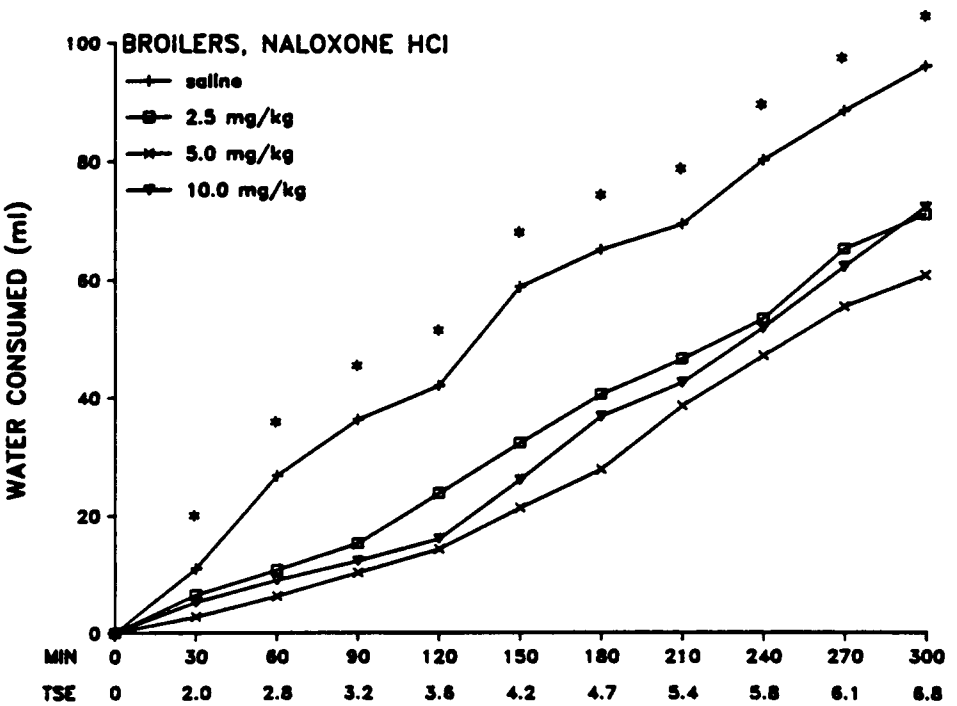


FIG. 1. Mean cumulative food and water intake of broiler cockerels in response to intramuscular injection of naloxone HCl. +, significant linear contrast ($P \leq .05$) within observation period. *, significant quadratic contrast ($P \leq .05$) within observation period. MIN, minutes; TSE, standard error of the treatment mean.

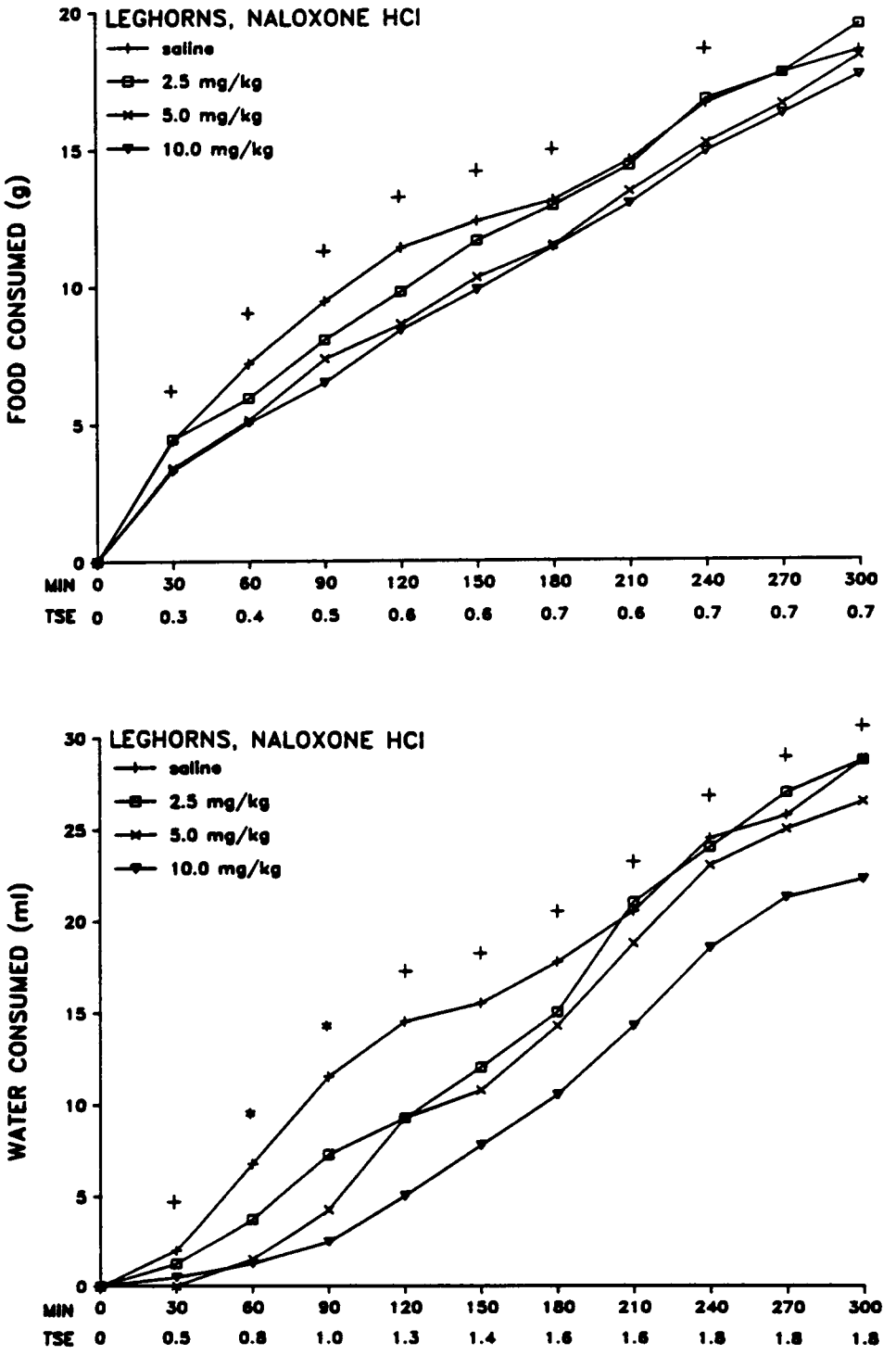
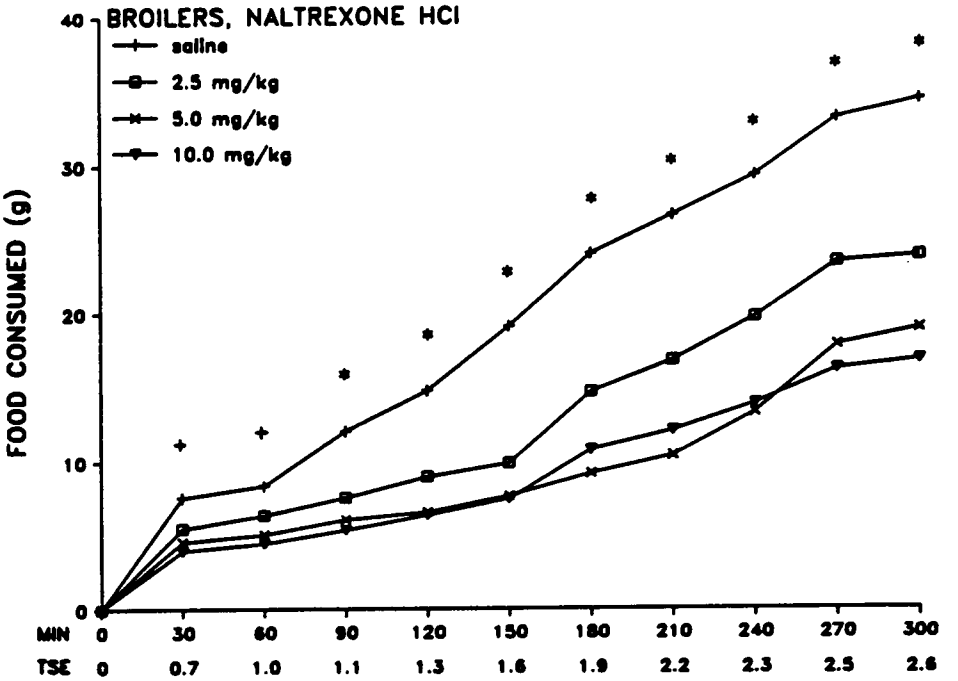


FIG. 2. Mean cumulative food and water intake of Leghorn cockerels in response to intramuscular injection of naloxone HCl. +, significant linear contrast ($P \leq .05$) within observation period. *, significant quadratic contrast ($P \leq .05$) within observation period. MIN, minutes; TSE, standard error of the treatment mean.



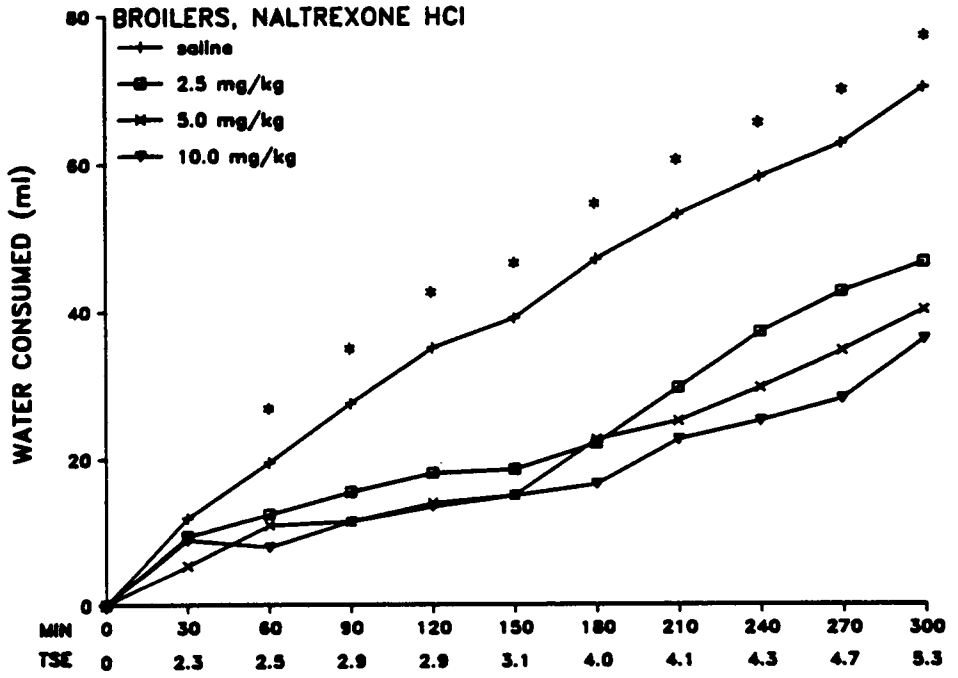
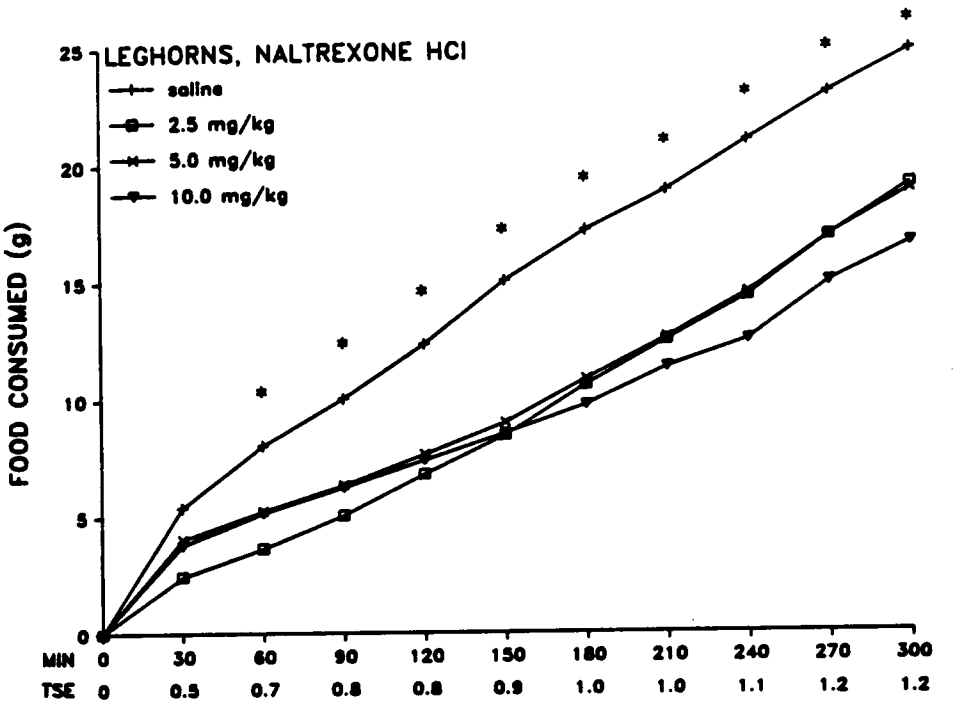


FIG. 3. Mean cumulative food and water intake of broiler cockerels in response to intramuscular injection of naltrexone HCl. +, significant linear contrast ($P \leq .05$) within observation period. *, significant quadratic contrast ($P \leq .05$) within observation period. MIN, minutes; TSE, standard error of the treatment mean.



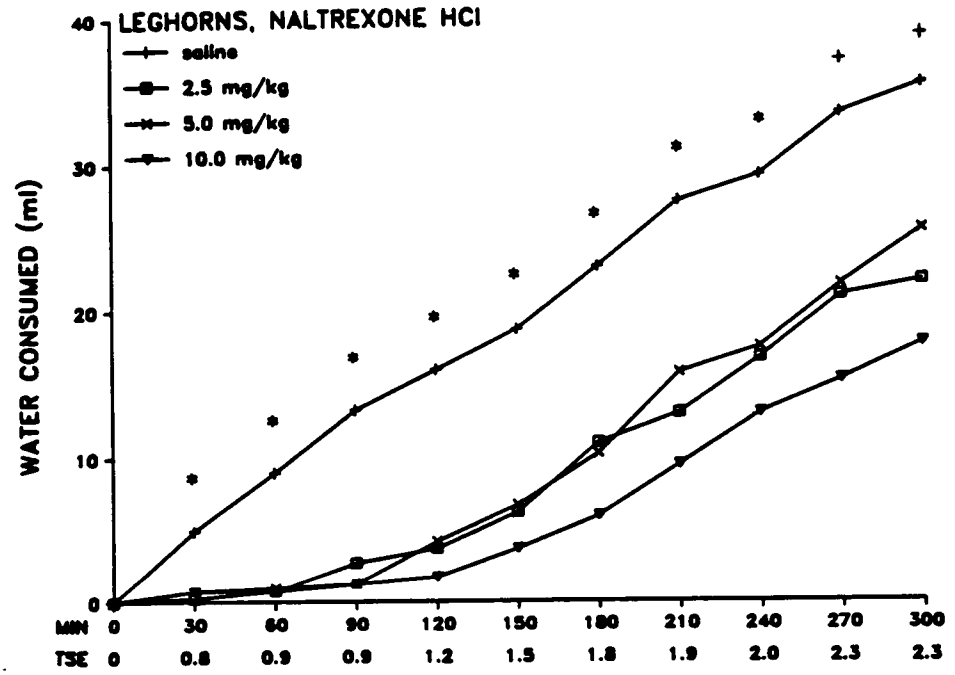


FIG. 4. Mean cumulative food and water intake of Leghorn cockerels in response to intramuscular injection of naloxone HCl. +, significant linear contrast ($P \leq .05$) within observation period. *, significant quadratic contrast ($P \leq .05$) within observation period. MIN, minutes; TSE, standard error of the treatment mean.

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CHAPTER 2

INGESTIVE BEHAVIOR OF MEAT AND EGG STOCKS OF CHICKENS: EQUAL SENSITIVITY TO NALOXONE

INTRODUCTION

There is substantial evidence supporting a role for endogenous opioid peptides in the regulation of ingestive behaviors. Opioid peptides such as β-endorphin, dynorphin, [D-Ala^2 , D-Leu^5]-enkephalin (DADL) and [D-Ala^2 , Met^5]-enkephalinamide (DALA) increase food and water intake (Grandison and Guidotti, 1977; McKay *et al.*, 1981; Morley and Levine, 1981; Woods *et al.*, 1981; McLean and Hoebel, 1983; Czech *et al.*, 1984) while opioid antagonists such as naloxone (N-allylnoroxymorphone) and naltrexone (N-cyclopropylmethylnoroxymorphone) attenuate these behaviors (Holtzman, 1974; Brown and Holtzman, 1979; Frenk and Rogers, 1979). Because of their association with hyperphagia and polydipsia, the opioids may be causative agents in the genesis of obesity and other eating disorders (Olson *et al.*, 1985).

Studies evaluating the relationship between opioid peptides and obesity have utilized either genetically obese animals or animals in which obesity was induced by lesions of the ventromedial hypothalamus (VMH). Increases in pituitary β-endorphin, [Leu^5]-enkephalin and dynorphin have been observed in genetically obese mice (*ob/ob*) and rats (*fa/fa*) (Margules *et al.*, 1978; Rossier *et al.*, 1979; Ferguson-Segall *et al.*, 1982). In addition, both plasma and pituitary β-endorphin increase in rats with lesions of the VMH (Matsumura *et al.*, 1984). In contrast, in other studies (Gunion and Peters, 1981; Morley *et al.*, 1982) no association was observed between levels of dynorphin or β-endorphin in the central nervous system and obesity syndromes in the rat or mouse.

Genetic and hypothalamic obesities also have been associated with increased sensitivity to opioid agonists and antagonists, with naloxone attenuation of food intake being greater in genetically obese mice than in their lean littermates (Margules *et al.*, 1978; Shimomura *et al.*, 1980). Naloxone is also more potent in attenuating ingestive behavior in VMH lesioned rats than in non-lesioned rats (Thornhill *et al.*, 1982). Furthermore, ethylketocyclazocine and Mr-2286, a

kappa opioid receptor agonist and antagonist, respectively, are more effective in altering feeding when administered to genetically obese mice than to their lean littermates (Ferguson-Segall *et al.*, 1982).

Opioids also influence ingestive behavior in *Aves* (Cooper and Turkish, 1981; Deviche and Schepers, 1984a,b; McCormack and Denbow, 1987 - Chapter 1). However, the role of opioids in the manifestation of obesity in *Aves* has not been evaluated. Because lipid metabolism in birds and humans is similar (Goodridge and Ball, 1966; 1967), the former seems to be a more suitable model than rodents for the study of obesity in humans.

Genetic selection of domestic fowl for either meat (Rock-Cornish, RC) or egg production (Single-Comb White Leghorn, SCWL) has resulted in the development of stocks which differ greatly in both growth rate and body composition. The RC stocks have been selected for rapid growth and have a relatively high percentage of body fat (Denbow and Kuenzel, 1978; McCarthy and Siegel, 1983; Siegel, 1984), while Leghorn stocks have been selected for egg production and consequently low body weight and low body fat during the juvenile growth phase (Kinney, 1969; Denbow and Kuenzel, 1978; Cherry *et al.*, 1987). Chickens selected for high body weight exhibit many characteristics common to genetic obesity in rodents; characteristics such as hyperphagia, hyperglycemia, hyperinsulinemia, insulin resistance and impaired fertility (McCarthy and Siegel, 1983; March, 1984; Nir, 1984; Siegel and Dunnington, 1985; Sinsigalli *et al.*, 1987). Therefore, RC and SCWL chickens may be considered obese and lean stocks, respectively (McCarthy and Siegel, 1983; Cherry *et al.*,1987). The purpose of the present study was to compare the effect of the opioid antagonist naloxone on ingestive behavior in RC and SCWL stocks of chickens.

MATERIALS AND METHODS

Animals. Commercial RC and SCWL cockerels were reared in heated batteries until 28 days-of-age, or 21 days-of-age for RC chicks in Exp. 1, then transferred to individual cages. The chicks were provided starter mash [20% protein, 2864 kcal/kg metabolizable energy (ME)] and water *ad libitum* and were exposed to continuous light. Birds were allowed to adapt to the individual cages and to handling for a minimum of one week prior to testing.

Direct comparison of RC and SCWL stocks are complicated by their differing growth patterns. To account for differences in age or body weight, two experiments were conducted. In the first experiment RC and SCWL chicks were tested at approximately the same body weight which resulted in the confounding of stock with age. In the second experiment RC and SCWL chicks were tested at the same age which resulted in the confounding of stock with body weight.

Experiment 1. The effect of naloxone HCl on food and water intake was evaluated in RC and SCWL cockerels of similar mean body weight, 858 ± 18 *vs.* 806 ± 15 ($\overline{X} \pm SE$) g, respectively. At these weights the RC chicks were 28 days-of-age while SCWL chicks were 56 days-of-age. Ten birds of each stock were randomly assigned to each of two groups receiving either 0 or 5 mg/kg body weight naloxone HCl (Endo Laboratories, Inc. Garden City, N.Y.). Therefore, the experiment was designed as a 2x2 factorial with stock and drug treatment as the main effects. Physiological saline (.9 % NaCl) served as a control solution and as a vehicle for the administration of naloxone HCl. All injections were administered intramuscularly in a total volume of 1 ml.

Immediately prior to initiating the experiment, feeders and waterers were removed and chicks were weighed to the nearest 1 g. Injections were administered in random order into the pectoral muscle 15 min prior to the return of food and water. Food and water intake was measured to the nearest 1 g and 5 ml, respectively, with measurements being made at 30 min intervals for 300 min and again at 1440 min.

Experiment 2. Protocol for this experiment was the same as that for Experiment 1 except that the effect of naloxone HCl on food and water intake was compared in RC and SCWL cockerels of the same age, 35 days. RC chicks weighed 1171 ± 26 ($\overline{X} \pm SE$) g while the SCWL chicks weighed 396 ± 7 ($\overline{X} \pm SE$) g.

Statistical Analysis. Cumulative food (g/kg body weight) and water intake (ml/kg body weight) at each observation period was analyzed using analysis of variance. The statistical model was:

$$Yijk = u + Si + Dj + (SD)ij + eijk$$

Where, S, D, and (SD) represent stock, drug and stock by drug interaction, respectively. When not significant, the variation attributable to the stock by drug interaction was pooled with the overall error. Significance implies $P \leq .05$.

RESULTS

Experiment 1. This experiment compared RC and SCWL stocks at similar body weights. Naloxone significantly attenuated cumulative food and water intake through 210 min (Tables 1 and 2). As evidenced by the absence of a significant stock by drug interaction, naloxone was equally effective in RC and SCWL. Although differences in consumption between the stocks were confounded with age and as a result may not strictly be attributed to genetic differences, RC chicks consumed a significantly greater quantity of food and water than the older SCWL chicks. When intake was expressed as g/kg body weight$^{.75}$ there were no differences in either food or water consumption attributable to stock while differences in intake due to drug treatment were maintained.

Experiment 2. This experiment compared RC and SCWL stocks at the same age. Naloxone significantly attenuated cumulative food intake through 300 min following the return of food and water while attenuating water intake at 60 and 120 min (Tables 3 and 4). As evidenced by the absence of a significant stock by drug treatment interaction, RC and SCWL stocks demonstrated equal sensitivity to naloxone. Due to the confounding of stock with body weight it was not possible to attribute the stock effect strictly to genetic differences. The lighter SCWL stock consumed more food and water than the heavier RC stock. As observed in Experiment 1, when intake was expressed on a metabolic body weight basis there was no stock effect while the effect of drug treatment remained significant.

DISCUSSION

Naloxone was equally effective in decreasing food and water intake in both RC and SCWL chickens. One possible explanation for the similarity in naloxone sensitivity is a lack of differential sensitivity at the dose examined. This explanation does not appear to be likely because previous experiments examining dose-response relationships have shown that the food and water intake of

RC or SCWL is attenuated by naloxone at doses from 2.5 to 10 mg/kg body weight with the most effective dose generally being either 5 or 10 mg/kg body weight (McCormack and Denbow, 1987 - Chapter 1). Naloxone may exhibit differential potency in RC and SCWL stocks at doses either slightly lower or higher than 5 mg/kg body weight, but this would require a differential response to emerge and wane over a dramatically narrow dose range. Furthermore, obese and lean rats have shown a differential response to naloxone at doses from .25 to 10 mg/kg body weight (Margules *et al.*, 1978; King *et al.*, 1980; Thornhill *et al.*, 1982).

A second possibility for a lack of differential naloxone sensitivity may be that the opioid mediated regulation of ingestive behavior has been conserved during genetic selection for meat and egg production in the domestic fowl. That naloxone was equally potent in both stocks is unusual because other putative regulators of ingestive behavior such as epinephrine (Denbow *et al.*, 1981; 1983), or serotonin, administered intracerebroventricularly (Denbow *et al.*, 1982; 1983), and glucose or fat, administered intrahepatically (Lacy *et al.*, 1985; 1986), affect these stocks differently.

Another possible reason that RC and SCWL stocks demonstrate equivalent sensitivity to naloxone may be that the body composition of the stocks had not diverged sufficiently. When compared at the same age (5 weeks) RC and SCWL stocks have approximately 12 and 8 % body fat, respectively, while when compared at the same body weight 4-week RC and 8-week-old SCWL have approximately 11 and 10 % body fat, respectively (Denbow and Kuenzel, 1978). However, young obese and lean rats demonstrate a much greater disparity in body composition with obese individuals having approximately 13% more body fat (Bell and Stern, 1977). Gunion and Peters (1981) proposed that the differential responses to naloxone observed in hypothalamic obese and normal rodents is dependent upon differences in body weight and composition. They observed that hypothalamic obese rats maintained at a body weight equivalent to that of normal rats exhibited an equal sensitivity to naloxone. These results contrasted with other studies in which body weight was not controlled and a differential sensitivity to naloxone was observed (King *et al.*, 1980; Margules *et al.*, 1978). Body weight and obesity have also been shown to influence the concentration of opioid peptides in the central nervous system. Rossier *et al.* (1979) demonstrated that body weight and increases in pituitary [Leu^5]-enkephalin were highly correlated, but β-endorphin

levels appeared to increase as a consequence of obesity. Therefore, it is possible that differential responses to opioid antagonists may result from the attenuation of obesity-induced opioid peptides.

SUMMARY

The efficacy of the opioid antagonist naloxone in attenuating ingestive behavior in stocks of chickens genetically selected for either meat (Rock-Cornish, RC) or egg production (Single-Comb White Leghorn, SCWL) was investigated. Because the stocks differ markedly in growth rate two experiments were conducted to compare RC and SCWL cockerels at similar body weight and at the same age. Birds were injected intramuscularly with either isotonic saline or naloxone HCl at a dose of 5 mg/kg body weight. Food and water were offered *ad libitum* 15 min post-injection.

In RC and SCWL stocks of similar body weight, naloxone significantly attenuated cumulative food and water intake through 210 min following the return of food and water. When administered to RC and SCWL stocks of the same age, naloxone significantly attenuated cumulative food intake for 300 min and cumulative water intake at 60 and 120 min. The relatively long-term depressions in cumulative food and water intake where attributable to significant decreases in incremental consumption within early time periods. There was no significant difference in the efficacy of naloxone in attenuating ingestive behavior when the stocks were compared at either similar body weight or the same age. The results demonstrate that genetic selection for meat or egg production has not significantly altered opioid mechanisms regulating food and water intake in the domestic fowl.

Chapter 2 has been accepted for publication in Poultry Science.

TABLE 1. Mean cumulative food intake (g/kg W[1]) by Rock-Cornish (RC) and Single-Comb White Leghorn (SCWL) cockerels of similar body weight following intramuscular injection of saline or naloxone hydrochloride (Exp. 1)

	Time (min)										
Main Effects [2]	30	60	90	120	150	180	210	240	270	300	1440
Stock Effect: [3]											
RC	7.9[a]	11.8[a]	13.8[a]	17.0[a]	21.0	23.9[a]	27.0[a]	28.4	32.1[a]	34.4	128.6[a]
SCWL	5.7	8.0	10.3	13.6	17.8	20.1	22.6	25.3	27.7	30.1	94.4
Drug Effect:											
Saline	8.0[b]	12.1[b]	14.1[b]	17.7[b]	21.7[b]	23.9[b]	27.1[b]	28.4	32.0	34.4	114.2
Naloxone HCl (5 mg/kg W)	5.6	7.7	10.0	13.6	17.1	20.1	22.5	25.3	27.8	30.1	108.8
Standard Error of the Treatment Mean	.6	.6	.7	.9	1.2	1.3	1.3	1.6	1.5	1.8	4.4

[1] W, body weight.

[2] Stock by drug interaction was not significant at any observation period.

[3] The RC chicks were 28 days-of-age and had a mean body weight of 858g. The SCWL chicks were 56 days-of-age and had a mean body weight of 806g.

[a] Significant stock effect ($P \leq .05$) at this observation period.

[b] Significant drug effect ($P \leq .05$) at this observation period.

TABLE 2. Mean cumulative water intake (ml/kg W[1]) by Rock-Cornish (RC) and Single-Comb White Leghorn (SCWL) cockerels of similar body weight following intramuscular injection of saline or naloxone hydrochloride (Exp. 1)

	Time (min)										
Main Effects [2]	30	60	90	120	150	180	210	240	270	300	1440
Stock Effect: [3]											
RC	9.7	13.7[a]	22.8[a]	30.1[a]	38.3[a]	43.5	50.5	57.2	63.0	69.9	286.8[a]
SCWL	5.7	6.6	11.1	16.2	23.2	30.6	37.7	47.9	56.2	58.2	207.0
Drug Effect:											
Saline	13.4[b]	16.1[b]	27.6[b]	34.0[b]	39.8[b]	45.1[b]	51.9[b]	59.8	65.9	69.2	241.9
Naloxone HCl (5 mg/kg W)	2.0	4.2	6.3	12.3	21.7	29.0	36.3	45.3	53.3	58.9	251.9
Standard Error of the Treatment Mean	2.0	2.2	2.7	3.0	4.1	4.5	4.5	5.2	5.9	6.0	18.3

[1] W, body weight

[2] Stock by drug interaction was not significant at any observation period.

[3] The RC chicks were 28 days-of-age and had a mean body weight of 858g. The SCWL chicks were 56 days-of-age and had a mean body weight of 806g.

[a] Significant stock effect ($P \leq .05$) at this observation period.

[b] Significant drug effect ($P \leq .05$) at this observation period.

TABLE 3. Mean cumulative food intake (g/kg W[1]) by Rock-Cornish (RC) and Single-Comb White Leghorn (SCWL) cockerels of equivalent age following intramuscular injection of saline or naloxone hydrochloride (Exp.2)

	Time (min)										
Main Effects [2]	30	60	90	120	150	180	210	240	270	300	1440
Stock Effect: [3]											
RC	7.1[a]	9.1[a]	11.5[a]	14.5[a]	18.0[a]	20.3[a]	22.3[a]	24.7[a]	27.8[a]	30.3[a]	108.8[a]
SCWL	11.0	13.9	18.0	22.5	25.9	29.7	32.5	36.0	39.2	42.2	140.8
Drug Effect:											
Saline	11.0[b]	13.9[b]	17.3[b]	21.2[b]	24.4[b]	27.7[b]	30.5[b]	33.3[b]	37.0[b]	39.7[b]	128.2
Naloxone HCl (5 mg/kg W)	7.1	9.1	12.2	15.8	19.5	22.3	24.3	27.4	30.0	32.8	121.4
Standard Error of the Treatment Mean	.5	.7	.8	.8	.8	.8	.9	1.0	1.2	1.2	3.1

[1] W, body weight.

[2] Stock by drug interaction was not significant at any observation period.

[3] The RC chicks were 35 days-of-age and had a mean body weightof 1171g.
The SCWL chicks were 35 days-of-age and had a mean body weight of 396g.

[a] Significant stock effect ($P \leq .05$) at this observation period.

[b] Significant drug effect ($P \leq .05$) at this observation period.

TABLE 4. Mean cumulative water intake (ml/kg W[1]) by Rock-Cornish (RC) and Single-Comb White Leghorn (SCWL) cockerels of equivalent age following intramuscular injection of saline or naloxone hydrochloride (Exp. 2)

	Time (min)										
Main Effects [2]	30	60	90	120	150	180	210	240	270	300	1440
Stock Effect: [3]											
RC	2.9	7.7	13.9	19.2	25.1[a]	32.7[a]	35.2[a]	40.4[a]	46.3[a]	53.2[a]	228.8[a]
SCWL	2.3	8.0	16.7	25.9	37.1	46.6	48.5	55.0	60.1	70.7	291.4
Drug Effect:											
Saline	2.7	11.1[b]	18.2	27.3[b]	34.3	43.1	45.2	51.2	57.2	67.1	266.1
Naloxone HCl (5 mg/kg W)	2.5	4.6	12.4	17.8	27.9	36.2	38.5	44.2	49.2	56.8	254.1
Standard Error of the Treatment Mean	1.0	1.6	2.5	3.0	2.8	3.4	3.3	3.6	4.5	5.2	19.8

[1] W, body weight.

[2] Stock by drug interaction was not significant at any observation period.

[3] The RC chicks were 35 days-of-age and had a mean body weight of 1171g. The SCWL chicks were 35 days-of-age and had a mean body weight of 396g.

[a] Significant stock effect ($P \leq .05$) at this observation period.

[b] Significant drug effect ($P \leq .05$) at this observation period.

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CHAPTER 3

NALOXONE ATTENUATES FOOD BUT NOT WATER INTAKE IN JAPANESE QUAIL (*COTURNIX COTURNIX JAPONICA*)

INTRODUCTION

Endogenous opioid peptides have been implicated as regulators or modulators of ingestive behavior in a variety of mammalian species including pigs (Baldwin and Parrott, 1985), sheep (Baile *et al.*, 1981), monkeys (Herling, 1981) and humans (Atkinson, 1982; Trenchard and Silverstone, 1983). The most extensive characterization has been conducted with rodents, where it was shown that opioid peptides stimulate ingestive behaviors while opioid antagonists attenuate such behaviors (Holtzman, 1974, 1975; Grandison and Guidotti, 1977). While both food and water intake are influenced by opioids, water intake seems to be more sensitive to the suppressant effects of opioid antagonists (Brown and Holtzman, 1979).

The role of opioids in the regulation of food and water intake in *Aves* is less well defined. In the pigeon, the opioid peptide β-endorphin stimulates food intake (Deviche and Schepers, 1984a) while the opioid antagonist naloxone attenuates food intake (Deviche and Schepers, 1984b). Water intake in the pigeon seems to be independent of opioid regulation (Deviche and Schepers, 1984a,b). In the domestic fowl, however, both food and water intake were decreased by naloxone (McCormack and Denbow, 1987 - Chapter 1). In Japanese quail (*Coturnix coturnix japonica*), intracranial injection of [Leu^5]-enkephalin depressed water intake (Uemura *et al.*, 1983; 1984). Administration of naloxone (3 mg/bird; 95 to 105g birds; ip) blocked the drinking attenuation produced by [Leu^5]-enkephalin and when administered alone substantially increased drinking. Therefore, opioid regulation of ingestive behavior in Japanese quail seems to be an anomaly and should prove useful for contrasting with opioid regulatory systems in other species. The purpose of the present study was to characterize the effects of the opioid antagonist naloxone on both food and water intake in Japanese quail.

MATERIALS AND METHODS

Animals. Male Japanese quail were reared in heated batteries until 4 weeks of age and then transferred to individual cages. The chicks were provided starter crumbles [26.7 % protein, 3047 kcal/kg metabolizable energy (ME)] and water *ad libitum* and were exposed to continuous light. Quail were adapted to the individual cages and handling for a minimum of 2 weeks prior to testing.

Procedure. Ninety-six birds were randomly assigned to four treatment groups consisting of 0, 3, 10, and 30 mg/kg body weight naloxone HCl (Endo Laboratories, Inc. Garden City, N.Y.) in a randomized complete block design with cage tier used as the blocking factor. The 3 and 10 mg/kg body weight doses are similar to those found to attenuate ingestive behavior in the chicken (McCormack and Denbow, 1987 - Chapter 1) while the 30 mg/kg body weight dose is similar to that used by Uemura *et al.* (1983; 1984) to examine opioid regulation of drinking behavior in Japanese quail. Physiological saline (.9 % NaCl) served as a control solution and as a vehicle for administration of naloxone. All injections were administered intramuscularly in a total volume of .5 ml. Immediately prior to initiating the experiment, feeders and waterers were removed and replenished, and chicks were weighed to the nearest .1 g. Injections were administered in random order into the pectoral muscle 15 min prior to the return of food and water. Food and water intake were measured to the nearest .1 g at 30, 60, 120, 180, 240 and 300 min following the return of food and water.

Statistical Analysis. Cumulative food and water intake at each observation period was analyzed using analysis of variance. The statistical model was:

$$Yijk = u + Bi + Tj + (BT)ij + eijk$$

Where, B, T and (BT) represent block, treatment, and block by treatment interaction, respectively. The block by treatment interaction was not significant at any time period; therefore, the variation attributable to the interaction was pooled with the overall error. Dose-relationships at each observation period were evaluated using linear and quadratic contrasts (Steel and Torrie, 1980). Significance implies $P \leq .05$.

RESULTS AND DISCUSSION

Naloxone attenuated food intake in a dose-dependent manner through 300 min following the return of food and water (Table 1). The dose-response relationship was quadratic with the 10 mg/kg body weight dose being the most effective. A similar "u"-type dose-response to naloxone was observed in chickens (McCormack and Denbow, 1987 - Chapter 1) in which a maximum attenuation of food intake was attained at a dose of 5 mg/kg body weight. The response to this opioid antagonist is the inverse to that typically observed with opioid agonists which cause an inverted "u" response (Morley and Levine, 1983; Gordon *et al.*, 1984). Naloxone did not affect drinking behavior at any of the levels tested (Table 2).

The possibility exists that naloxone may have reduced food intake by non-specific actions such as inducing malaise or impairing motor function. However, no overt signs of malaise or alterations in motor function were observed. Furthermore, if reductions in food intake were attributable to non-specific effects the expectation would be for drinking to be affected as well. This was not the case. Therefore, it seems that food, but not water, intake in Japanese quail is influenced by an opioid, naloxone-sensitive mechanism.

Several lines of evidence support a role for endogenous opioid peptides as regulators or modulators of feeding behavior in *Aves*. First, endogenous opioids are present in birds. Endogenous opioid peptides have been isolated and sequenced from the pituitary of both the ostrich and turkey (Chang *et al.*, 1980; Naude *et al.*, 1980, 1981). Second, naloxone attenuates feeding in Japanese quail, pigeons (Deviche and Schepers, 1984b) and the domestic fowl (McCormack and Denbow, 1987 - Chapter 1). Lastly, intracerebroventricular injection of ostrich β-endorphin in the pigeon stimulates food intake (Deviche and Schepers, 1984a).

The effects of opioid agonists and antagonists on water intake in *Aves* are less consistent than their effects on food intake. Water intake in pigeons seems to be independent of opioid regulation (Deviche and Schepers 1984a,b) while drinking behavior in the domestic fowl is attenuated by naloxone (McCormack and Denbow, 1987 - Chapter 1). Uemura *et al.* (1983; 1984) reported that in Japanese quail opioid agonists inhibit drinking and opioid antagonists stimulate water intake, results opposite to that found in both mammals and other birds. The data obtained in the

present investigation demonstrate that water intake in Japanese quail, like that of the pigeon, is insensitive to naloxone. The discrepancy between our results and those of Uemura *et al.* (1983; 1984) may be due to genetic differences in the stocks of quail used, experimental protocol or physiological condition of the subjects. Nonetheless, we find no evidence to indicate that endogenous opioid peptides are involved in the regulation of water intake in Japanese quail.

SUMMARY

The effect of the opioid antagonist naloxone on food and water intake in male Japanese quail (*Coturnix coturnix japonica*) was investigated. Birds were injected intramuscularly with 0, 3, 10 and 30 mg/kg body weight of naloxone hydrochloride. Food and water were offered *ad libitum* 15 min post-injection. Food intake was attenuated in a dose-dependent fashion through 300 min following the return of food and water. The dose-reponse relationship was quadratic with the greatest reduction in food intake occuring at the 10 mg/kg body weight dose. Water intake was not affected by naloxone. The results demonstrate that endogenous opioid peptides are involved in the regulation of food, but not water, intake in Japanese quail.

Chapter 3 has been accepted for publication in Poultry Science.

TABLE 1. Mean cumulative food intake (g) by male Japanese quail (Coturnix coturnix japonica) following intramuscular injection of saline or naloxone hydrochloride

	Time (min)					
Treatment [1]	30	60	120	180	240	300
Saline	.65	.93	1.71	2.40	3.07	3.85
Naloxone HCl (3 mg/kg W[2])	.37	.68	1.39	2.06	2.59	3.34
Naloxone HCl (10 mg/kg W)	.16	.35	.98	1.52	2.10	2.81
Naloxone HCl (30 mg/kg W)	.21	.52	1.16	1.91	2.60	3.41
Standard error of the treatment mean	.10	.12	.17	.20	.23	.27
F value of contrasts						
Linear	7.22*	3.58	3.69	1.64	.76	.34
Quadratic	8.55*	7.96*	7.08*	7.94*	8.26*	7.05*

[1] All treatments were administered intramuscularly in a total volume of .5 ml.

[2] W, body weight.

* Critical F (1, 85) = 3.97, P ≤ .05.

TABLE 2. Mean cumulative water intake (ml) by male Japanese quail (Coturnix coturnix japonica) following intramuscular injection of saline or naloxone hydrochloride

	Time (min)					
Treatment [1]	30	60	120	180	240	300
Saline	2.00	3.11	4.72	6.54	7.86	9.14
Naloxone HCl (3 mg/kg W[2])	1.87	2.81	4.85	6.48	7.84	9.64
Naloxone HCl (10 mg/kg W)	2.09	2.88	4.94	6.45	7.69	9.40
Naloxone HCl (30 mg/kg W)	2.12	3.25	5.00	6.54	7.68	9.15
Standard error of the treatment mean	.29	.36	.54	.72	.83	1.00
F value of contrasts [3]						
Linear	.22	.37	.12	0	.03	.03
Quadratic	.02	.37	.04	.01	.01	.04

[1] All treatments were administered intramuscularly in a total volume of .5 ml.

[2] W, body weight.

[3] Critical F (1, 85) = 3.97, $P \leq .05$.

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CHAPTER 4

OPIOID REGULATION OF INGESTIVE BEHAVIOR IN THE DOMESTIC FOWL: CENTRAL *vs* PERIPHERAL NERVOUS SYSTEM SITES OF ACTION

INTRODUCTION

The hypothesis that endogenous opioid peptides have an important function in the physiological mediation of ingestive behavior is supported by a considerable body of research. Most investigations have utilized synthetic or endogenous opioid receptor ligands to examine the influence of opioid receptor stimulation or blockade on food and water intake. One of the more common techniques has been the use of pure narcotic antagonists such as naloxone and naltrexone. These compounds bind to opioid receptors and, while blocking the effects of opioid agonists, elicit little if any effect of their own (Blumberg and Dayton, 1974). Therefore, the resultant attenuation of ingestive behavior is believed to be a result of interruption in the activity of endogenous opioid systems.

Receptors at which these antagonists can exert their effects have been found in the central nervous system as well as peripheral tissues such as the pituitary, myenteric plexus of the gastrointestinal tract and the adrenal medulla (Pasternak and Childers, 1983). The typically used tertiary forms of opioid antagonists are non-polar and lipid soluble and, therefore, readily traverse the blood-brain barrier. However, it is the ability of these compounds to cross the blood-brain barrier which has precluded the determination of location(s) at which they influence ingestion. One solution to this problem has been the use of quaternized opioid antagonists. These quaternary derivatives contain an additional methyl group on the nitrogen atom in the ring structure resulting in greater polarity and reduced lipid solubility. The reduction in lipid solubility, in turn, greatly reduces their ability to traverse the blood-brain barrier and, therefore, limits their activity to sites outside the blood-brain barrier (Brown and Goldberg, 1985).

Additionally, quaternization reduces the affinity of opioid antagonists for opioid receptors. Quaternary opioid antagonists have been estimated to bind to opioid receptors with only 2 to 4

% the affinity of their tertiary congeners (Killian *et al.*, 1981; Valentino *et al.*, 1983). The substantially lower receptor affinity may be attributed to the stearic hinderence caused by the addition of the methyl substituent or perhaps, to a loss in hydrogen binding potential (Opheim and Cox, 1976). Therefore, to achieve an equivalent degree of antagonism greater quantities of quaternary opioid antagonist are required (Brown and Goldberg, 1985). Brown and Goldberg (1985) have suggested that a prime consideration in studies comparing tertiary and quaternary opioid antagonists should be that the comparison be made at levels which reflect their differential affinity for opioid receptors.

Few studies have been conducted comparing the effects of tertiary and quaternary antagonists on ingestive behavior. Brown and Holtzman (1981) administered tertiary and quaternary antagonists to 24-hr food-deprived rats. Subcutaneous (sc) injection of tertiary naloxone and naltrexone, dose-dependently reduced water intake while their quaternary congeners failed to affect drinking. Upon intracerebroventricular (ICV) administration, the quaternary derivatives suppressed drinking. Similar observations were made by Cooper and Turkish (1983) who examined the effects of tertiary and quaternary naloxone on the consumption of water, saline and saccharin solutions in water- deprived rats. Quaternary naloxone (sc) had no affect on fluid intake while its tertiary counterpart (sc) caused a dose-dependent suppression of all solutions. In contrast, Hemmer *et al.* (1982) observed an attenuation of drinking when 50 mg/kg body weight quaternary naloxone was administered intraperitoneally (ip).

Feeding in rats also seems to be insensitive to peripheral administration of quaternary naloxone. Carr and Simon (1983) tested the effects of tertiary and quaternary naloxone on the frequency threshold for stimulation-induced feeding in rats. Rats were stimulated to feed by electrical stimulation of the lateral hypothalamus. Tertiary naloxone produced a dose-dependent elevation of the frequency threshold while quaternary naloxone had no effect.

Endogenous opioids have been demonstrated to influence food and water intake in *Aves* (Deviche and Schepers, 1984a, 1984b; McCormack and Denbow, 1987 - Chapter 1). Additionally, the depression in water intake has been shown to be independent of the effects on food intake (McCormack and Denbow, 1987 - Chapter 1). However, the site(s) at which opioid antagonists

exert their effects have not been elucidated. Deviche *et al.* (1984) compared the effects of tertiary and quaternary naloxone in pigeons. Intramuscular injection of tertiary naloxone decreased food intake in pigeons while an equimolar dose of quaternary naloxone was without effect. However, this may be an expected result given the difference in potency between equimolar doses of the congeners. Therefore, the objective of the present investigation was to determine if the actions of opioid antagonists in the domestic fowl are mediated at sites within the central and/or peripheral nervous system.

MATERIALS AND METHODS

Animals. Commercial Rock-Cornish (RC) cockerels were reared in heated batteries until 3 weeks of age and then transferred to individual cages. The chicks were provided starter mash [20 % protein, 2864 kcal/kg metabolizable energy (ME)] and water *ad libitum* and exposed to continuous light. Birds were adapted to the individual cages and handling for a minimum of one week prior to testing.

Experiment 1. The effect of tertiary (naloxone hydrochloride, NHCl; Endo Laboratories, Inc., Garden City, NY) and quaternary naloxone (naloxone methobromide, NMBr; Boehringer Ingelheim, Ingelheim, FRG) on food and water intake were compared using six-week old RC cockerels. Fifteen chicks (5/replicate) received each of five treatments, i.e., 0, 25, 50, 100 mg/kg body weight NMBr and 2.5 mg/kg body weight NHCl, in a replicated 5 x 5 Latin square design with birds and days used as blocking factors. The levels of tertiary and quaternary naloxone used were equilibrated for molarity and receptor affinity. Because quaternary antagonists exhibit only 1 to 4 % the receptor affinity of their tertiary congeners (Brown and Goldberg, 1985) an intermediate estimate of 2.5 % was chosen for equilibration. Therefore, 2.5 mg/kg body weight of NHCl may be considered equipotent to 100 mg/kg body weight of NMBr. Physiological saline (0.9 % NaCl) served as a control solution and as a vehicle for the administration of the antagonists. All injections were made intramuscularly in a total volume of 2 ml.

Immediately prior to initiating the experiment, feeders and waterers were removed and chicks were weighed to the nearest 1 g. Injections were administered into the pectoral muscle 15

min prior to the return of food and water. Food and water intake were measured at 15, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 1440 min following the return of food and water.

Experiment 2. In this experiment the effect of tertiary naloxone on food and water intake was evaluated when administered intracerebroventricularly (ICV). Five-week old RC cockerels were anesthetized with sodium pentobarbitol (25 mg/kg body weight, intravenously; iv) and a 23-guage thin-walled stainless steel guide cannula was stereotaxically implanted into the right lateral cerebral ventricle by the method of Denbow *et al.* (1981). Cannula location was verified by welling of cerebrospinal fluid (CSF) in the guide cannula during surgery and by post-surgical testing with norepinephrine (67 ug, ICV) (Denbow *et al.*, 1981). A one-week recovery period was allowed prior to initiating experimentation.

Chicks were housed in individual cages and provided food and water *ad libitum.* Sixteen chicks (4/replicate) received each of 4 treatments, i.e., 0, 10, 20, and 50 ug NHCl, in a replicated 4 x 4 Latin square design with birds and days used as blocking factors. Immediately prior to initiating the experiment, feeders and waterers were removed and chicks were weighed to the nearest 1 g. All injections were administered ICV in a total volume of 10 ul with artificial cerebrospinal fluid (aCSF; Anderson and Heisey, 1972) serving as a control solution and as a vehicle for the administration of the antagonist. Food and water intake were measured as in Experiment 1.

Experiment 3. This experiment was conducted to determine if the effects observed following the ICV administered NHCl were due to actions outside the central nervous system. The experiment was conducted similar to Experiment 2 except that injections were made into the pectoral muscle 15 min prior to the return of food and water. Eight chicks (4/replicate) were used and the injection volume was 1 ml.

Statistical Analysis. In all experiments, mean cumulative food and water intake within each observation period were analyzed using analysis of variance. Dose-response relationships (NMBr treatments in Experiment 1) within each observation period were evaluated using orthogonal linear and quadratic contrasts (Steele and Torrie, 1980). In Experiment 1, means within each observation period were separated by Duncan's multiple range test (Duncan, 1955). The statistical models for analysis of variance were as follows:

Experiment 1. $Yijkl = \mu + Ri + B(i)j + D(i)k + T(l) + eijkl$

Experiment 2 and 3. $Yijkl = \mu + Ri + B(i)j + Dk + T(l) + eijkl$

Where R, B, D, and T represent the replicate, bird, day and treatment, respectively.

RESULTS AND DISCUSSION

The im administration of tertiary naloxone or quaternary naloxone (Experiment 1) significantly decreased food and water intake (Table 1 and 2). Quaternary naloxone significantly attenuated food intake in a linear fashion from 15 through 300 min following the return of food and water. As evidenced by a significant quadratic contrast some curvature in the response to quaternary naloxone was detected at 180 min. Tertiary naloxone also induced a significant decline in food intake from 15 through 300 min. At levels of tertiary naloxone and quaternary naloxone balanced for potency, 2.5 and 100 mg/kg body weight, respectively, quaternary naloxone was significantly more effective than tertiary naloxone in attenuating food intake. Furthermore, the attenuation of food intake produced by tertiary naloxone was not significantly different from that induced by administration of 50 mg/kg body weight quaternary naloxone from 15 through 300 min or that produced by 25 mg/kg body weight quaternary naloxone at 15 min and from 90 through 300 min. There were no differences in food intake at 1440 min.

Quaternary naloxone significantly attenuated water intake in a linear manner from 30 through 300 min following the return of food and water. A significant quadratic response was observed at 15 min and from 90 through 150 min. The quadratic response at 15 min was different from the quadratic response at latter time periods. At 15 min the low and intermediate quaternary naloxone groups drank more water than either the control or the 100 mg/kg body weight quaternary naloxone treatments. This relationship seems to be the result of low water intake on the part of the control group and perhaps due to the high food consumption of that group during this initial time period. The curvilinear nature of the response from 90 through 300 min is more typical of the effects of opioid antagonists on intake with the response plateauing at higher levels of quaternary naloxone. Equipotent doses of the quaternary naloxone and tertiary naloxone did not differ in their

effectiveness in suppressing drinking behavior. In addition, there was no significant difference in the attenuation produced by the lower doses of quaternary naloxone and tertiary naloxone. No treatment differences in water intake were observed at 1440 min.

While it is possible that quaternary naloxone may have attenuated food and water intake through the production of malaise or impairment of motor function, there were no overt symptoms of illness, discomfort or motor function problems. Furthermore, the depression in food and water intake induced by quaternary naloxone was of the same magnitude as that induced by 2.5 mg/kg body weight of tertiary naloxone. This low level of tertiary naloxone is generally considered to produce no ill effects (Segal *et al.*, 1979). Therefore, if quaternary naloxone was suppressing intake by malaise or motor impairment a more dramatic difference between the intake of quaternary and tertiary treatments would be expected.

In the few studies comparing the effects of tertiary and quaternary opioid antagonists on ingestive behavior peripherally administered quaternary antagonists have generally been without effect (Brown and Goldberg, 1985). In these studies, the tertiary and quaternary antagonists were administered at equimolar levels or at levels above equimolar but still below equipotent. For example, Brown and Holtzman (1981) compared the effect of 1 mg/kg body weight tertiary naloxone with 10 mg/kg body weight quaternary naloxone on drinking in rats. The tertiary form significantly suppressed drinking while the quaternary form was ineffective. In another study, Hemmer *et al.* (1982) compared the effects of equimolar and equipotent levels of peripherally administered tertiary and quaternary naloxone on food and water intake in rats. An equimolar dose of quaternary naloxone was ineffective in attenuating food or water consumption while an equipotent dose produced a significant suppression of drinking. The authors attributed the suppression in drinking to possible movement of quaternary naloxone across the blood-brain barrier and action at sites within the central nervous system. However, Smith *et al.* (1982) examined the ability of radiolabled quaternary morphine and nalorphine to cross the blood-brain barrier and observed that the two quaternary derivatives did not penetrate the blood-brain barrier and did not accumulate within the central nervous system to any appreciable extent at levels up to 22.5 mg/kg body weight. While it

may be possible for higher levels of quaternary compounds to traverse the blood-brain barrier there is little evidence to support this hypothesis.

The attenuation of food and water intake produced by quaternary naloxone appears to be the result of action at sites outside the blood-brain barrier. The very highly significant linear dose-response indicates that the attenuation of food and water intake occurred over the entire dose range. Secondly, even at the lowest level of quaternary naloxone (25 mg/kg body weight; 10 times the tertiary dose) a significant reduction in feeding and drinking was observed. In the rat, a quaternary to tertiary naloxone ratio of 10 to 1 has been ineffective in altering ingestive behavior (Brown and Holtzman, 1981). The most compelling evidence that quaternary naloxone is acting at peripheral sites is provided by the results of Experiment 2 and 3. Experiment 2 examined the effect of ICV administered tertiary naloxone on food and water consumption. ICV injection of tertiary naloxone produced a significant linear suppression of water intake from 15 through 60 min post-injection while having no effect on food intake (Tables 3 and 4). In Experiment 3 these effects were confirmed to be actions of tertiary naloxone within the central nervous system and not to movement of the compound across the blood-brain barrier to sites in the periphery. The im administration of tertiary naloxone at levels found to attenuate water intake when injected ICV did not effect either food or water consumption (Tables 5 and 6). Therefore, if quaternary naloxone was altering food intake by traversing the blood-brain barrier and acting at sites within the central nervous system it would be expected that ICV injection of an opioid antagonist would also attenuate feeding. However, the absence of a central response to tertiary naloxone does not totally exclude the possibility that endogenous opioids influence food intake in the central nervous system. It is possible that the delta opioid receptor, for which naloxone has relatively low affinity (Lord *et al.* 1977), may be involved in food intake regulation while receptor subclasses for which naloxone has higher affinity regulate water intake. Nonetheless, if tertiary naloxone fails to alter food intake centrally it seems improbable that peripherally administered quaternary naloxone would achieve high enough central concentrations to affect feeding.

GENERAL DISCUSSION

The attenuation of food and water consumption produced by the intramuscular injection of quaternary naloxone demonstrates that ingestive behavior in the domestic fowl is at least, in part, mediated at sites outside the blood-brain barrier. In order for quaternary naloxone to influence food intake at sites outside the blood-brain barrier there must be peripheral tissue(s) which are associated with food ingestion and whose function is influenced by opioids. The gastrointestinal tract meets these criteria and may, therefore, be a possible site of action. The gastrointestinal tract has a substantial degree of enkephalin releasing neurons, receptors and cell bodies (Polak *et al.*, 1977; Epstein *et al.*, 1981; Epstein and Dahl, 1982). Receptors in the gastrointestinal tract are predominately of the mu and delta subtypes (Lord *et al.*, 1976, 1977). The actions of opioids on intestinal motility differ among species (Olson *et al.*, 1985). Administration of mu and delta agonists increases motility in the canine (Hirning *et al.*, 1984) while opioids decrease gastrointestinal motility in rats, (Galligan *et al.*, 1984), mice (Pillai and Bhargava, 1984) and sheep (Ruckebusch *et al.*, 1984). In general, enteric opioid action appears to be spasmogenic, increasing the tone of the intestinal wall and sphincters and decreasing intestinal secretions (Polak et al., 1977; Ambinder and Schuster, 1979). Since these actions are antipropulsive it is unlikely that opioids would stimulate feeding via their influence on gastrointestinal motility. However, the effects of opioids on intestinal motility in birds has not been investigated.

An alternative scenario for the action of opioids at the level of the gastrointestinal tract is their possible interaction with enteric hormones such as cholecystokinin (CCK). Faris (1985) proposed reciprocal roles for endogenous opioid peptides and CCK in the regulation of energy balance and food intake. According to this hypothesis satiety and perhaps many of the behavioral effects of CCK are due to CCK's ability to block the actions of endogenous opioids. Support for this hypothesis has come from studies in which CCK blocked opioid-induced analgesias, but not non-opioid analgesia (Faris *et al.*, 1983). Morphine-induced analgesia is also potentiated by autoimmunization against CCK (Faris *et al.*, 1984). Furthermore, Schiller *et al.* (1978) reported that a des-CCK-7 is a specific ligand of opioid receptors. Opioids also antagonize the actions of CCK. In mice, the satiety effect of cerulein, a peptide closely related to CCK, was antagonized by

the enkephalin analogue FK 33-824 and enhanced by naloxone (Zetler and Morsdorf, 1984). Similarly, pretreatment of rats with morphine reduced, whereas pretreatment naloxone enhanced CCK-induced satiety (Wilson *et al.*, 1983). A problem which has confronted the theory of interaction between opioids and CCK has been that the peptides appear to have different sites of action. For example, in the rat, CCK-induced satiety appears to be mediated at peripheral sites (Smith *et al.*, 1981), whereas opioid stimulation of feeding seems to be mediated at sites within the central nervous system (Leibowitz and Hor, 1982). However, in the fowl, CCK attenuates food intake peripherally (Savory and Gentle, 1980), and as the present investigation suggests, opioids appear to stimulate feeding peripherally. Therefore, in the fowl, opioids and CCK may reciprocally influence feeding by interaction at the level of the gastrointestinal tract.

Opioids may possibly influence food intake peripherally by actions on the endocrine pancreas. The presence of enkephalin-like- immunoreactivity in the pancreas was originally demonstrated by Polak *et al.* (1977). β-endorphin, β-lipotropin, [Leu^5]-enkephalin and [Met^5]-enkephalin have subsequently been detected by immunohistochemical techniques in the rat and human pancreas and localized in the islets of Langerhans by immunoflourescent techniques (Grube *et al.*, 1978; Bruni *et al.*, 1979). Evidence for a direct action of opioids on the endocrine pancreas was shown by Ipp *et al.* (1978) where β-endorphin and morphine inhibited somatostatin release and increased insulin release. Green *et al.* (1980) demonstrated that opioid peptides may have both stimulatory and inhibitory effects on insulin and glucagon secretion depending on the concentration of the peptide.

Further evidence of a close association between pancreatic hormones and opioids has been found in studies by Recant *et al.* (1984) using models with abnormal glucoregulatory conditions. The pancreata of genetically obese (*ob/ob*) mice, which are hyperphagic and hyperinsulinemic, contained higher levlels of opioids than lean mice. In addition, naloxone blocked the release of insulin in *ob/ob* mice but not their lean controls. Hypoinsulinemia in mice is associated with a 63 to 73 % decline in pancreatic bioactive opioids. Immunocytochemical analyses of pancreata from normal mice revealed that enkephalin-immunoreactivity, like insulin, was distributed throughout the islets of Langerhans. No definitive opioid staining was observed in the pancreases of

hypoinsulinemic animals. However, hypoinsulinemia was associated with increased levels of somatostatin. Although, a direct causal relationship between opioid stimulation of insulin secretion and/or inhibition of somatostatin secretion has not been clearly established there appears to be considerable circumstantial evidence supporting the possibility that opioids may induce alterations in the these hormones. Furthermore, it is well established that peripheral administration of insulin will induce eating (Silverstone and Kyriakides, 1982) and that somatostatin acts peripherally to inhibit food intake (Lotter *et al.*, 1981). Therefore, it seems plausible that opioids may act peripherally to alter the levels of these hormones which, in turn, influence food consumption.

Based on the present investigation there appears to be both a peripheral and central components to endogenous opioid mediation of water intake in the domestic fowl. The attenuation of water intake induced by im administration of quaternary naloxone indicates that peripheral tissues may be involved while the attenuation of drinking produced by ICV administration of tertiary naloxone indicates that there is also a central component. However, these observations are complicated by the possible involvement of circumventricular structures such as the subfornical organ (SFO) or the organum vasculosum laminae terminalis (OVLT). These structures are not isolated from the peripheral circulation by a blood-brain barrier and have been shown to be associated with drinking behavior in birds (de Caro and Massi, 1983). Therefore, it is possible that peripherally administered quaternary naloxone may act at these structures to influence water consumption. Nonetheless, the present study raises the possibility that water consumption may be influenced by opioids in the periphery.

As with peripheral opioid regulation of food intake a scenario for peripheral opioid influence on water intake requires tissues which may be influenced by opioids and which are involved in water balance. One possible site of action is the kidney, which contains high affinity opioid binding sites (Simantov *et al.*, 1978). A number of opioids possess strong antidiuretic action upon iv administration (Bisset *et al.*, 1978; Tseng *et al.*, 1978). The mode of action of these peptides has not been clearly elucidated but it has been theorized that opioids bind to the smooth muscle of the renal vasculature (Szekely, 1982). Therefore, a small dilation of the arteriolae efferens or constriction of the arteriolae afferens would induce changes in renal blood pressure and increase the

activity of the renin-angiotensin system which, in turn, could induce polydipsia. A second peripheral tissue which may also be involved with increased water intake is the adrenal gland. There is evidence indicating that opioids play a role in the regulation of aldosterone production in animals and man (Palmore and Mulroe, 1967; McCaa *et al.*, 1974; Brown *et al.*, 1979; Sen *et al.*, 1981). β-endorphin and the [Met^5]-enkephalin analogue FK 33-824 have been found to increase aldosterone secretion both *in vitro* and *in vivo* (Bevilacqua *et al.*, 1982; Gullner and Gill, 1983; Loche *et al.*, 1983). The stimulatory effects of FK 33-824 on aldosterone secretion was blocked by naloxone, suggesting the effect was mediated by opioid receptors. However, Loche *et al.* (1984) were unable to inhibit the release of aldosterone by administration of naloxone alone suggesting that the steroidogenic properties of opioids may not be linked to their opioid activity but perhaps to action at a different receptor site or by inducing the synthesis of another factor which, in turn, increases aldosterone. If indeed aldosterone synthesis and release are increased by opioids it is possible that increases in plasma osmolarity and polydipsia may occur. However, increases in plasma osmolarity following opioid administration has not been demonstrated.

The actual peripheral tissues at which quaternary naloxone may be exerting its effects on food and water intake are unknown. The aforementioned proposed sites of action extend from a limited number of studies examining the effects of opioids on peripheral tissues. It is possible that peripheral opioid mechanisms play a more important role in ingestive behavior regulation in birds than in mammals. Most of the possible sites of action, i.e., gastrointestinal tract, pancreas, and kidney, are structures in which there are anatomical and(or) physiological differences between birds and mammals (see Sturkie, 1986). Whether or not these differences account for a greater or different role for opioids in peripheral ingestive regulation in birds is not known. Further investigation will be needed to evaluate the importance of opioids on the functioning of these tissues and the regulation ingestive behavior.

SUMMARY

Three experiments were conducted to determine whether opioid regulation of ingestive behavior in the domestic fowl is mediated at sites within the central nervous system or peripheral tissues. In the first experiment, food and water intake as affected by two opioid antagonists differing in their ability to cross the blood-brain barrier was investigated. Rock-Cornish (RC) cockerels were injected intramuscularly (im) with naloxone hydrochloride (NHCl) or naloxone methobromide (NMBr) which have high and low ability, respectively, to cross the blood-brain barrier. NHCl at 2.5 mg/kg body weight was compared with three levels of NMBr, 25, 50 and 100 mg/kg body weight, the highest of which was equilibrated for molarity and receptor affinity with NHCl. NMBr and NHCl induced a significant depression in food intake from 15 through 300 min following the return of food and water. At equipotent levels, NMBr was significantly more effective in suppressing food intake than NHCl. NMBr attenuated water intake from 30 through 300 min while NHCl decreased drinking from 45 through 300 min. There were no differences in efficacy between equipotent levels of the congeners.

In the second experiment, RC cockerels were administered NHCl intracerebroventricularly (ICV) at levels of 0, 10, 25 and 50 ug. Water intake was significantly attenuated in a linear fashion from 15 through 60 min post-injection. No significant effect on food intake was noted. A third experiment was conducted to confirm that the attenuating effects of ICV administered NHCl were due to actions at sites within the central nervous system. NHCl was administered im at levels found to decrease water intake when injected ICV (Experiment 2). There was no effect on either food or water intake.

These results demonstrate that there is a significant peripheral component to opioid regulation of food intake while opioid regulation of water intake appears to be mediated at peripheral sites as well as within the central nervous system. These findings are discussed in relation to possible peripheral sites of action for opioids.

TABLE 1. Mean cumulative food intake (g) of Rock-Cornish cockerels following intramuscular injection of naloxone hydrochloride or naloxone methobromide (Exp. 1)

	Time (min)										
Treatment [1]	15	30	45	60	90	120	150	180	240	300	1440
Isotonic saline	7.3[a]	9.9[a]	11.5[a]	13.1[a]	16.5[a]	20.1[a]	22.6[a]	26.4[a]	33.5[a]	39.2[a]	133.7[a]
Naloxone MBr (25mg/kg W[2])	6.1[ab]	8.5[a]	9.5[a]	10.8[ab]	12.7[b]	16.2[b]	18.9[b]	21.7[b]	28.8[b]	34.1[b]	131.5[a]
Naloxone MBr (50mg/kg W)	3.7[c]	5.4[b]	7.0[b]	8.3[b]	11.1[bc]	14.2[bc]	15.9[bc]	18.5[bc]	26.0[bc]	30.6[bc]	134.9[a]
Naloxone MBr (100mg/kg W)	1.3[d]	2.9[c]	4.5[c]	5.7[c]	8.4[c]	11.7[c]	13.3[c]	16.5[c]	22.5[c]	28.7[c]	127.7[a]
Naloxone HCl (2.5mg/kg W)	4.6[bc]	5.9[b]	7.3[b]	8.7[b]	12.0[b]	16.1[b]	18.9[b]	22.1[b]	27.9[b]	33.6[b]	135.4[a]
Standard error of the treatment mean	.7	.8	.8	.8	1.0	1.0	1.1	1.2	1.3	1.4	3.4
F value of contrasts [3]											
Linear	49.25***	39.57***	46.26***	42.45***	33.61***	34.56***	36.53***	33.18***	35.16***	27.46***	1.36
Quadratic	0.22	0.37	0.87	1.00	2.17	2.81	2.61	4.58*	2.05	4.39*	0.55

[1] All treatments administered in a volume of 2ml.

[a,b,c,d] Means within an observation period followed by different superscripts differ significantly ($P \leq .05$).

[2] W, body weight.

[3] Dose relationship of 0, 25, 50 and 100mg/kg naloxone MBr treatments.

* Critical F (1, 44) $\cong$ 4.06, $P \leq .05$, therefore, these contrasts are significant.

*** Critical F (1, 44) $\cong$ 12.5, $P \leq .001$, therefore, these contrasts are very highly significant.

TABLE 2. Mean cumulative water intake (ml) of Rock-Cornish cockerels following intramuscular injection of naloxone hydrochloride or naloxone methobromide (Exp. 1)

	Time (min)										
Treatment [1]	15	30	45	60	90	120	150	180	240	300	1440
Isotonic saline	1.0[a]	5.0[a]	11.0[a]	18.0[a]	25.3[a]	31.7[a]	38.0[a]	44.3[a]	57.7[a]	70.7[a]	248.3[a]
Naloxone MBr (25mg/kg W[2])	2.3[a]	5.7[a]	9.0[ab]	10.0[b]	12.3[b]	16.7[b]	24.0[b]	31.0[b]	45.0[b]	57.7[b]	238.7[a]
Naloxone MBr (50mg/kg W)	2.7[a]	4.0[a]	5.3[bc]	10.0[b]	12.7[b]	17.3[b]	21.0[bc]	28.0[bc]	37.3[bc]	49.3[bc]	248.3[a]
Naloxone MBr (100mg/kg W)	1.3[a]	1.7[a]	2.7[c]	5.0[b]	7.0[c]	11.3[b]	15.3[c]	19.7[c]	31.7[c]	42.3[c]	240.3[a]
Naloxone HCl (2.5mg/kg W)	1.7[a]	2.7[a]	4.7[bc]	6.0[b]	9.3[bc]	16.0[b]	21.0[bc]	27.0[bc]	41.0[bc]	53.0[bc]	249.3[a]
Standard error of the treatment mean	.7	1.2	1.5	1.8	1.7	2.1	2.4	2.9	3.6	4.3	8.0
F value of contrasts [3]											
Linear	0.01	5.50*	16.99***	23.37***	48.05***	37.04***	39.13***	32.43***	25.03***	21.89***	0.21
Quadratic	4.28*	0.59	0.38	2.00	8.64**	7.73**	6.87*	2.76	3.38	2.32	0

[1] All treatments were administered in a volume of 2ml.

[2] W, body weight.

[a,b,c] Means within an observation period followed by different superscripts differ significantly ($P \leq .05$).

[3] Dose relationship of 0, 25, 50 and 100mg/kg naloxone MBr treatments.

* Critical F (1, 44) $\cong$ 4.06, $P \leq .05$, therefore, these contrasts are significant.

** Critical F (1, 44) $\cong$ 7.26, $P \leq .01$, therefore, these contrasts are highly significant.

*** Critical F (1, 44) $\cong$ 12.5, $P \leq .001$, therefore, these contrasts are very highly significant.

TABLE 3. Mean cumulative food intake (g) of Rock-Cornish cockerels following intracerebroventricular injection of naloxone hydrochloride (Exp. 2)

	Time (min)										
Treatment [1]	15	30	45	60	90	120	150	180	240	300	1440
aCSF [2]	8.9	11.6	14.1	16.1	18.8	22.1	25.7	28.6	35.3	41.8	136.2
Naloxone HCl (10μg)	7.8	10.2	12.9	14.5	18.4	21.6	25.0	28.6	33.9	40.3	138.9
Naloxone HCl (20μg)	8.9	10.9	13.7	15.1	19.4	23.1	27.4	29.8	34.1	41.4	141.8
Naloxone HCl (50μg)	7.7	9.8	12.4	14.2	17.8	21.8	24.6	27.9	34.4	41.0	139.1
Standard error of the treatment mean	0.8	0.8	1.0	1.1	1.2	1.2	1.4	1.5	1.6	1.6	3.3
F value of contrasts[3]											
Linear	0.79	1.75	1.23	0.97	0.34	0	0.24	0.11	0.03	0.02	0.24
Quadratic	0.01	0.11	0	0.14	0.31	0.32	1.02	0.51	0.31	0.05	1.13

[1] All treatments administered in a volume of 10μl.

[2] Artificial cerebrospinal fluid (aCSF) consisting of (in meq/l) Na^+, 155; K^+, 3.7; Ca^{+2}, 2.5; Mg^{+2}, 2.1; Cl^-, 140 and HCO_3^-, 23.

[3] Critical F (1, 46) $\cong$ 4.06, $P \leq .05$, therefore, there was no significant dose-response relationship at any time period.

TABLE 4. Mean cumulative water intake (ml) of Rock-Cornish cockerels following intracerebroventricular injection of naloxone hydrochloride (Exp. 2)

	Time (min)										
Treatment [1]	15	30	45	60	90	120	150	180	240	300	1440
aCSF [2]	8.9	17.8	25.3	31.3	40.6	50.6	55.9	67.2	83.1	105.6	419.7
Naloxone HCl (10μg)	9.4	18.4	24.7	30.0	38.4	49.4	58.8	70.0	77.2	107.2	410.3
Naloxone HCl (20μg)	6.6	13.4	19.7	30.0	41.9	53.4	60.6	71.3	89.7	111.6	430.3
Naloxone HCl (50μg)	4.1	9.7	18.1	21.6	32.5	42.2	49.7	62.8	80.9	103.4	415.6
Standard error of the treatment mean	1.7	2.5	2.6	2.9	3.1	3.7	3.8	4.4	5.9	5.4	13.9
F value of contrasts											
Linear	5.22*	7.11*	4.52*	6.51*	3.75	3.05	2.31	0.94	0	0.16	0
Quadratic	0.01	0.01	0.42	0.46	0.94	1.40	2.44	1.23	0.41	0.90	0.21

[1] All treatments administered in a volume of 10μl.

[2] Artificial cerebrospinal fluid (aCSF) consisting of (in meq/l) Na^+, 155; K^+, 3.7; Ca^{+2}, 2.5; Mg^{+2}, 2.1; Cl^-, 140 and HCO_3^-, 23.

* Critical F (1, 46) $\cong$ 4.06, P $\leq$ .05, therefore, these contrasts are significant.

TABLE 5. Mean cumulative food intake (mg) of Rock-Cornish cockerels following intramuscular injection of naloxone hydrochloride at levels effective in attenuating water intake when administered intracerebroventricularly (Exp. 3)

	Time (min)										
Treatment [1]	15	30	45	60	90	120	150	180	240	300	1440
Isotonic saline	8.4	11.4	12.9	13.9	16.8	20.3	23.4	26.8	30.9	38.1	128.9
Naloxone HCl (10μg)	9.3	12.3	14.0	15.1	18.9	22.1	25.5	28.4	32.1	37.5	126.1
Naloxone HCl (20μg)	7.9	11.6	13.6	15.5	19.1	21.9	25.2	29.8	33.5	39.9	135.5
Naloxone HCl (50μg)	6.9	9.4	10.8	11.9	15.6	18.9	22.1	25.0	30.8	38.0	128.9
Standard error of the treatment mean	1.0	1.0	1.1	1.1	1.6	1.7	1.7	1.6	1.5	1.7	4.2
F value of contrasts[2]											
Linear	2.07	3.37	3.35	2.93	0.88	0.92	1.02	1.25	0.08	0	0.03
Quadratic	0.12	1.05	1.80	3.32	2.57	1.44	2.01	3.37	1.92	0.41	0.86

[1] All treatments administered intramuscularly in a total volume of 1ml.

[2] Critical F (1, 18) $\cong$ 4.41, $P \leq .05$, therefore, there was no significant dose-response relationship at any time period.

TABLE 6. Mean cumulative water intake (ml) of Rock-Cornish cockerels following intramuscular injection of naloxone hydrochloride at levels effective in attenuating water intake when administered intracerebroventricularly (Exp. 3)

	Time (min)										
Treatment [1]	15	30	45	60	90	120	150	180	240	300	1440
Isotonic saline	6.9	10.0	17.5	21.9	34.4	40.6	50.0	58.1	68.8	99.4	334.4
Naloxone HCl (10μg)	11.3	20.0	23.8	29.4	45.6	53.8	66.3	76.9	89.4	92.5	331.3
Naloxone HCl (20μg)	9.4	12.5	20.0	25.6	34.4	48.1	54.4	61.3	73.8	103.8	357.5
Naloxone HCl (50μg)	10.0	13.8	16.3	24.4	39.4	46.3	56.3	63.8	83.1	102.5	347.5
Standard error of the treatment mean	3.7	3.9	4.2	3.8	5.0	5.8	6.0	6.3	7.0	10.0	12.4
F value of contrasts[2]											
Linear	0.13	0.01	0.41	0	0.04	0.03	0.01	0.01	0.74	0.19	0.84
Quadratic	0.22	0.56	0.75	0.90	0.05	1.26	0.66	0.58	0.28	0	0.78

[1] All treatments administered intramuscularly in a total volume of 1ml.

[2] Critical F (1, 18) $\cong$ 4.41, $P \leq .05$, therefore, there was no significant dose-response relationship at any time period.

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CHAPTER 5

INGESTIVE RESPONSES TO MU AND DELTA OPIOID RECEPTOR AGONISTS IN THE DOMESTIC FOWL

INTRODUCTION

The discovery of numerous opioid peptides and opioid receptor subtypes has led to vigorous investigation into their physiological functions. One function which has received considerable attention is the influence of opioids on ingestive behavior (Olson *et al.*, 1984). The role of opioids in the regulation of food and water intake is supported by studies using opioid antagonists, such as naloxone. Opioid antagonists bind to and block endogenous opioid actions at most opioid receptor subtypes. However, naloxone has relatively low affinity for the delta subtype (Lord *et al.*, 1977) and blockade of this opioid receptor subtype occurs only with relatively high levels of naloxone.

Opioid antagonists attenuate food and water intake in mammals and birds; however, in birds there are differences among species as to which behaviors are affected. In the pigeon (Deviche and Schepers, 1984) and Japanese quail (McCormack and Denbow, 1987b - Chapter 3) food, but not water, intake is attenuated by naloxone while in the domestic fowl, both food and water intake are suppressed by naloxone (McCormack and Denbow, 1987a - Chapter 1). The sites at which opioids influence ingestive behavior in birds have been studied using naloxone congeners with differing ability to traverse the blood- brain barrier (bbb). In the pigeon, systemic administration of quaternary naloxone, which does not cross the bbb, has no effect on ingestive behavior (Deviche *et al.*, 1984). However, systemic administration of quaternary naloxone in the domestic fowl attenuates food and water intake indicating that there is a peripheral component to opioid regulation of ingestive behavior (Chapter 4). Intracerebroventricular (ICV) administration of tertiary naloxone attenuates water intake but not food intake (Chapter 4). Therefore, food intake seems to be influenced by opioids at sites outside the bbb while water intake appears to be influenced at sites within and outside the bbb.

While naloxone's ability to block numerous opioid receptor subtypes is useful in establishing possible opioid influence on a behavior it also precludes detection of the identity of the receptor subtypes mediating a behavior. Therefore, to further understand how opioids influence ingestive behavior it is necessary to identify which receptor subtypes are involved.

The mu and delta subtypes are the prototypic opioid receptors and their binding properties and distribution are well characterized (Herz, 1984). Naloxone has the highest affinity for the mu opioid receptor. Therefore, its agonists would likely influence many of the same behaviors as naloxone but, in the opposite manner. The delta receptor is not readily blocked by naloxone and therefore, administration of its agonists should reveal any behavioral affects which are not readily discernible by administration of naloxone.

[Met^5]- and [Leu^5]-enkephalin have been identified as highly specific natural ligands of the delta receptor (Herz, 1984). A highly specific natural ligand for the mu receptor has not been identified. β-endorphin exhibits affinity for the mu receptor but also has affinity for the delta and the putative epsilon receptors (Lord *et al.*, 1977).

The influence of the mu receptor on ingestive behavior has been examined using the exogenous mu agonist, morphine. Injection of morphine into the paraventricular nucleus (PVN) (Woods and Leibowitz, 1985) or ventromedial hypothalamus (VMH) (Tepperman and Hirst, 1982) increases food intake in the rat. Intramuscular or subcutaneous injection of morphine in the rat increases both food and water intake (Czirr and Reid, 1986). Subcutaneous injection of morphine in the pigeon increases water but not food intake (Cooper and Turkish, 1981). This finding contradicts results with naloxone in which only food intake was attenuated (Deviche and Scheppers, 1984).

The role of the delta receptor in ingestive behavior regulation is less well understood. McLean and Hoebel (1983) observed an increase in food but not water intake when the synthetic enkephalin analogue [D-Ala^2-D-Met^5]-enkephalinamide (DALA) was injected into the PVN of rats. The only examination of a delta receptor agonist in birds was conducted by Uemura *et al.* (1983) in Japanese quail. Intracranial injections of [Leu^5]- enkephalin did not influence drinking at low levels (1 and 10 μg) but decreased drinking at higher levels (30 and 60 μg). These results are

difficult to evaluate because of the very high levels (30 and 60 μg) required to observe this depression.

The objective of the present investigation was to characterize the influence of mu and delta opioid receptor agonists on food and water intake in the domestic fowl. The novel tetrapeptide morphiceptin (β-casomorphin 1-4 amide; H2N-Tyr-Pro-Phe-Pro-NH2) was used as a mu receptor agonist. Morphiceptin is highly specific for the mu opioid receptor, exhibiting 1000 times greater affinity for the mu receptor than the delta receptor (Chang *et al.*, 1981). [Met^5]-enkephalin was used to evaluate the influence of delta receptor agonists on ingestive behavior. Both peptides were administered intracerebroventricularly (ICV) and intramuscularly (im) in order to study the influence of the mu and delta receptors within and outside the bbb.

MATERIALS AND METHODS

Animals. Commercial Rock-Cornish (RC) cockerels were reared in heated batteries until 3 weeks of age and then transferred to individual cages. The chicks were provided starter mash [20 % protein, 2864 kcal/kg metabolizable energy (ME)] and water *ad libitum* and exposed to continuous light. Birds were adapted to the individual cages and to handling for a minimum of one week prior to testing.

Experiment 1. This experiment evaluated the effect of ICV administered morphiceptin (Bachem Inc., Torrance, CA) on food and water intake. Five-week old RC cockerels were anesthetized with sodium pentobarbitol (25 mg/kg body weight, intravenously; iv) and a 23-guage thin-walled stainless steel guide cannula was stereotaxically implanted into the right lateral cerebral ventricle by the method of Denbow *et al.* (1981). Cannula location was verified by welling of cerebrospinal fluid (CSF) in the guide cannula during surgery and by post-surgical testing with norepinephrine (67 μg, ICV) (Denbow *et al.*, 1981). A one-week recovery period was allowed prior to initiating experimentation.

Ten chicks (5/replicate) received each of 5 treatments, i.e., 0, .625, 1.25, 2.5, and 5.0 μg morphiceptin, in a replicated 5 x 5 Latin square design with birds and days used as blocking factors. Immediately prior to initiating the experiment, feeders and waterers were removed and replenished. All injections were administered ICV in a volume of 10 ul with artificial cerebrospinal fluid (aCSF;

Anderson and Heisey, 1972) serving as a control solution and as a vehicle for the administration of the peptide. Food and water intake were recorded at 15, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 1440 min post-injection.

Experiment 2. In this experiment the effect of peripherally (im) administered morphiceptin on food and water intake was evaluated. Twelve five-week old chicks (4/replicate) received each of 4 treatments, i.e., 0, .5, 1.5, and 3.0 mg/kg body weight morphiceptin, in a replicated 4 x 4 Latin square design with days and birds used as blocking factors. Physiological saline (0.9 % NaCl) served as a control solution and as a vehicle for the administration of the peptide. Immediately prior to initiating the experiment, feeders and waterers were removed and replenished. Chicks were weighed to the nearest 1g and administered the appropriate treatment. Injections were administered in a volume of 1 ml into the pectoral muscle 15 min prior to the return of food and water. Food and water intake were recorded at 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 1440 min following the return of food and water.

Experiment 3. In this experiment the effect of [Met^5]-enkephalin (Sigma Chemical Company, St. Louis, MO) on food and water. The protocol was similar to Experiment 1 except that only one replicate was conducted. The levels of [Met^5]-enkephalin used were identical to that of morphiceptin in Experiment 1 and were based on the acetate salt of the peptide.

Experiment 4. In this experiment the effect of im administration [Met^5]-enkephalin on food and water intake was evaluated. The experimental protocol was similar to Experiment 2 except that 2 replicates were conducted. The levels of [Met^5]-enkephalin used were identical to the levels of morphiceptin used in Experiment 2 and were based on the acetate salt of the peptide.

Statistical Analysis. In all experiments, mean cumulative food and water intake within each observation period were analyzed using analysis of variance. Dose-response relationships within each observation period were evaluated using orthogonal linear and quadratic contrasts (Steele and Torrie, 1980). The statistical models for analysis of variance were as follows:

Experiment 1, 2 and 4:

$$Yijkl = u + Ri + B(i)j + Dk + T(l) + eijkl$$

Experiment 3:

$$Yijkl = u + Bi + Dj + T(k) + eijkl$$

Where R, B, D, and T represent the replicate, bird, day and treatment, respectively.

RESULTS

The ICV administration of morphiceptin (Experiment 1) produced a significant dose-dependent increase in water intake but did not affect food intake (Table 1 and 2). The dose-response relationship for water intake was linear from 15 through 45 min and again at 150 min post-injection. Also, as evidenced by a significant quadratic contrast, a curvilinear dose-response was observed at 90 and 180 min post-injection.

Intramuscular administration of morphiceptin (Experiment 2) produced a significant dose-dependent increase in food intake while having no effect on water intake (Table 3 and 4). The dose-response relationship was quadratic from 45 through 120 min following the return of food and water. The 1.5 mg/kg body weight dose of morphiceptin produced the greatest increase in feeding while .5 and 3 mg/kg body weight were less effective.

ICV administration of [Met^5]-enkephalin (Experiment 3) produced a dose-dependent increase in food intake from 30 through 240 min post-injection (Table 5 and 6). The dose-response relationship was quadratic at all time periods with the 2 μg dose producing the greatest increase.

Intramuscular administration of [Met^5]-enkephalin (Experiment 4) also produced a significant dose-dependent increase in feeding while not affecting drinking behavior (Table 7 and 8). Feeding was increased in a curvilinear manner from 15 through 90 min following the return of food and water. The maximum increase in feeding was attained with the intermediate 1.5 mg/kg body weight dose.

DISCUSSION

The results of the present investigation suggest that within the central nervous system of the fowl mu and delta opioid receptor agonists enhance water and food consumption, respectively. Furthermore, both mu and delta receptor agonists stimulate food consumption at sites outside the bbb. The possibility that these peripheral effects may be the result of the peptides traversing the bbb and acting centrally is remote since opioid peptides do not readily traverse the bbb (Conford *et al.*, 1978). In addition, the morphiceptin stimulated different behaviors when administered either centrally or peripherally.

The inverted "u" type dose-response seen following administration of both morphiceptin or [Met^5]-enkephalin is typical of physiological responses to opioid agonists (Gordon *et al.*, 1984; Morley and Levine, 1983) The lower efficacy observed at higher levels of the agonists may be due to down regulation of the receptor (Blanchard *et al.*, 1982) or perhaps to stimulation of alternate sites which have an opposite effect on these behaviors.

The results attained by central and peripheral administration of morphiceptin and peripheral administration of [Met^5]-enkephalin confirm earlier studies using opioid antagonists (Chapter 4) in which water intake was attenuated by ICV administration of naloxone and food intake was suppressed by im injection of quaternary naloxone, a congener of naloxone that does not cross the bbb. The insensitivity of food intake to centrally administered (ICV) naloxone is likely due to the naloxone's low affinity for the delta receptor (Lord *et al.*, 1977). Furthermore, the observation that food and water intake are mediated by separate receptor subclasses supports the observation that opioid antagonists attenuate feeding and drinking independently (McCormack and Denbow, 1987a - Chapter 1).

The ingestive responses following ICV administration of morphiceptin and [Met^5]-enkephalin in the fowl are consistant with the central nervous system effects of mu and delta receptor agonists observed in other species. In the rat, enkephalins have been reported to be potent stimulators of feeding while exerting little influence on drinking (McLean and Hoebel, 1983). Alternatively, mu receptor agonists have been associated with polydipsia. Cooper and Turkish (1981) reported increases in water intake following administration of morphine sulphate to pigeons.

The actions of opioid peptides outside the bbb are poorly understood and they have received far less attention than their actions within the CNS. There is little question that opioids can exert a profound influence on the function of peripheral tissues (Herz and Millian, 1984). Indeed, many organs, including the pancreas, adrenal gland, and gastrointestinal tract contain opioid receptors, the activation of which may modify their activity (Pasternak and Childers, 1983). Increases in feeding following peripheral administration of an enkephalin analogue have been reported by Sanger and McCarthy (1981). The question, therefore, becomes where outside the bbb do opioids act to influence feeding? Two organs which are involved in food intake and energy balance and whose function may also be influenced by opioids are the pancreas and the gut. It has been well established, in what has now become a classic preparation, that opioids induce contractions of the ileum (Olson *et al.*, 1984). Their effect on gastrointestinal motility *in vivo* has also been studied, with the general finding that opioids inhibit motility and decrease transit time (Galligan *et al.*, 1984; Ruckebusch *et al.*, 1984). Therefore, it is unlikely that opioids exert a stimulatory effect on feeding through their effects on intestinal motility.

Another possibility for peripheral opioid action on feeding is that opioids interact with enteric hormones such as cholecystokinin (CCK). Faris (1985) has hypothesized that opioid peptides and CCK have reciprocal roles in the regulation of energy balance and food intake. This hypothesis is supported by studies which have demonstrated that CCK antagonizes the analgesic effects of opioids (Faris *et al.*, 1983) and that opioid antagonists enhance and opioid agonists antagonize CCK-induced satiety (Wilson *et al.*, 1983). A problem which has deferred acceptance of the hypothesis is that opioids and CCK seem to have different sites of action. In the rat, CCK induces satiety outside the bbb (Smith *et al.*, 1981) whereas opioids stimulate ingestion at sites within the CNS (Leibowitz and Hor, 1982). However, in the fowl, CCK induces satiety at sites outside the blood-brain barrier (Savory and Gentle, 1980) and within the CNS (Denbow and Myers, 1982). As the present investigation suggests, opioids also appear to stimulate feeding at sites outside and within the bbb. Therefore, in the fowl, CCK and opioids may reciprocally influence feeding by interacting at sites in the periphery and in the CNS.

Another possible scenario for the peripheral effects of opioid agonists is that they may influence the actions of the endocrine pancreas. Opioids act directly on the endocrine pancreas to increase the release of insulin and decrease the release of somatostatin (Ipp *et al.*, 1978) while the opioid antagonist naloxone increases blood glucose levels following insulin-induced hypoglycemia (Levine and Morley, 1981). Immunocytochemical studies have shown that the concentration of opioids and insulin in the Islets of Langerhans increase in hyperinsulinemic conditions (Recant et al., 1984). Furthermore, hypoinsulinemia was associated with reduced pancreatic opioid concentration and an increase in pancreatic somtatostatin (Recant *et al.*, 1984). Insulin (Silverstone and Kyriakides, 1982) and somatostatin (Lotter *et al.*, 1981) have been shown to have stimulatory and inhibitory effects on feeding, respectively. Therefore, opioid-induced alterations in these hormones may induce changes in food consumption.

The results of the present investigation indicate that, in the fowl, there is both a central and peripheral component to opioid influence on ingestive behavior. Feeding is stimulated by delta receptor agonists in the CNS and both mu and delta receptor agonists outside the bbb. Water intake is stimulated by centrally administered mu agonist but not affected by a delta or mu agonists outside the bbb. Other less well characterized receptors, such as the kappa, epsilon and sigma, may also influence ingestion in the fowl. Further studies will be needed to evaluate the role of other receptor agonists and to elucidate the exact site and mode of action of opioids outside the bbb.

SUMMARY

Four experiments were conducted using the highly specific mu and delta opioid receptor agonists morphiceptin (β-casomorphin 1-4, amide) or [Met^5]-enkephalin, respectively, to evaluate the effect of mu and delta opioid receptor agonists on ingestive behavior in the domestic fowl. In the first experiment six-week old Rock-Cornish (RC) cockerels were injected intracerebroventricularly (ICV) with 0, .625, 1.25, 2.5 and 5.0 μg morphiceptin. In the second experiment, five-week old RC cockerels were injected intramuscularly (im) with 0, .5, 1.5 and 3.0 mg/kg body weight morphiceptin. The third and fourth experiments were conducted using the same protocol as the first and second experiments, respectively, except [Met^5]-enkephalin was used.

ICV administration of morphiceptin significantly stimulated water intake from 15 through 90 min and from 150 through 180 min post-injection. Food intake was not altered. Intramuscular injection of morphiceptin induced a significant increase in food consumption from 45 through 120 min following the return of food and water whereas drinking was not effected. ICV administration of [Met^5]-enkephalin significantly stimulated feeding from 30 through 240 min post-injection while having no effect on water intake. Intramuscular administration of [Met^5]-enkephalin significantly stimulated feeding from 15 through 90 min following the return of food and water but did not alter water intake.

The results of this investigation suggest that, in the central nervous system, mu opioid receptor agonists stimulate water intake and delta opioid receptor agonists stimulate feeding behavior. At sites outside the blood-brain barrier, both mu and delta opioid receptor agonists stimulate feeding. Furthermore, these results confirm earlier studies which suggest that feeding and drinking are influenced independently by opioids and that there is both a peripheral and central component to opioid regulation of ingestive behavior in the fowl.

TABLE 1. Mean cumulative food intake (g) of Rock-Cornish cockerels following intracerebroventricular injection of morphiceptin (Exp. 1)

	Time (min)										
Treatment [1]	15	30	45	60	90	120	150	180	240	300	1440
aCSF [2]	7.6	10.7	12.5	14.4	17.9	22.0	24.7	27.7	32.0	36.7	131.0
Morphiceptin (.625 µg)	8.6	11.7	14.6	16.0	19.8	22.8	27.2	30.1	33.6	37.2	128.3
Morphiceptin (1.25 µg)	8.7	11.8	14.0	15.4	18.9	22.7	27.2	30.3	35.1	39.8	137.0
Morphiceptin (2.5 µg)	7.4	11.2	13.9	15.7	20.4	24.5	27.9	29.7	33.4	38.5	131.9
Morphiceptin (5.0 µg)	7.5	10.8	12.8	14.7	18.7	22.9	25.4	28.2	32.1	38.3	124.7
Standard error of the treatment mean	0.6	0.7	1.0	1.2	1.3	1.5	1.5	1.6	1.7	2.0	4.3
F value of contrasts [3]											
Linear	1.0	0.1	0.2	0	0	0.2	0	0.1	0.1	0.2	1.3
Quadratic	0.1	0.7	1.5	0.7	1.4	0.9	2.8	1.5	1.1	0.5	1.4

[1] All treatments administered in a volume of 10 µl.

[2] Artificial cerebrospinal fluid (aCSF) consisting of (in meq/l) Na^+, 155; K^+, 3.7; Ca^{+2}, 2.5; Mg^{+2}, 2.1; Cl^-, 140 and HCO_3^-, 23.

[3] Critical F (1, 77) $\cong$ 3.98, $P \leq .05$, therefore, there was no significant dose-response relationship at any time period.

TABLE 2. Mean cumulative water intake (ml) of Rock-Cornish cockerels following intracerebroventricular injection of morphiceptin (Exp. 1)

	Time (min)										
Treatment [1]	15	30	45	60	90	120	150	180	240	300	1440
aCSF [2]	1.8	6.8	14.0	21.5	35.0	45.0	53.8	64.5	78.8	91.3	366.8
Morphiceptin (.625 µg)	3.8	13.3	19.5	27.8	39.3	50.0	56.3	69.0	84.3	99.5	364.8
Morphiceptin (1.25 µg)	3.8	10.8	18.0	26.5	44.3	52.8	61.3	73.0	84.0	99.0	394.8
Morphiceptin (2.5 µg)	5.0	15.5	25.3	33.3	47.5	59.8	69.8	83.3	94.0	109.3	392.3
Morphiceptin (5.0 µg)	7.0	15.8	24.8	29.8	44.0	55.0	66.5	74.3	89.0	105.5	372.5
Standard error of the treatment mean	1.4	2.1	2.6	2.9	3.4	4.0	4.3	4.8	5.2	5.8	16.1
F value of contrasts											
Linear	7.1**	7.3**	8.7**	3.7	3.3	3.3	5.9*	2.6	2.2	3.0	0.1
Quadratic	.2	2.2	2.4	3.9	4.7*	3.8	3.1	5.0*	1.9	2.0	2.1

[1] All treatments administered in a volume of 10 µl.

[2] Artificial cerebrospinal fluid (aCSF) consisting of (in meq/l) Na^+, 155; K^+, 3.7; Ca^{+2}, 2.5; Mg^{+2}, 2.1; Cl^-, 140 and HCO_3^-, 23.

* Critical F (1, 77) $\cong$ 3.98, P $\leq$ .05, therefore, these contrasts are significant.

** Critical F (1, 77) $\cong$ 7.01, P $\leq$ .01, therefore, these contrasts are highly significant.

TABLE 3. Mean cumulative food intake (g) of Rock-Cornish cockerels following intramuscular injection of morphiceptin (Exp. 2)

	Time (min)										
Treatment [1]	15	30	45	60	90	120	150	180	240	300	1440
Saline	4.3	5.7	7.0	8.5	11.4	13.6	16.1	18.4	22.1	25.9	98.0
Morphiceptin (.5 mg/kg W [2])	4.6	7.2	8.9	10.6	13.5	16.2	18.7	20.8	24.4	28.2	100.7
Morphiceptin (1.5 mg/kg W)	5.8	7.7	9.3	11.2	14.1	17.2	18.8	20.8	25.0	28.0	99.1
Morphiceptin (3.0 mg/kg W)	5.3	7.3	8.4	9.8	11.9	14.3	16.3	18.3	22.7	27.0	95.7
Standard error of the treatment mean	0.5	0.5	0.5	0.6	0.9	1.1	1.2	1.1	1.2	1.4	2.2
F value of contrasts											
Linear	2.7	3.4	2.0	1.0	0	0	0.1	0.3	0	0	1.4
Quadratic	2.4	3.9	9.0**	10.9**	6.2*	6.9*	3.7	3.8	4.0	1.1	1.0

[1] All treatments administered in a volume of 1 ml.

[2] W, body weight.

* Critical F (1, 30) $\cong$ 4.17, P $\leq$ .05, therefore, these contrasts are significant.

** Critical F (1, 30) $\cong$ 7.56, P $\leq$ .01, therefore, these contrasts are highly significant.

TABLE 4. Mean cumulative water intake (ml) of Rock-Cornish cockerels following intramuscular injection of morphiceptin (Exp. 2)

	Time (min)										
Treatment [1]	15	30	45	60	90	120	150	180	240	300	1440
Saline	4.6	10.0	12.9	16.3	20.8	27.9	34.2	38.3	46.7	54.6	219.6
Morphiceptin (.5 mg/kg W [2])	2.9	7.5	13.3	16.7	23.3	29.2	34.2	38.8	47.1	55.0	217.1
Morphiceptin (1.5 mg/kg W)	5.0	9.2	13.3	17.1	25.0	30.8	37.5	43.8	50.8	61.7	242.9
Morphiceptin (3.0 mg/kg W)	3.8	9.2	13.3	15.4	23.3	31.3	35.4	41.3	51.7	59.6	225.0
Standard error of the treatment mean	1.4	1.7	1.8	1.8	2.1	2.3	3.1	3.1	3.3	3.9	11.2
F value of contrasts [3]											
Linear	0	0	0	0.1	0.5	1.1	0.2	0.7	1.6	1.3	0.4
Quadratic	0.1	0.2	0	0.3	1.4	0.2	0.4	0.9	0.1	0.8	1.7

[1] All treatments administered in a volume of 1 ml.

[2] W, body weight.

[3] Critical F (1, 30) $\cong$ 4.17, $P \leq .05$, therefore, there was no significant dose-response relationship at any time period.

TABLE 5. Mean cumulative food intake (g) of Rock-Cornish cockerels following intracerebroventricular injection of [Met^5]-enkephalin ([Met^5]-enk) (Exp. 3)

	Time (min)										
Treatment [1]	15	30	45	60	90	120	150	180	240	300	1440
aCSF [2]	10.6	15.6	17.2	19.0	20.8	25.8	32.0	32.0	37.6	46.0	148.6
[Met^5]-enk (1 µg)	13.2	18.2	19.6	21.4	23.2	26.6	30.0	31.4	33.4	34.6	136.4
[Met^5]-enk (2 µg)	12.4	19.0	28.4	32.6	35.6	39.6	44.4	46.2	51.2	54.2	155.4
[Met^5]-enk (4 µg)	13.8	18.8	24.4	26.8	28.6	30.8	35.8	38.8	46.4	49.6	153.0
[Met^5]-enk (8 µg)	10.4	14.6	17.0	19.6	20.8	26.2	32.2	33.8	40.4	47.6	145.0
Standard error of the treatment mean	1.3	1.6	1.3	1.9	2.2	2.7	2.7	2.9	2.8	3.2	3.8
F value of contrasts											
Linear	0.3	1.0	1.3	0.4	0.7	0.2	0	0	1.0	1.7	0
Quadratic	4.0	4.9*	39.0***	23.1***	20.3***	8.0*	6.0*	8.9*	10.9**	2.3	3.5

[1] All treatments administered in a volume of 10 µl; levels of [Met^5]-enk based on acetate salt form of the peptide.

[2] Artificial cerebrospinal fluid (aCSF) consisting of (in meq/l) Na^+, 155; K^+, 3.7; Ca^{+2}, 2.5; Mg^{+2}, 2.1; Cl^-, 140 and HCO_3^-, 23.

* Critical F (1, 12) $\cong$ 4.75, P $\leq$ .05, therefore, these contrasts are significant.

** Critical F (1, 12) $\cong$ 9.33, P $\leq$ .01, therefore, these contrasts are highly significant.

*** Critical F (1, 12) $\cong$ 18.60, P $\leq$ .001, therefore, these contrasts are very highly significant.

TABLE 6. Mean cumulative water intake (ml) Of Rock-Cornish cockerels following intracerebroventricular injection of [Met^5]-enkephalin ([Met^5]-enk) (Exp. 3)

	Time (min)										
Treatment [1]	15	30	45	60	90	120	150	180	240	300	1440
aCSF [2]	8.0	16.0	29.0	49.0	64.0	67.0	80.0	86.0	119.0	167.0	536.0
[Met^5]-enk (1 µg)	6.0	14.0	34.0	45.0	56.0	72.0	79.0	85.0	99.0	110.0	474.0
[Met^5]-enk (2 µg)	7.0	17.0	42.0	56.0	72.0	97.0	106.0	121.0	141.0	164.0	535.0
[Met^5]-enk (4 µg)	6.0	15.0	24.0	31.0	59.0	80.0	96.0	105.0	134.0	155.0	533.0
[Met^5]-enk (8 µg)	13.0	23.0	33.0	43.0	60.0	75.0	85.0	104.0	138.0	163.0	535.0
Standard error of the treatment mean	3.1	4.1	8.6	11.1	10.7	11.2	13.6	15.8	16.7	19.7	15.0
F value of contrasts [3]											
Linear	2.0	2.0	0	0.4	0.1	0	0	0.6	1.5	0.5	1.5
Quadratic	1.3	0.6	0	0.4	0	1.8	1.6	1.1	0.4	0.1	0

[1] All treatments administered in a volume of 10 µl; levels of [Met^5]-enk based on acetate salt form of the peptide.

[2] Artificial cerebrospinal fluid (aCSF) consisting of (in meq/l) Na^+, 155; K^+, 3.7; Ca^{+2}, 2.5; Mg^{+2}, 2.1; Cl^-, 140 and HCO_3^-, 23.

[3] Critical F (1, 12) $\cong$ 4.75, $P \leq .05$, therefore, there were no significant dose-response relationships at any time period.

TABLE 7. Mean cumulative food intake (g) of Rock-Cornish cockerels following intramuscular injection of [Met^5]-enkephalin ([Met^5]-enk) (Exp. 4)

	Time (min)										
Treatment [1]	15	30	45	60	90	120	150	180	240	300	1440
Saline	6.1	8.2	9.6	10.9	13.5	16.4	18.6	20.4	26.1	30.4	107.4
[Met^5]-enk (0.5 mg/kg W [2])	6.4	8.1	10.1	11.7	14.3	17.5	19.6	21. 7	27.1	31.4	109.5
[Met^5]-enk (1.5 mg/kg W)	7.6	10.0	12.2	13.6	15.9	18.0	20.1	22.2	27.2	31.7	110.9
[Met^5]-enk (3.0 mg/kg W)	6.0	8.1	9.2	10.8	13.0	16.2	17.9	20.5	25.5	30.9	108.7
Standard error of the treatment mean	0.4	0.6	0.6	0.6	0.6	0.8	1.0	0.9	0.8	1.0	1.8
F value of contrasts											
Linear	0	0	0	0	0.3	0.1	0.5	0	0.7	0	0.2
Quadratic	7.1*	5.5*	13.6**	15.2**	10.4**	2.9	2.5	3.1	2.3	1.0	1.9

[1] All treatments administered in a volume of 1 ml; levels of [Met^5]-enk based on acetate salt form of the peptide.

[2] W, body weight.

* Critical F (1, 18) $\cong$ 4.41, P $\leq$ .05, therefore, these contrasts are significant.

** Critical F (1, 18) $\cong$ 8.29, P $\leq$ .01, therefore, these contrasts are highly significant.

TABLE 8. Mean cumulative water intake (ml) of Rock-Cornish cockerels following intramuscular injection of [Met^5]-enkephalin ([Met^5]-enk) (Exp. 4)

	Time (min)										
Treatment [1]	15	30	45	60	90	120	150	180	240	300	1440
Saline	4.4	13.8	20.6	23.1	28.1	36.9	43.1	50.0	59.4	70.0	265.0
[Met^5]-enk (0.5 mg/kg W [2])	3.1	8.1	15.6	21.2	28.1	37.5	43.1	48.7	58.1	69.4	265.6
[Met^5]-enk (1.5 mg/kg W)	3.1	7.5	15.6	19.4	26.3	33.1	36.2	44.4	55.0	64.4	248.7
[Met^5]-enk (3.0 mg/kg W)	2.5	7.5	15.0	18.7	29.4	31.9	38.1	44.4	55.6	68.1	267.5
Standard error of the treatment mean	0.8	2.6	2.6	3.3	3.1	3.0	3.4	3.4	3.1	2.9	7.6
F value of contrasts [3]											
Linear	2.0	1.9	1.5	0.9	0.1	2.2	1.8	1.7	0.9	0.4	0
Quadratic	0.2	1.4	0.8	0.2	0.4	0	0.8	0.4	0.4	1.5	2.9

[1] All treatments administered in a volume of 1 ml.

[2] W, body weight.

[3] Critical F (1, 18) $\cong$ 4.41, P $\leq$ .05, therefore, there were no significant dose-response relationships at any time period.

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CHAPTER 6

FEEDING, DRINKING AND TEMPERATURE RESPONSES TO INTRACEREBROVENTRICULAR β-ENDORPHIN IN THE DOMESTIC FOWL

INTRODUCTION

The endogenous opioid peptide β-endorphin has been shown to influence numerous physiological functions such as ingestion (Olson *et al.*, 1985), thermoregulation (Kastin *et al.*, 1984), gonadotropin release (Besser and Grossman, 1984) and immunocompetence (Margules, 1984). β-endorphin (β-END) is derived, along with melanocyte stimulating hormone and adrenocorticotrophic hormone, from the precursor peptide pro-opiomelanocortin. β-END does not exhibit specificity for any particular opioid receptor subtype but has equal affinity for the mu and delta receptor subtypes (Lord *et al.*, 1977) as well as the putative epsilon receptor (Schulz *et al.*, 1981).

Grandison and Guidotti (1977) first suggested that β-END was involved in the regulation of ingestive behavior when they observed increases in feeding after β-END was injected into the ventromedial hypothalamus of the rat. Similar increases in feeding have been noted when β-END was administered into the paraventricular nucleus (PVN) of the hypothalamus (Leibowitz and Hor, 1982) and intracerebroventricularly (ICV) (McKay *et al.*, 1981). Elevations in pituitary β-END concentration also have been associated with hyperphagia and the pathogenesis of obesity (Margules *et al.*, 1978) whereas food deprivation has been shown to reduce hypothalamic β-END (Gambert *et al.*, 1980). There has been only one investigation of the influence of β-END on feeding in birds. Deviche and Schepers (1984) observed an increase in food, but not water, intake following ICV administration of ostrich β-END to pigeons.

Thermoregulatory responses to β-END are less consistent and not as well understood as ingestive responses. β-END has been shown to induce hyperthermia (Gordon *et al.*, 1983), hypothermia (Wong and Tse, 1984) or a biphasic temperature response (Holaday *et al.*, 1978) depending on the species, route of administration and dose. Feldberg and Smyth (1977) found only

a hyperthermic response when β-END was administered into the third ventricle of cats. Similarly, injection of β-END into the preoptic/anterior hypothalamus induced hyperthermia (Gordon *et al.*, 1984; Thornhill and Saunders, 1984). The ICV administration of β-END produced hypothermia in anesthetized rats (Wong and Tse, 1984). In unanesthetized rats, β-END produced hyperthermia at levels less than 10 μg whereas higher levels induced hypothermia (Holaday et al., 1978). Hypothermia was also observed in the only study examining the thermoregulatory response of birds to β-END (Nistico *et al.*, 1980). However, this study was conducted using 1 to 2-week old chicks which do not possess a fully mature thermoregulatory system (Freeman, 1965; Myhre, 1978).

The objective of the present investigation was to examine the ingestive and thermoregulatory responses of meat (Rock-Cornish; RC) and egg (Single-Comb White Leghorn; SCWL) stocks of chickens to ICV administered β-END. RC stocks have been selected for rapid growth (McCarthy and Siegel, 1983), while SCWL stocks have been selected for egg production and consequently low body weight (Kinney, 1969).

MATERIALS AND METHODS

Animals. RC and SCWL cockerels were reared in heated batteries until 4 and 7 weeks of age, respectively, and then transferred to individual cages. The chicks were provided starter mash [20% protein, 2864 kcal/kg metabolizable energy (ME)] and water *ad libitum*, and were exposed to continuous light. Birds were adapted to the individual cages and handling for a minimum of one week prior to surgery.

Surgical preparation. At 5 and 8 weeks of age RC and SCWL cockerels, respectively, were anesthetized with sodium pentobarbitol (25 mg/kg body weight, intravenously; iv) and a 23-gauge thin-walled stainless steel guide cannula was stereotaxically implanted into the right lateral cerebral ventricle by the method of Denbow *et al.* (1981). Cannula location was verified by welling of cerebrospinal fluid (CSF) in the guide cannula during surgery and by post-surgical testing with norepinephrine (67 μg, ICV) (Denbow *et al.*, 1981). A one-week recovery period was allowed prior to initiating experimentation. During the last five days of the recovery period chicks were adapted

to the thermistor probes (Model 423, Yellow Springs Instrument Co., Yellow Springs, OH) used to measure colonic temperature during subsequent experiments.

Experiment 1. This experiment evaluated the effect of ICV administered β-END (Bachem Inc., Torrance, CA) on food intake, water intake and colonic temperature in RC cockerels. Sixteen chicks (4/replicate) received each of 4 treatments, i.e., 0, 1.5, 3.0 and 6.0 μg β-END, in a replicated 4 x 4 Latin square design with birds and days used as blocking factors. Thermistor probes were inserted in the colon, immobilized and connected to a telethermometer (Model 46 TUC, Yellow Springs Instrument Co., Yellow Springs, OH) thirty minutes prior to injection of the peptide in order to establish a consistent baseline temperature. Immediately prior to injection feeders and waterers were removed and replenished. All injections were administered ICV in a volume of 10 ul with artificial cerebrospinal fluid (aCSF; Anderson and Heisey, 1972) serving as a control solution and a vehicle for the administration of the peptide. Food, water and colonic temperature were recorded at 15, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 1440 min post-injection.

All injection equipment was stored in 70 % ethanol and the glassware was heated to 250 C for 1 hr to render materials pyrogen-free. Solutions were prepared daily and aCSF was filtered through a .2 μm filter (Gelman Instrument Co., Ann Arbor, MI) just prior to injection.

Experiment 2. This experiment was conducted to examine the effects of lower levels of β-END, i.e., 0, .5, 1.0, and 2.0 μg, on food intake, water intake and colonic temperature in RC cockerels. The protocol was similar to Experiment 1 except that eight chicks were used.

Experiment 3. This experiment was conducted to examine the effects of higher dosages of β-END, i.e., 0, 1.5, 3.0 and 6.0 μg, on food intake, water intake and colonic temperature in SCWL chicks. The protocol was similar to Experiment 1 except that eight chicks were used.

Experiment 4. This experiment was conducted to examine the effects of the lower dose range of β-END, i.e., 0, .5, 1.0 and 2.0 μg, on food intake, water intake and colonic temperature in SCWL chicks. The protocol was similar to Experiment 2.

Statistical Analysis. Mean cumulative food and water intake and change in colonic temperature from baseline (colonic temperature at injection) within each observation period were analyzed using analysis of variance. Dose-response relationships within each time period were analyzed

using orthogonal linear and quadratic contrasts (Steel and Torrie, 1980). The statistical models for analysis of variance were as follows:

Experiment 1: $Yijkl = u + Ri + B(i)j + D(i)k + T(l) + eijkl$

Experiment 2,3 and 4: $Yijkl = u + Ri + B(i)j + Dk + T(l) + eijkl$

Where R, B, D, and T represent the replicate, bird, day and treatment, respectively.

RESULTS

Food Intake. The ICV administration of β-END to RC cockerels (Experiment 1 and 2) resulted in a significant increase in food intake (Figure 1). At the higher dose range (1.5 to 6 μg; Experiment 1) β-END produced an initial suppression of feeding at 15 min while inducing a significant curvilinear increase in feeding from 45 min and from 90 through 240 min post-injection (Figure 1a). The maximum increase in feeding was induced by the 1.5 μg dose. The 3 μg dose produced an increase in feeding over the control, whereas the response to the 6 μg dose was similar to the control. This inverted "u" type dose-response seems to be typical of both ingestive (Morley and Levine, 1983) and thermoregulatory (Gordon *et al.*, 1984) responses to opioid agonists. Experiment 2 (Figure 1b) tested the effect of lower levels (.5 to 2 μg) of β-END on feeding in RC cockerels. β-END at levels from .5 to 2 μg produced a significant linear increase in food intake from 180 through 300 min post-injection. The maximum increase in feeding was attained at the 2 μg level. From the results of Experiment 1 and 2 it seems that in RC cockerels feeding behavior is maximally stimulated between 1.5 to 2 μg β-END.

The ICV administration of β-END to SCWL cockerels (Experiments 3 and 4) also produced a significant increases in feeding (Figure 2). The injection of higher levels (1.5 to 6 μg; Experiment 3) produced a significant curvilinear increase in food intake from 90 through 300 min post-injection (Figure 2a). As was observed in Experiment 1 with RC chicks, the maximum stimulation of intake was attained with 1.5 μg while the 3 μg dose produced higher intake than the control but less than the 1.5 μg dose. The 6 μg dose of β-END did not alter feeding from the

control level. At lower levels (.5 to 2.0 μg; Experiment 4) of β-END feeding was stimulated in a curvilinear manner with the greatest stimulation occurring at the .5 μg level (Figure 2b). Food intake was also stimulated at the 1 and 2 μg level but to lesser degree than at .5 μg dose.

Water Intake. Drinking behavior in RC chicks was significantly decreased at 60 and 90 min post-injection (Figure 3a) by β-END at levels from 1.5 to 6 μg (Experiment 1). However, at levels between .5 and 2 μg β-END (Experiment 2) water intake was significantly stimulated in a linear manner from 120 through 300 min post-injection (Figure 3b). The drinking responses of SCWL chicks to β-END was similar to those of RC chicks. At higher levels (1.5 to 6 μg; Experiment 3) β-END initially depressed water intake from 30 through 60 min post-injection (Figure 4a). At 180 and 300 min post-injection β-END produced a significant curvilinear increase in drinking. Lower levels (.5 to 2 μg; Experiment 4) of β-END significantly depressed drinking from 45 through 90 min post-injection (Figure 4b) while significantly increasing water intake from 150 through 180 min post-injection.

Temperature. ICV administration of β-END at levels from 1.5 to 6.0 μg (Experiment 1) induced a significant increase in colonic temperature from 30 through 240 min post-injection in RC chicks (Figure 5a). The dose-response relationship was generally linear with some curvature detected from 30 through 60 min post-injection and again at 120 min post-injection. At levels between .5 and 2.0 μg (Experiment 2) β-END also produced a dose-dependent hyperthermia from 15 through 240 min post injection (Figure 5b). The dose-relationship was linear from 15 through 180 min with some curvature in the response evident at 45, 90 and 240 min post-injection.

The effects of β-END on colonic temperature in SCWL chicks was similar to that noted in RC chicks. At levels from 1.5 to 6.0 μg (Experiment 3) β-END induced a significant dose-dependent hyperthermia from 15 through 180 min post-injection (Figure 6a). The dose-response relationship was linear with some curvature detected at 60 and 90 min post-injection. A significant hyperthermia was also induced at levels from .5 to 2.0 μg β-END (Experiment 4). The dose-response relationship was linear from 30 through 180 min post-injection with some curvature detected at 30 min and from 90 through 120 min post-injection.

DISCUSSION

The results of this investigation suggest that β-END influences ingestive behavior and colonic temperature through actions within the central nervous system. These physiological functions seem to be influenced independently by opioids. For example, although food and water intake in the fowl are affected in a similar fashion, alterations in water intake do not seem to be a consequence of altered food intake. Previous investigations using opioid antagonists have demonstrated that water intake in the domestic fowl is attenuated by naloxone even when food is not available (McCormack and Denbow, 1987 - Chapter 1). Secondly, ICV administration of the highly specific mu opioid receptor agonist morphiceptin stimulates water but not food intake, whereas administration of the delta receptor agonist [Met^5]-enkephalin stimulates only food intake (Chapter 5). Evidence for independent opioid mediation of food and water intake also exists in the present investigation. In Experiment 2 significant increases in water intake occurred prior to increases in feeding. Therefore, food and water intake in the domestic fowl seem to be influenced by independent opioid receptor systems, i.e., delta and mu, respectively. The present results using β-END conform to this paradigm. β-endorphin has equal affinity for both the mu and delta opioid receptors (Paterson *et al.*, 1983) and, therefore, could be expected to stimulate both food and water intake in the domestic fowl.

The hyperthermia induced by ICV administration of β-endorphin seems to be a specific effect and not related to increases food consumption or feeding activity. For example the dose-response relationships of the two responses were often different. In Experiment 1 and 3 food consumption was stimulated in a curvilinear manner whereas colonic temperature was increased in a linear manner. Secondly, in all experiments significant increases in colonic temperature occurred prior to increases in feeding. Typically, food intake and body temperature are inversly related (Brobeck, 1948). However, opioids have been reported to induce simultaneous stimulation of ingestive behavior and hyperthermia in other species. Tepperman *et al.* (1981) observed increases in feeding and core temperature when the mu opioid agonist morphine was injected into the ventromedial hypothalamus (VMH) of rats. In a latter study, Tepperman and Hirst (1982) pro-

posed that the hyperthermic and ingestive responses to morphine are not coupled and result from the stimulation of different receptor subtypes.

The ingestive behavior of both RC and SCWL was enhanced by central administration of β-END. At levels of β-END between 1.5 and 6.0 μg (Experiment 1 and 3) nearly identical curvilinear increases in feeding were observed in RC and SCWL. However, when β-END was administered at lower levels (.5 to 2.0 μg; Experiment 2 and 4) maximum stimulation of feeding occurred at .5 μg in SCWL and at 2.0 μg in RC. β-END influenced water intake and body temperature in a similar manner to food intake. Because the stocks were tested in separate experiments it is not possible to discern whether the greater response of SCWL to low levels of β-END is indicative of a greater sensitivity of SCWL to opioids. Nonetheless, this is an interesting occurrence because previous studies have demonstrated that SCWL are relatively insensitive to substances which enhance feeding in RC chicks (Denbow, 1985).

SUMMARY

Four experiments were conducted to evaluate the effect of β-endorphin (β-END) on feeding, drinking and colonic temperature in rapidly growing (Rock-Cornish; RC) and slow growing (Single-Comb White Leghorn; SCWL) stocks of chickens. In the first experiment RC cockerels were injected intracerebroventricularly (ICV) with 0, 1.5, 3.0 and 6.0 μg β-END. In the second experiment RC cockerels were injected ICV with .5, 1.0, and 2.0 μg β-END. Experiments 3 and 4 were conducted identically to Experiment 1 and 2, respectively, except SCWL were used.

Administration of β-END at levels between 1.5 and 6.0 μg produced a significant curvilinear increase in feeding in both RC and SCWL chicks. In RC chicks, feeding was significantly elevated at 45 min and from 90 through 240 min post-injection whereas in SCWL chicks feeding was increased from 90 through 300 min post-injection. Water intake was depressed in RC and SCWL from 60 through 90 min and from 30 through 60 min post-injection, respectively. Significant increases in water intake occurred at 180 and 300 min post-injection in SCWL. β-END also induced a significant hyperthermia in RC and SCWL from 30 through 240 min and from 15 through 180 min post-injection, respectively.

At lower levels of β-END, i.e., 0, .5, 1.0 and 2.0 μg, feeding, drinking and body temperature were significantly increased in both stocks. Feeding in RC chicks was stimulated in a linear fashion from 180 through 300 min post-injection while feeding in SCWL was stimulated in a curvilinear manner from 180 through 240 min post-injection. Water intake in RC chicks was increased in a linear manner from 120 through 240 min post-injection while drinking in SCWL was initially decreased from 45 through 90 min but significantly increased from 150 through 180 post-injection. Lower levels of β-END also induced a significant increases in body temperature. A significant hyperthermic response was observed from 15 through 240 min and from 30 through 180 min post-injection in RC and SCWL, respectively.

These results suggest that β-END acts at sites within the central nervous system of the domestic fowl to increase feeding, drinking, and body temperature. Furthermore, these responses are similar in stocks of chickens which exhibit vast differences in growth.

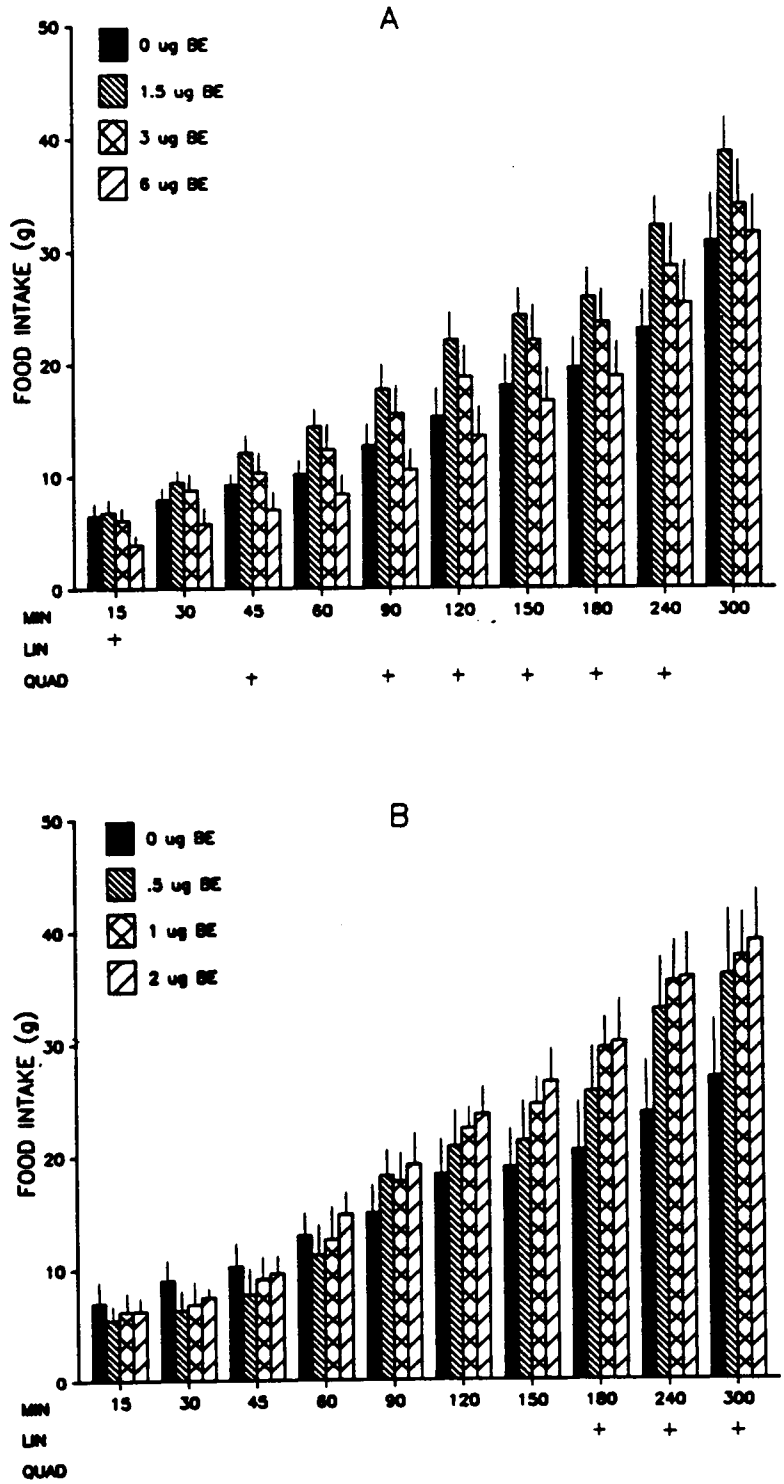

FIG. 1. Mean (±SE) cumulative food intake of Rock-Cornish cockerels following intracerebroventricular injection of β-endorphin (BE); MIN, minutes; LIN, linear contrast; QUAD, quadratic contrast; + $P \leq .05$.

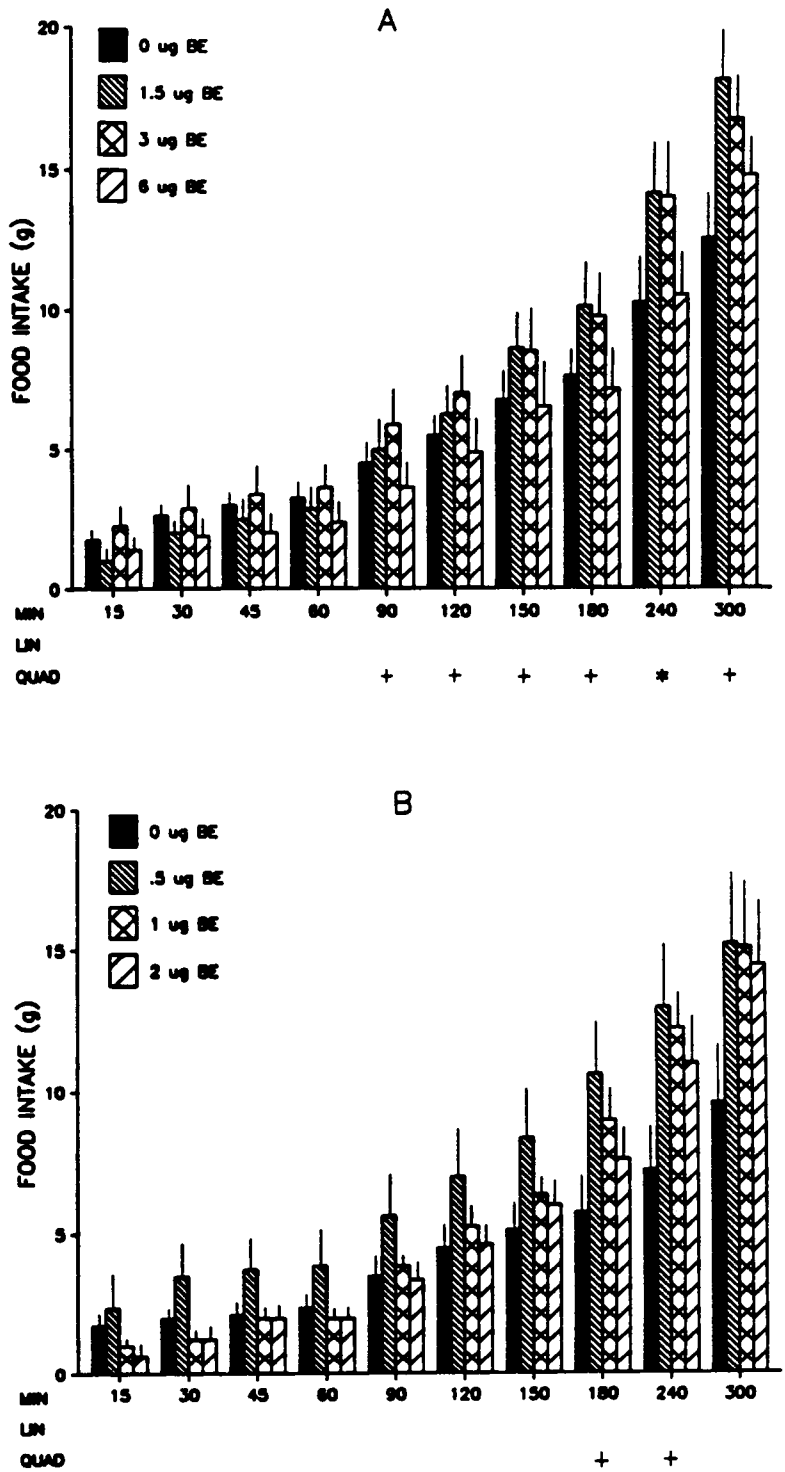

FIG. 2. Mean (± SE) cumulative food intake of Single-Comb White Leghorn cockerels following intracerebroventricular injection of β-endorphin (BE); MIN, minutes; LIN, linear contrast; QUAD, quadratic contrast; + $P \leq .05$; * $P \leq .01$.

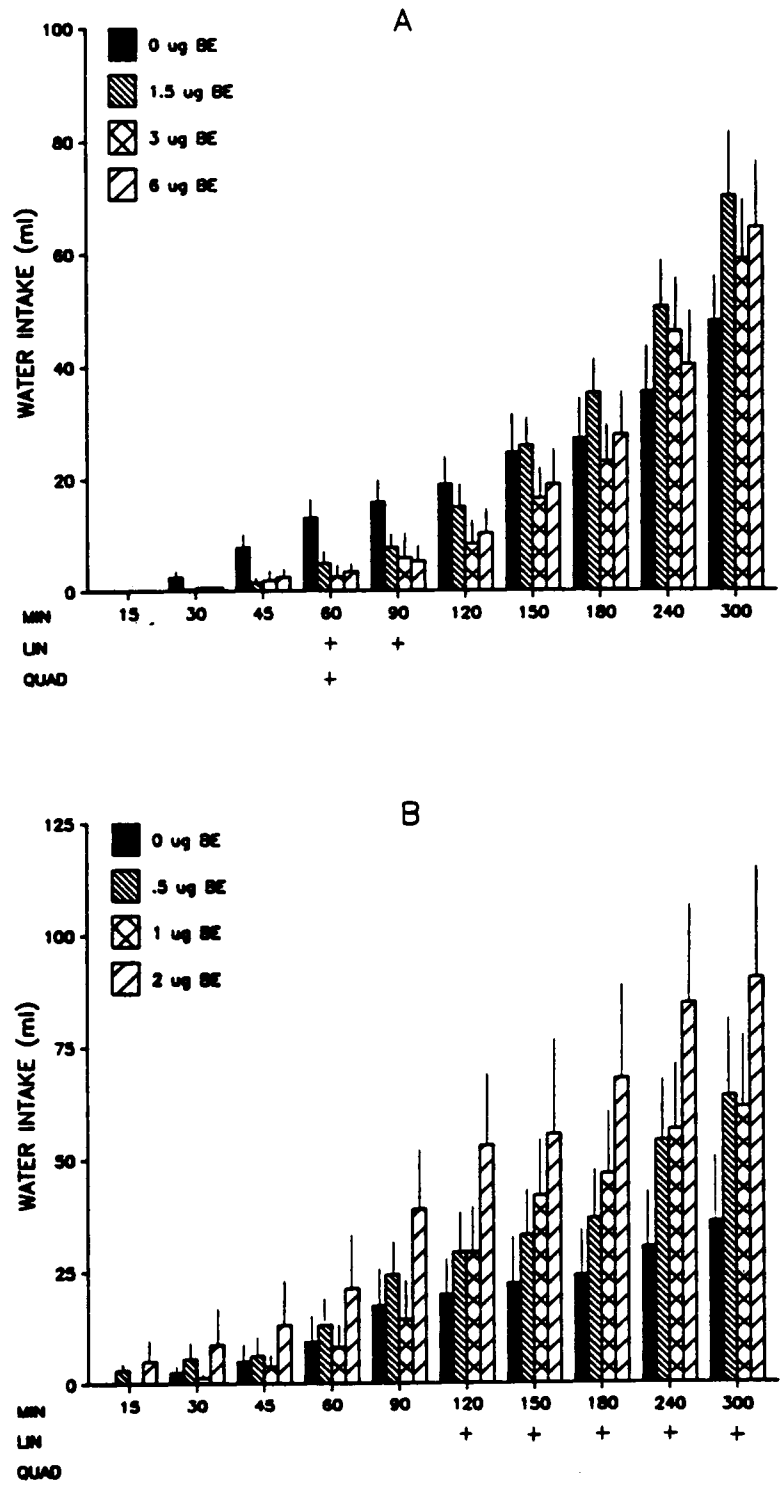

FIG. 3. Mean (±SE) cumulative water intake of Rock-Cornish cockerels following intracerebroventricular injection of β-endorphin (BE); MIN, minutes; LIN, linear contrast; QUAD, quadratic contrast; + $P \leq .05$.

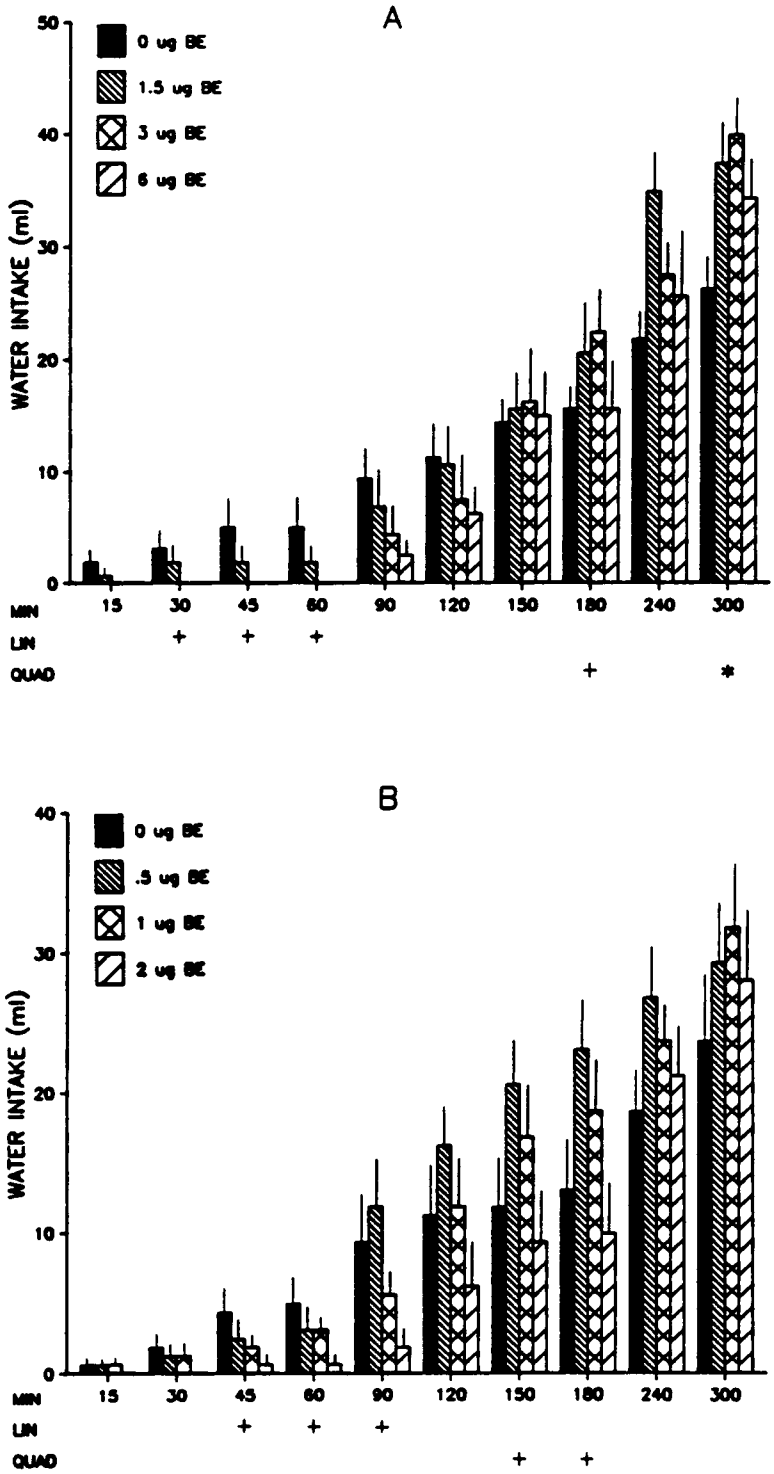

FIG. 4. Mean (± SE) cumulative water intake of Single-Comb White Leghorn cockerels following intracerebroventricular injection of β-endorphin (BE); MIN, minutes; LIN, linear contrast; QUAD, quadratic contrast; + $P \leq .05$; * $P \leq .01$.

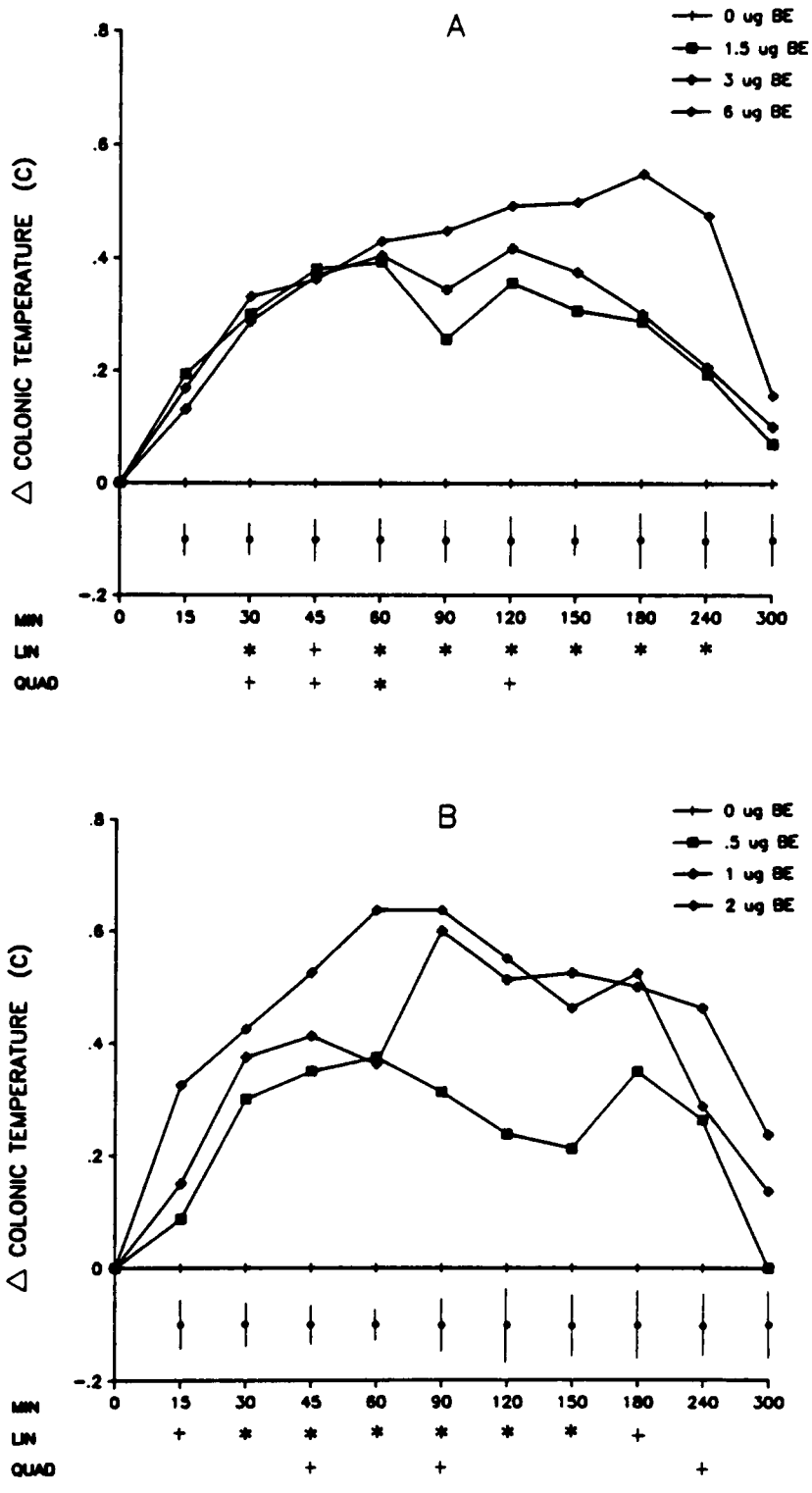

FIG. 5. Change (Δ) in colonic temperature (C; adjusted to control = 0 change) of Rock-Cornish cockerels following intracerebroventricular injection of β-endorphin (BE); MIN, minutes; LIN, linear contrast; QUAD, quadratic contrast; (ϕ) Standard error of the treatment mean; + $P \leq .05$; * $P \leq .01$.

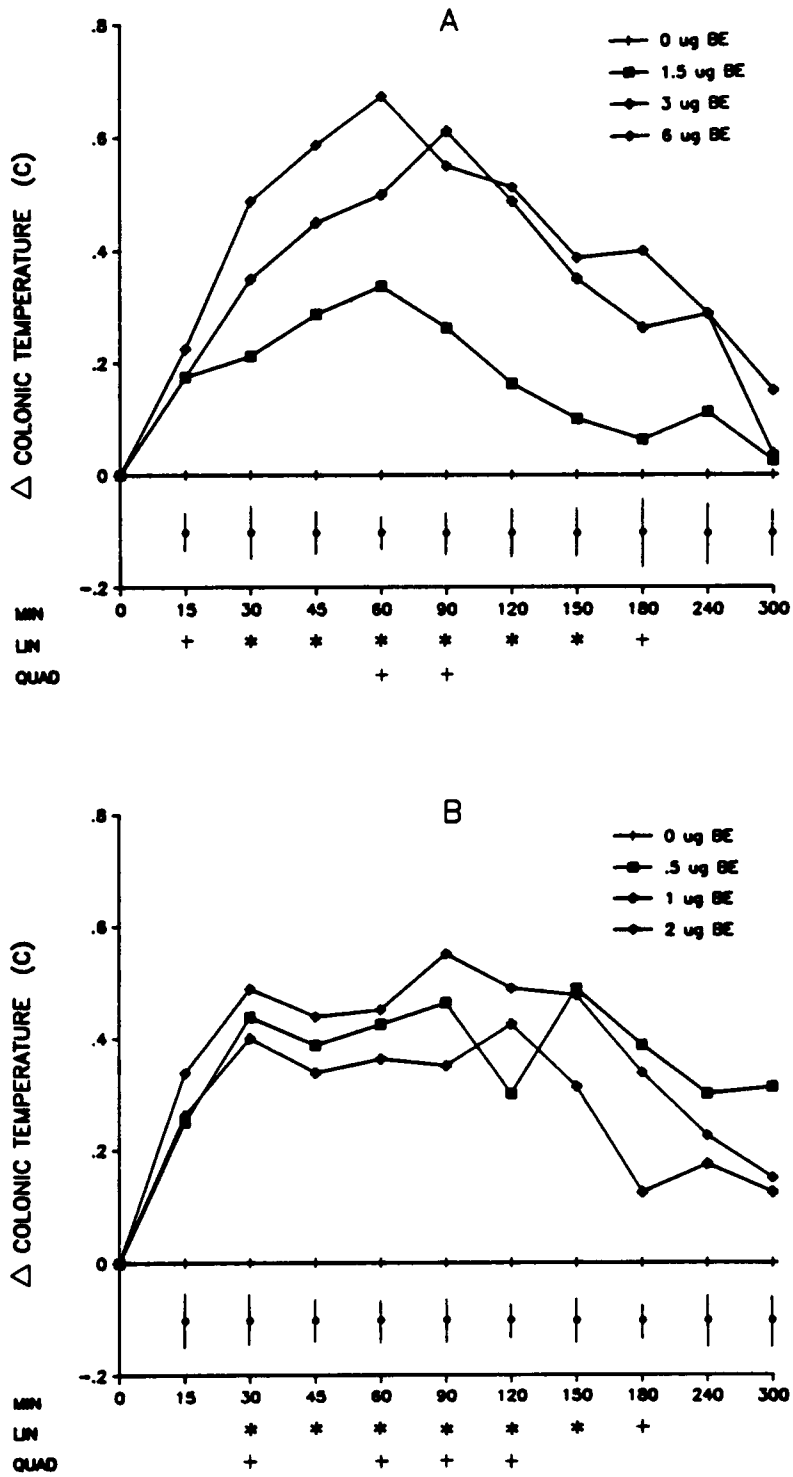

FIG. 6. Change (Δ) in colonic temperature (C; adjusted to control = 0 change) of Single-Comb White Leghorn cockerels following intracerebroventricular injection of β-endorphin (BE); MIN, minutes; LIN, linear contrast; QUAD, quadratic contrast; (⧫)Standard error of the treatment mean; + $P \leq .05$; * $P \leq .01$.

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General Synthesis

The six studies which comprise this dissertation characterize several aspects of opioid regulation of ingestive behavior in the domestic fowl. These experiments indicate that endogenous opioid peptides act at sites within the central nervous system (CNS) and peripheral tissues to influence the food and water consumption of meat (Rock-Cornish; RC) and egg (Single-Comb White Leghorn; SCWL) stocks of chickens. As with most scientific endeavors these studies have provoked additional questions and opened new avenues of inquiry.

The initial experiments of this investigation were conducted using opioid antagonists. These compounds bind to opioid receptors and, while blocking the effects of endogenous opioid peptides, elicit little if any effect of their own (Blumberg and Dayton, 1973). Therefore, alterations in behavior following administration of these compounds may be attributed to interruption in the activity of an endogenous opioid system. Administration of the opioid antagonists, naloxone and naltrexone, decreased food and water consumption in RC and SCWL stocks. When measured independent of food intake, water intake in normally hydrated and saline-loaded chicks was attenuated by naloxone. Therefore, naloxone-induced depressions in water intake were not merely an artifact of decreased food intake, but were due to blockade of opioid receptors. Accordingly, the attenuation of ingestive behavior produced by these opioid antagonists indicates that food and water intake in RC and SCWL are independently influenced by endogenous opioid peptides.

Genetic selection of the domestic fowl for meat or egg production has resulted in the development of stocks which differ greatly in both growth rate and body composition. However, the naloxone-sensitive opioid systems which influence ingestive behavior in these stocks does not appear to have been altered. When RC and SCWL stocks were tested at the same age or similar body weight there was no difference in the efficacy of naloxone in attenuating ingestive behavior. These results differ from those obtained with genetically obese and lean mice in which obesity is associated with increased sensitivity to opioid agonists and antagonists (Margules *et al.*, 1978; Fergusun-Segall *et al.*, 1982). However, it is not known whether these alterations in opioid mechanisms are a cause

or an effect of obesity (Gunion and Peters, 1981). Rossier *et al.* (1979) reported that increases in pituitary [Leu^5]-enkephalin where correlated with obesity whereas, pituitary β-endorphin increased as a consequence of obesity. Furthermore, Gunion and Peters (1981) have proposed that differential sensitivity to naloxone is dependent upon differences in body weight and composition. Therefore, it is possible that the differential naloxone sensitivity demonstrated by obese and lean rats is attributable to interruption in the activity of obesity-induced opioid systems. Since RC and SCWL chickens do not normally demonstrate the great disparity in percent body fat that obese and lean rats do, it would be interesting to compare the naloxone sensitivity of RC and SCWL stocks when differences in percent body fat were similar to that of obese and lean rats.

Opioid antagonists appear to influence food intake at sites outside the blood-brain barrier (bbb) since intracerebroventricular (ICV) administration of naloxone does not alter food intake. Intramuscular (im) administration of its quaternary congener, which does not cross the bbb, attenuates food intake. Water intake, on the other hand, was attenuated by ICV injection of naloxone and im injection of its quaternary congener, suggesting that water intake may be regulated at sites within and outside the bbb.

These results suggest that opioids may stimulate food consumption at sites outside the bbb. The mechanism(s) by which this occurs remains to be elucidated. Two possible areas of investigation should be explored. First, opioids may influence the release of hormones from the endocrine pancreas. There is evidence that opioid peptides increase the release of insulin and inhibit the release of somatostatin from the pancreas (Ipp *et al.*, 1978). Furthermore, increases in insulin (Silverstone and Kyriakides, 1982) and somatostatin (Lotter *et al.*, 1981) have been shown to have stimulatory and inhibitory effects on feeding, respectively. Therefore, opioid-induced alterations in these hormones may induce changes in food consumption. The second and perhaps more intriguing possibility for peripheral opioid action on feeding is that opioids interact with enteric hormones such as cholecystokinin (CCK). This theory was proposed by Faris (1985) and is supported by studies in which CCK and opioids were found to be mutually antagonistic (Faris *et al.*, 1983; Wilson *et al.*, 1983). The problem which has deferred acceptance of this hypothesis is that, in the rat, opioids are believed to influence ingestion solely through actions within the CNS, while

CCK influences feeding at sites outside the CNS. However, in the domestic fowl, CCK induces satiety when administered peripherally (Savory and Gentle, 1980) and centrally (Denbow and Myers, 1982). As the studies presented in this dissertation suggest, opioids act peripherally and in the CNS. Therefore, it would be interesting to determine if these peptides act as a continuously reciprocating system, communicating between the periphery and the CNS, to regulate feeding behavior.

While the ability of opioid antagonists to block numerous opioid receptor subtypes is useful for initial characterization of opioid regulated behaviors it also precludes determination of the opioid receptor subtype mediating the behavior. Therefore, in the latter studies of this investigation specific opioid receptor agonists were used to determine the identity of the opioid receptor subtypes mediating ingestive behavior.

The mu and delta subtypes were examined because they are the receptors for which naloxone has the highest and lowest affinity, respectively (Lord *et al.*, 1977). Mu agonists would be expected to influence many of the same behaviors as naloxone, but in an opposite manner, while delta agonists would be expected to reveal any behavioral effects which were not readily discernible by naloxone administration. ICV administration of the mu agonist morphiceptin stimulated drinking whereas im administration stimulated feeding. The delta receptor agonist [Met^5]-enkephalin stimulated feeding by both routes of administration. ICV administration of β-endorphin, which exhibits equal affinity for the mu and delta subtypes, induced increases in both feeding and drinking.

The results obtained with opioid agonists generally confirm the results of studies using opioid antagonists. The only discrepancy being the stimulation of food intake produced by ICV administration of the delta receptor agonist, [Met^5]-enkephalin. Central opioid regulation of feeding was probably not detected with antagonists because of the resistance of the delta receptor to naloxone blockade.

The delta opioid receptor's influence on food intake provokes an interesting question regarding the naloxone sensitivity of RC and SCWL stocks of chickens. The effect of genetic selection for meat or egg production on opioid regulation of ingestive behavior was evaluated using

naloxone. However, the delta opioid receptor, which mediates food intake, is resistant to naloxone antagonism. Therefore, it is possible that genetic selection for meat or egg production has induced changes in the influence of the delta opioid receptor which can not be detected by naloxone antagonism. Accordingly, it would be interesting to compare the sensitivity of RC and SCWL stocks to delta receptor agonists.

In conclusion, these studies provide evidence that opioid regulation of behavior in the domestic fowl is a multi-faceted phenomenon, involving a number of opioid receptors and peptides which act at sites within the central nervous system and peripheral tissues. There are several reasons why manipulation of these systems may become practical as a means by which food intake and growth in poultry may be controlled. First, there are vast number of commercially available compounds which may be used to either inhibit or stimulate opioid systems. Second, these systems are not exclusive to the CNS and are, therefore, readily accessible. Lastly, because opioids appear to influence food and water intake via separate receptors it would be possible to manipulate these behaviors independently.

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Appendix A Table 1. Analysis of variance for the effect of intramuscular injection of naloxone hydrochloride on food intake of Rock-Cornish cockerels (Chapter 1).

Source of variation	df	Sum of squares	F value	P
30 min				
Rep	1	92.45	5.76	.0189
Trt	3	306.55	6.36	.0007
Error	75	1204.55		
Total	79	1603.55		
Linear contrast	1	138.17	8.60	.0044
Quadratic contrast	1	168.34	10.48	.0018
60 min				
Rep	1	6.61	0.26	.6142
Trt	3	474.14	6.13	.0009
Error	75	1934.94		
Total	79	2415.69		
Linear contrast	1	238.39	9.24	.0033
Quadratic contrast	1	235.72	9.14	.0034
90 min				
Rep	1	15.31	0.33	.5668
Trt	3	788.14	5.68	.0015
Error	75	3469.04		
Total	79	4272.49		
Linear contrast	1	553.58	11.97	.0009
Quadratic contrast	1	228.61	4.94	.0292
120 min				
Rep	1	9.11	0.17	.6807
Trt	3	909.24	5.68	.0015
Error	75	4004.64		
Total	79	4922.99		
Linear contrast	1	636.51	11.92	.0009
Quadratic contrast	1	255.51	4.79	.0318
150 min				
Rep	1	7.20	0.10	.7525
Trt	3	1118.65	5.19	.0026
Error	75	390.10		
Total	79	6515.95		
Linear contrast	1	738.52	10.28	.0020
Quadratic contrast	1	341.19	4.75	.0325
180 min				
Rep	1	0.61	0.93	.9305
Trt	3	1069.84	4.46	.0061
Error	75	5990.74		
Total	79	7061.19		
Linear contrast	1	781.23	9.78	.0025
Quadratic contrast	1	237.97	2.98	.0885
210 min				
Rep	1	122.51	1.35	.2494
Trt	3	1030.64	3.78	.0139
Error	75	6819.34		
Total	79	7972.49		
Linear contrast	1	789.70	8.69	.0043
Quadratic contrast	1	154.18	1.70	.1968

240 min				
Rep	1	281.25	2.79	.0991
Trt	3	835.85	2.76	.0479
Error	75	7564.85		
Total	79	8681.95		
Linear contrast	1	609.16	6.04	.0163
Quadratic contrast	1	164.18	1.63	.2060
270 min				
Rep	1	661.25	6.68	.0117
Trt	3	1131.10	3.81	.0134
Error	75	7421.85		
Total	79	9214.20		
Linear contrast	1	873.61	8.83	.0040
Quadratic contrast	1	151.07	1.53	.2205
300 min				
Rep	1	806.45	8.28	.0052
Trt	3	992.50	3.40	.0221
Error	75	7305.85		
Total	79	9104.80		
Linear contrast	1	633.65	6.50	.0128
Quadratic contrast	1	270.11	2.77	.1000
1440 min				
Rep	1	1312.20	2.68	.1055
Trt	3	1717.05	1.17	.3265
Error	75	36654.70		
Total	79	39683.95		
Linear contrast	1	393.75	0.81	.3723
Quadratic contrast	1	1301.41	2.66	.1069

Appendix A Table 2. Analysis of variance for the effect of intramuscular injection of naloxone hydrochloride on water intake of Rock-Cornish cockerels (Chapter 1).

Source of variation	df	Sum of squares	F value	P
30 min				
Rep	1	661.25	3.11	.0816
Trt	3	472.50	0.74	.5304
Error	75	15921.25		
Total	79	17055.00		
Linear contrast	1	217.29	1.02	.3149
Quadratic contrast	1	2.92	0.01	.9069
60 min				
Rep	1	690.31	2.48	.1194
Trt	3	3873.44	4.64	.0050
Error	75	20860.94		
Total	79	25424.69		
Linear contrast	1	2223.22	7.99	.0060
Quadratic contrast	1	815.93	2.93	.0909
90 min				
Rep	1	320.00	1.04	.3113
Trt	3	7023.00	7.60	.0002
Error	75	23095.00		
Total	79	30438.75		
Linear contrast	1	4201.75	13.64	.0004
Quadratic contrast	1	1629.61	5.29	.0242
120 min				
Rep	1	245.00	0.62	.4349
Trt	3	7581.25	6.36	.0007
Error	75	29812.50		
Total	79	37638.75		
Linear contrast	1	5685.75	14.30	.0003
Quadratic contrast	1	1432.35	3.60	.0615
150 min				
Rep	1	227.81	0.51	.4784
Trt	3	13668.44	10.15	.0001
Error	75	33663.44		
Total	79	47559.69		
Linear contrast	1	8592.51	19.14	.0001
Quadratic contrast	1	4314.78	9.61	.0027
180 min				
Rep	1	254.00	0.43	.5145
Trt	3	11636.25	6.79	.0004
Error	75	42837.50		
Total	79	54718.75		
Linear contrast	1	6330.04	11.08	.0014
Quadratic contrast	1	4926.68	8.63	.0044
210 min				
Rep	1	7.81	0.01	.9163
Trt	3	9010.94	4.27	.0077
Error	75	52715.94		
Total	79	61734.69		
Linear contrast	1	5474.01	7.79	.0067
Quadratic contrast	1	2665.74	3.79	.0552

240 min				
Rep	1	0	0	1.0000
Trt	3	6.25	4.47	.0061
Error	75	822.50		
Total	79	71698.75		
Linear contrast	1	5685.75	7.01	.0099
Quadratic contrast	1	3743.12	4.62	.0349
270 min				
Rep	1	661.25	0.77	.3835
Trt	3	9472.50	3.67	.0159
Error	75	64546.25		
Total	79	74680.00		
Linear contrast	1	5266.29	6.12	.0156
Quadratic contrast	1	3568.75	4.15	.0452
300 min				
Rep	1	911.25	0.87	.3547
Trt	3	9921.25	3.15	.0299
Error	75	78806.25		
Total	79	89638.75		
Linear contrast	1	3960.32	3.77	.0560
Quadratic contrast	1	5365.89	5.11	.0267
1440 min				
Rep	1	7411.25	0.88	.3524
Trt	3	31187.50	1.23	.3055
Error	75	634896.25		
Total	79	673495.00		
Linear contrast	1	5049.14	0.60	.4424
Quadratic contrast	1	20035.06	2.37	.1282

Appendix A Table 3. Analysis of variance for the effect of intramuscular injection of naloxone hydrochloride on food intake of Single-Comb White Leghorn cockerels (Chapter 1).

Source of variation	df	Sum of squares	F value	P
30 min				
Rep	1	4.51	2.11	.1503
Trt	3	23.24	3.63	.0167
Error	75	160.24		
Total	79	187.99		
Linear contrast	1	17.44	8.16	.0055
Quadratic contrast	0.69	0.32	.5722	
60 min				
Rep	1	4.51	1.09	.2988
Trt	3	59.24	4.79	.0043
Error	75	309.14		
Total	79	372.89		
Linear contrast	1	44.50	10.80	.0015
Quadratic contrast	1	14.73	3.57	.0625
90 min				
Rep	1	9.11	1.72	.1931
Trt	3	93.44	5.89	.0012
Error	75	396.34		
Total	79	498.89		
Linear contrast	1	85.40	16.16	.0001
Quadratic contrast	1	7.60	1.44	.2341
120 min				
Rep	1	2.45	0.34	.5633
Trt	3	114.20	5.24	.0026
Error	75	545.15		
Total	79	661.80		
Linear contrast	1	89.29	12.28	.0008
Quadratic contrast	1	24.74	3.40	.0690
150 min				
Rep	1	0.61	0.09	.7708
Trt	3	81.04	3.77	.0140
Error	75	537.24		
Total	79	618.89		
Linear contrast	1	72.00	10.05	.0022
Quadratic contrast	1	5.83	0.81	.3697
180 min				
Rep	1	2.81	0.31	.5794
Trt	3	50.04	1.84	.1459
Error	75	680.54		
Total	79	733.39		
Linear contrast	1	36.34	4.01	.0490
Quadratic contrast	1	4.91	0.54	.4641
210 min				
Rep	1	6.61	0.79	.3773
Trt	3	34.94	1.39	.2517
Error	75	628.64		
Total	79	670.19		
Linear contrast	1	31.93	3.81	.0547
Quadratic contrast	1	0.52	0.06	.8040

240 min				
Rep	1	1.51	0.15	.6989
Trt	3	57.24	1.90	.1348
Error	75	752.14		
Total	79	810.89		
Linear contrast	1	44.50	4.44	.0385
Quadratic contrast	1	0.66	0.07	.7985
270 min				
Rep	1	4.51	0.48	.4927
Trt	3	36.34	1.28	.2884
Error	75	712.04		
Total	79	752.89		
Linear contrast	1	30.66	3.23	.0763
Quadratic contrast	1	0.38	0.04	.8428
300 min				
Rep	1	19.01	1.77	.1870
Trt	3	34.94	1.09	.3607
Error	75	803.94		
Total	79	857.89		
Linear contrast	1	17.76	1.66	.2020
Quadratic contrast	1	5.83	0.54	.4632
1440 min				
Rep	1	3112.51	48.18	.0001
Trt	3	341.64	1.76	.1615
Error	75	4818.74		
Total	79	8439.39		
Linear contrast	1	214.51	3.32	.0724
Quadratic contrast	1	59.74	0.92	.3393

Appendix A Table 4. Analysis of variance for the effect of intramuscular injection of naloxone hydrochloride on water intake of Single-Comb White Leghorn cockerels (Chapter 1).

Source of variation	df	Sum of squares	F value	P
30 min				
Rep	1	7.81	4.17	.0446
Trt	3	45.94	2.55	.0613
Error	75	450.94		
Total	79	504.69		
Linear contrast	1	25.08	4.17	.0446
Quadratic contrast	1	17.18	2.86	.0951
60 min				
Rep	1	15.31	1.05	.3082
Trt	3	390.94	8.96	.0001
Error	75	1090.94		
Total	79	1497.19		
Linear contrast	1	299.01	20.56	.0001
Quadratic contrast	1	91.23	6.27	.0144
90 min				
Rep	1	80.00	3.78	.0556
Trt	3	931.25	14.67	.0001
Error	75	1587.50		
Total	79	2598.75		
Linear contrast	1	814.32	38.47	.0001
Quadratic contrast	1	116.94	5.52	.0214
120 min				
Rep	1	11.25	0.34	.5600
Trt	3	907.50	9.22	.0001
Error	75	2461.25		
Total	79	3380.00		
Linear contrast	1	803.57	24.49	.0001
Quadratic contrast	1	31.23	0.95	.3324
150 min				
Rep	1	61.25	1.59	.2114
Trt	3	617.50	5.34	.0023
Error	75	2891.25		
Total	79	3570.00		
Linear contrast	1	585.14	15.18	.0002
Quadratic contrast	1	22.82	0.59	.4441
180 min				
Rep	1	125.00	2.56	.1136
Trt	3	536.25	3.67	.0159
Error	75	3657.50		
Total	79	4318.75		
Linear contrast	1	522.89	10.72	.0016
Quadratic contrast	1	0.99	0.02	.8873
210 min				
Rep	1	125.00	2.40	.1256
Trt	3	566.25	3.62	.0167
Error	75	3907.50		
Total	79	4598.75		
Linear contrast	1	505.75	9.71	.0026
Quadratic contrast	1	48.10	0.92	.3397

240 min				
Rep	1	11.25	0.18	.6728
Trt	3	450.00	2.40	.0734
Error	75	4688.00		
Total	79	5150.00		
Linear contrast	1	416.57	6.66	.0118
Quadratic contrast	1	33.23	0.53	.4683
270 min				
Rep	1	11.25	0.18	.6702
Trt	3	367.50	1.99	.1211
Error	75	4616.25		
Total	79	4995.00		
Linear contrast	1	289.29	4.70	.0333
Quadratic contrast	1	60.01	0.98	.3266
300 min				
Rep	1	15.31	0.23	.6349
Trt	3	563.44	2.79	.0456
Error	75	5050.94		
Total	79	5629.69		
Linear contrast	1	527.22	7.83	.0065
Quadratic contrast	1	27.29	0.41	.5264
1440 min				
Rep	1	2101.25	6.28	.0144
Trt	3	1745.00	1.74	.1646
Error	75	25083.75		
Total	79	28930.00		
Linear contrast	1	195.57	0.58	.4469
Quadratic contrast	1	10.52	0.03	.8597

Appendix A Table 5. Analysis of variance for the effect of intramuscular injection of naltrexone hydrochloride on food intake of Rock-Cornish cockerels (Chapter 1).

Source of variation	df	Sum of squares	F value	P
30 min				
Trt	3	74.47	4.67	.0074
Error	36	191.30		
Total	39	265.77		
Linear contrast	1	60.49	11.38	.0018
Quadratic contrast	1	13.47	2.53	.1201
60 min				
Trt	3	89.40	2.96	.0450
Error	36	362.20		
Total	39	451.60		
Linear contrast	1	74.98	7.45	.0097
Quadratic contrast	1	14.42	1.43	.2390
90 min				
Trt	271.80	7.27	.0006	
Error	36	448.60		
Total	39	720.40		
Linear contrast	1	199.13	15.98	.0003
Quadratic contrast	1	68.61	5.51	.0246
120 min				
Trt	3	460.00	9.51	.0001
Error	36	580.40		
Total	39	1040.40		
Linear contrast	1	314.93	19.53	.0001
Quadratic contrast	1	142.26	8.82	.0053
150 min				
Trt	3	899.50	11.41	.0001
Error	36	946.40		
Total	39	1845.90		
Linear contrast	1	555.66	21.14	.0001
Quadratic contrast	1	318.26	12.11	.0013
180 min				
Trt	3	1319.47	11.65	.0001
Error	36	1359.30		
Total	39	2678.77		
Linear contrast	1	798.03	21.14	.0001
Quadratic contrast	1	521.42	13.81	.0007
210 min				
Trt	3	1586.67	11.20	.0001
Error	36	1699.30		
Total	39	3285.97		
Linear contrast	1	982.81	20.82	.0001
Quadratic contrast	1	602.32	12.76	.0010
240 min				
Trt	3	1650.70	10.35	.0001
Error	36	1913.20		
Total	39	3563.90		
Linear contrast	1	1130.40	21.27	.0001
Quadratic contrast	1	519.11	9.77	.0035

270 min				
Trt	3	1769.90	9.20	.0001
Error	36	2309.20		
Total	39	4079.10		
Linear contrast	1	180.07	21.52	.0001
Quadratic contrast	1	389.14	6.07	.0187
300 min				
Trt	3	1849.47	9.08	.0001
Error	36	2444.50		
Total	39	4239.97		
Linear contrast	1	1442.31	21.24	.0001
Quadratic contrast	1	399.26	5.88	.0205
1440 min				
Trt	3	1695.00	1.26	.3037
Error	36	16182.60		
Total	39	17877.60		
Linear contrast	1	1134.00	2.52	.1210
Quadratic contrast	1	96.30	0.21	.6463

Appendix A Table 6. Analysis of variance for the effect of intramuscular injection of naltrexone hydrochloride on water intake of Rock-Cornish cockerels (Chapter 1).

Source of variation	df	Sum of squares	F value	P
30 min				
Trt	3	215.00	1.33	.2809
Error	36	1945.00		
Total	39	2160.00		
Linear contrast	1	48.29	0.89	.3508
Quadratic contrast	1	143.45	2.66	.1119
60 min				
Trt	3	712.50	3.74	.0194
Error	36	2285.00		
Total	39	2997.50		
Linear contrast	1	320.64	5.05	.0308
Quadratic contrast	1	390.08	6.15	.0180
90 min				
Trt	3	1720.00	6.79	.0010
Error	36	3040.00		
Total	39	4760.00		
Linear contrast	1	1098.29	13.01	.0009
Quadratic contrast	1	598.59	7.09	.0115
120 min				
Trt	3	3071.87	12.49	.0001
Error	36	2952.50		
Total	39	6024.37		
Linear contrast	1	1817.16	22.16	.0001
Quadratic contrast	1	1183.71	14.43	.0005
150 min				
Trt	3	3991.87	13.52	.0001
Error	36	3542.50		
Total	39	7534.50		
Linear contrast	1	2275.87	23.13	.0001
Quadratic contrast	1	1540.35	15.65	.0003
180 min				
Trt	3	5555.00	11.62	.0001
Error	36	5735.00		
Total	39	11290.00		
Linear contrast	1	3584.00	22.50	.0001
Quadratic contrast	1	1337.58	8.40	.0064
210 min				
Trt	3	5855.00	11.62	.0001
Error	36	6045.00		
Total	39	11900.00		
Linear contrast	1	3844.57	22.90	.0001
Quadratic contrast	1	1779.07	10.59	.0025
240 min				
Trt	3	6406.87	11.49	.0001
Error	36	6692.50		
Total	39	13099.37		
Linear contrast	1	4921.87	26.48	.0001
Quadratic contrast	1	1403.65	7.55	.0093

270 min				
Trt	3	6726.87	10.34	.0001
Error	36	7807.50		
Total	39	14534.37		
Linear contrast	1	5540.16	25.55	.0001
Quadratic contrast	1	1123.31	5.18	.0289
300 min				
Trt	3	6941.87	8.25	.0003
Error	36	10092.50		
Total	39	17034.37		
Linear contrast	1	5035.02	17.96	.0001
Quadratic contrast	1	1747.03	6.23	.0173
1440 min				
Trt	3	13202.50	1.20	.3222
Error	36	131585.00		
Total	39	144787.50		
Linear contrast	1	5480.64	1.50	.2287
Quadratic contrast	1	2507.75	0.69	.4130

Appendix A Table 7. Analysis of variance for the effect of intramuscular injection of naltrexone hydrochloride on food intake of Single-Comb White Leghorn cockerels (Chapter 1).

Source of variation	df	Sum of squares	F value	P
30 min				
Rep	1	0.45	0.08	.7748
Trt	3	87.65	5.35	.0023
Error	75	409.45		
Total	79	497.55		
Linear contrast	1	6.80	1.25	.2679
Quadratic contrast	1	27.69	5.07	.0272
60 min				
Rep	1	0.05	0.01	.9413
Trt	3	202.15	7.35	.0003
Error	75	687.75		
Total	79	889.95		
Linear contrast	1	35.21	3.84	.0538
Quadratic contrast	1	84.46	9.21	.0033
90 min				
Rep	1	0.05	0.00	.9494
Trt	3	281.35	7.61	.0002
Error	75	924.15		
Total	79	1205.55		
Linear contrast	1	74.92	6.08	.0160
Quadratic contrast	1	121.63	9.87	.0024
120 min				
Rep	1	0.20	0.01	.9042
Trt	3	392.70	9.54	.0001
Error	75	1028.90		
Total	79	1421.80		
Linear contrast	1	151.82	11.07	.0014
Quadratic contrast	1	159.58	11.63	.0010
150 min				
Rep	1	9.11	0.56	.4550
Trt	3	614.64	12.68	.0001
Error	75	1211.74		
Total	79	1835.49		
Linear contrast	1	286.08	17.71	.0001
Quadratic contrast	1	232.43	14.39	.0003
180 min				
Rep	1	48.05	2.52	.1166
Trt	3	706.90	12.36	.0001
Error	75	1430.25		
Total	79	2185.20		
Linear contrast	1	410.42	21.52	.0001
Quadratic contrast	1	207.33	10.87	.0015
210 min				
Rep	1	15.31	0.71	.4020
Trt	3	702.74	10.87	.0001
Error	75	1616.84		
Total	79	2334.89		
Linear contrast	1	432.93	20.08	.0001
Quadratic contrast	1	190.06	8.82	.0040

240 min				
Rep	1	27.61	1.12	.2923
Trt	3	830.84	11.28	.0001
Error	75	1840.94		
Total	79	2699.39		
Linear contrast	1	567.90	23.14	.0001
Quadratic contrast	1	169.31	6.90	.0105
270 min				
Rep	1	14.45	0.54	.4665
Trt	3	744.30	9.20	.0001
Error	75	2023.05		
Total	79	2781.80		
Linear contrast	1	524.62	19.45	.0001
Quadratic contrast	1	144.06	5.34	.0236
300 min				
Rep	1	84.05	2.71	.1040
Trt	3	742.50	7.97	.0001
Error	75	2327.65		
Total	79	3154.20		
Linear contrast	1	567.00	18.27	.0001
Quadratic contrast	1	114.99	3.71	.0580
1440 min				
Rep	1	1901.25	17.90	.0001
Trt	3	216.10	0.68	.5717
Error	75	7965.45		
Total	79	10082.80		
Linear contrast	1	159.37	1.50	.2244
Quadratic contrast	1	39.08	0.37	.5460

Appendix A Table 8. Analysis of variance for the effect of intramuscular injection of naltrexone hydrochloride on water intake in Single-Comb White Leghorn cockerels (Chapter 1).

Source of variation	df	Sum of squares	F value	P
30 min				
Rep	1	0.31	0.02	.8757
Trt	3	295.94	7.78	.0002
Error	75	950.94		
Total	79	1247.19		
Linear contrast	1	114.01	8.99	.0037
Quadratic contrast	1	130.94	10.33	.0019
60 min				
Rep	1	5.00	0.33	.5667
Trt	3	1001.15	22.10	.0001
Error	75	1132.75		
Total	79	2138.75		
Linear contrast	1	472.32	31.28	.0001
Quadratic contrast	1	403.73	26.74	.0001
90 min				
Rep	1	5.00	0.31	.5809
Trt	3	2013.75	41.27	.0001
Error	75	1220.00		
Total				
Linear contrast	1	972.32	59.77	.0001
Quadratic contrast	1	673.41	41.40	.0001
120 min				
Rep	1	15.31	0.55	.4604
Trt	3	2508.44	30.06	.0001
Error	75	2085.94		
Total	79	4609.69		
Linear contrast	1	1522.94	54.76	.0001
Quadratic contrast	1	668.77	24.05	.0001
150 min				
Rep	1	61.25	1.44	.2335
Trt	3	2703.75	21.23	.0001
Error	75	3183.75		
Total	79	5948.75		
Linear contrast	1	1712.89	40.35	.0001
Quadratic contrast	1	654.88	15.43	.0002
180 min				
Rep	1	37.81	0.58	.4476
Trt	3	3195.94	16.42	.0001
Error	75	4865.94		
Total	79	8099.69		
Linear contrast	1	2405.01	37.07	.0001
Quadratic contrast	1	548.92	8.46	.0048
210 min				
Rep	1	52.81	0.70	.4051
Trt	3	3655.94	16.17	.0001
Error	75	5650.94		
Total	79	9359.69		
Linear contrast	1	2423.58	32.17	.0001
Quadratic contrast	1	499.44	6.63	.0120

240 min				
Rep	1	245.00	3.07	.0836
Trt	3	2966.25	12.41	.0001
Error	75	5977.50		
Total	79	9188.75		
Linear contrast	1	2074.32	26.03	.0001
Quadratic contrast	1	511.57	6.42	.0134
270 min				
Rep	1	781.25	7.52	.0076
Trt	3	3516.25	11.28	.0001
Error	75	7791.25		
Total	79	12088.75		
Linear contrast	1	2740.32	26.38	.0001
Quadratic contrast	1	361.56	3.48	.0660
300 min				
Rep	1	1087.81	10.27	.0020
Trt	3	3438.44	10.82	.0001
Error	75	7945.94		
Total	79	12472.19		
Linear contrast	1	2386.51	22.53	.0001
Quadratic contrast	1	266.08	2.51	.1172
1440 min				
Rep	1	9452.81	36.49	.0001
Trt	3	4300.94	1.78	.1573
Error	75	60530.94		
Total	79	94284.69		
Linear contrast	1	8.58	0.01	.9182
Quadratic contrast	1	2970.91	3.68	.0588

Appendix A Table 9. Analysis of variance for the effect of naloxone hydrochloride on water intake in Rock-Cornish cockerels with and without saline pre-loads (data transformed to $\sqrt{Y} + .5$) (Chapter 1).

Source of variation	df	Sum of squares	F value	P
30 min				
Rep	1	2.67	0.93	.3425
Trt	3	49.88	5.77	.0026
Error	35	100.77		
Total	39	153.32		
A vs B	1	5.17	1.80	.1889
C vs D	1	44.64	15.50	.0004
AB vs CD	1	0.07	0.02	.8798
60 min				
Rep	1	0.00	0.00	.9680
Trt	3	111.28	12.04	.0001
Error	35	107.86		
Total	39	219.14		
A vs B	1	13.34	4.33	.0448
C vs D	1	93.40	30.31	.0001
AB vs CD	1	4.54	1.47	.2330
90 min				
Rep	1	8.06	3.05	.0897
Trt	3	197.07	24.83	.0001
Error	35	92.59		
Total	39	297.72		
A vs B	1	24.45	9.24	.0045
C vs D	1	155.82	58.90	.0001
AB vs CD	1	16.79	6.35	.0165
120 min				
Rep	1	7.49	2.68	.1108
Trt	3	198.93	23.71	.0001
Error	35	97.90		
Total	39	304.32		
A vs B	1	28.21	10.09	.0031
C vs D	1	153.84	55.00	.0001
AB vs CD	1	16.89	6.04	.0191
150 min				
Rep	1	25.01	3.44	.0719
Trt	3	140.56	6.45	.0014
Error	35	254.23		
Total	39	419.80		
A vs B	1	32.90	4.53	.0404
C vs D	1	71.76	9.88	.0034
AB vs CD	1	35.90	4.94	.0328
180 min				
Rep	1	10.24	0.93	.3415
Trt	3	135.03	4.09	.0137
Error	35	385.27		
Total	39	530.54		
A vs B	1	38.86	3.53	.0686
C vs D	1	26.00	2.36	.1333
AB vs CD	1	70.17	6.37	.0163

210 min				
Rep	1	30.67	2.53	.1205
Trt	3	106.78	2.94	.0466
Error	35	423.82		
Total	39	561.27		
A vs B	1	25.51	2.11	.1555
C vs D	1	8.13	0.67	.4182
AB vs CD	1	73.14	6.04	.0191
240 min				
Rep	1	17.14	1.35	.2530
Trt	3	108.89	2.86	.0508
Error	35	444.14		
Total	39	570.17		
A vs B	1	13.68	1.08	.3062
C vs D	1	0.81	0.06	.8015
AB vs CD	1	94.40	7.44	.0099
270 min				
Rep	1	16.75	1.32	.2589
Trt	3	106.28	2.79	.0551
Error	35	445.16		
Total	39	568.19		
A vs B	1	9.23	0.73	.4000
C vs D	1	0.09	0.01	.9343
AB vs CD	1	96.96	7.62	.0091
300 min				
Rep	1	6.02	0.56	.4581
Trt	3	143.34	4.47	.0093
Error	35	374.31		
Total	39	523.67		
A vs B	1	7.52	0.68	.4157
C vs D	1	5.65	0.53	.4722
AB vs CD	1	130.44	12.20	.0013

Appendix B Table 1. Analysis of variance for the effect of intramuscular injection of naloxone hydrochloride on food intake of Rock-Cornish and Single-Comb White Leghorn cockerels of the same age (Chapter 2).

Source of variation	df	Sum of squares	F value	P
30 min				
Drug Trt	1	136.90	20.54	.0001
Stock	1	184.90	27.74	.0001
Error	37	246.60		
Total	39	568.40		
60 min				
Drug Trt	1	156.02	15.02	.0004
Stock	1	297.02	28.60	.0001
Error	37	384.33		
Total	39	837.37		
90 min				
Drug Trt	1	202.50	12.26	.0012
Stock	1	462.40	28.00	.0001
Error	37	611.10		
Total	39	1276.00		
120 min				
Drug Trt	1	235.22	13.25	.0008
Stock	1	714.02	40.23	.0001
Error	37	656.73		
Total	39	1605.97		
150 min				
Drug Trt	1	164.02	11.37	.0018
Stock	1	1311.02	90.89	.0001
Error	37	533.73		
Total	39	2008.77		
180 min				
Drug Trt	1	198.02	11.21	.0019
Stock	1	1651.22	93.46	.0001
Error	37	653.73		
Total	39	2502.97		
210 min				
Drug Trt	1	255.02	11.82	.0015
Stock	1	20002.22	92.77	.0001
Error	37	798.53		
Total	39	3055.77		
240 min				
Drug Trt	1	260.10	8.50	.0060
Stock	1	2433.60	79.53	.0001
Error	37	1132.20		
Total	39	3825.90		
270 min				
Drug Trt	1	308.02	9.37	.0041
Stock	1	3294.22	100.19	.0001
Error	37	1216.53		
Total	39	4818.77		
300 min				
Drug Trt	1	280.90	8.69	.0055
Stock	1	4040.10	124.92	.0001
Error	37	1196.60		
Total	39	5517.60		
1440 min				
Drug Trt	1	313.60	0.62	.4347
Stock	1	61465.60	122.27	.0001
Error	37	18600.30		
Total	39	80379.50		

Appendix B Table 2. Analysis of variance for the effect of intramuscular injection of naloxone hydrochloride on water intake of Rock-Cornish and Single-Comb White Leghorn cockerels of the same age (Chapter 2).

Source of variation	df	Sum of squares	F value	P
30 min				
Drug Trt	1	2.50	0.19	.6640
Stock	1	62.50	4.79	.0350
Error	37	482.50		
Total	39	547.50		
60 min				
Drug Trt	1	422.50	12.74	.0010
Stock	1	360.00	10.85	.0022
Error	37	1227.50		
Total	39	2010.00		
90 min				
Drug Trt	1	422.50	5.55	.0239
Stock	1	1000.00	13.13	.0009
Error	37	2817.50		
Total	39	4240.00		
120 min				
Drug Trt	1	680.62	6.28	.0168
Stock	1	1500.62	13.84	.0007
Error	37	4013.13		
Total	39	6194.37		
150 min				
Drug Trt	1	360.00	3.96	.0542
Stock	1	2250.00	24.72	.0001
Error	37	3367.50		
Total	39	5977.50		
180 min				
Drug Trt	1	225.62	2.46	.1253
Stock	1	4305.62	46.95	.0001
Error	37	3393.13		
Total	39	7924.37		
210 min				
Drug Trt	1	275.62	2.78	.1037
Stock	1	5405.62	54.60	.0001
Error	37	3663.13		
Total	39	9344.37		
240 min				
Drug Trt	1	302.50	1.78	.1902
Stock	1	7290.00	42.92	.0001
Error	37	6285.00		
Total	39	13877.50		
270 min				
Drug Trt	1	302.50	1.50	.2286
Stock	1	10240.00	50.74	.0001
Error	37	7467.50		
Total	39	18010.00		
300 min				
Drug Trt	1	330.62	1.28	.2653
Stock	1	3140.62	50.84	.0001
Error	37	9563.13		
Total	39	23034.37		
1440 min				
Drug Trt	1	105.62	0.02	.8775
Stock	1	273075.62	62.30	.0001
Error	37	162178.13		
Total	39	435359.37		

Appendix B Table 3. Analysis of variance for the effect of intramuscular injection of naloxone hydrochloride on food intake of Rock-Cornish and Single-Comb White Leghorn cockerels of similar weight (Chapter 2).

Source of variation	df	Sum of squares	F value	P
30 min				
Drug Trt	1	28.90	7.94	.0077
Stock	1	40.00	10.99	.0021
Error	37	134.70		
Total	39	203.60		
60 min				
Drug Trt	1	105.62	20.34	.0001
Stock	1	133.22	25.66	.0001
Error	37	192.13		
Total	39	430.97		
90 min				
Drug Trt	1	81.22	12.86	.0010
Stock	1	126.02	19.95	.0001
Error	37	233.73		
Total	39	440.97		
120 min				
Drug Trt	1	65.02	5.79	.0212
Stock	1	180.62	16.09	.0003
Error	37	415.33		
Total	39	660.97		
150 min				
Drug Trt	1	78.40	4.52	.0403
Stock	1	136.90	7.89	.0079
Error	37	642.30		
Total	39	857.60		
180 min				
Drug Trt	1	46.22	2.15	.1508
Stock	1	189.22	8.81	.0052
Error	37	794.53		
Total	39	1029.97		
210 min				
Drug Trt	1	70.22	2.99	.0923
Stock	1	255.02	10.84	.0022
Error	37	870.13		
Total	39	1195.37		
240 min				
Drug Trt	1	16.90	0.52	.4775
Stock	1	168.10	5.12	.0296
Error	37	1214.10		
Total	39	1399.10		
270 min				
Drug Trt	1	42.02	1.36	.2516
Stock	1	286.22	9.24	.0043
Error	37	1146.13		
Total	39	1474.37		
300 min				
Drug Trt	1	44.10	1.06	.3089
Stock	1	291.60	7.04	.0117
Error	37	1532.70		
Total	39	1868.40		
1440 min				
Drug Trt	1	13.22	0.04	.8333
Stock	1	12006.22	40.78	.0001
Error	37	10894.33		
Total	39	22913.77		

Appendix B Table 4. Analysis of variance for the effect of intramuscular injection of naloxone hydrochloride on water intake of Rock-Cornish and Single-Comb White Leghorn cockerels of similar weight (Chapter 2).

Source of variation	df	Sum of squares	F value	P
30 min				
Drug Trt	1	855.62	15.92	.0003
Stock	1	105.62	1.97	.1692
Error	37	1988.13		
Total	39	2949.37		
60 min				
Drug Trt	1	902.50	13.64	.0007
Stock	1	360.00	5.44	.0252
Error	37	2447.50		
Total	39	3710.00		
90 min				
Drug Trt	1	2890.00	31.27	.0001
Stock	1	1000.00	10.82	.0022
Error	37	3420.00		
Total	39	7310.00		
120 min				
Drug Trt	1	2890.00	23.37	.0001
Stock	1	1562.50	12.64	.0011
Error	37	4575.00		
Total	39	9027.50		
150 min				
Drug Trt	1	1822.50	7.72	.0085
Stock	1	1822.50	7.72	.0085
Error	37	8732.50		
Total	39	12377.50		
180 min				
Drug Trt	1	1322.50	4.78	.0353
Stock	1	1440.00	5.20	.0285
Error	37	10247.50		
Total	39	13010.00		
210 min				
Drug Trt	1	1155.62	4.12	.0496
Stock	1	1500.62	5.35	.0264
Error	37	10378.13		
Total	39	13034.37		
240 min				
Drug Trt	1	855.62	2.23	.1434
Stock	1	950.62	2.48	.1236
Error	37	14168.13		
Total	39	15974.37		
270 min				
Drug Trt	1	562.50	1.14	.2931
Stoc	1	640.00	1.29	.2626
Error	37	18295.00		
Total	39	19497.50		
300 min				
Drug Trt	1	562.50	1.14	.2931
Stock	1	640.00	1.29	.2626
Error	37	18295.00		
Total	39	19497.50		
1440 min				
Drug Trt	1	302.50	0.58	.4494
Stock	1	1562.50	3.02	.0906
Error	37	19145.00		
Total	39	21010.00		

Appendix C Table 1. Analysis of variance for the effect of intramuscular injection of naloxone hydrochloride on food intake in male Japanese quail (Chapter 3).

Source of variation	df	Sum of squares	F value	P
30 min				
Block	7	2.75	1.81	.0017
Trt	3	3.59	5.50	.0017
Error	85	18.48		
Total	95	24.82		
Linear contrast	1	1.57	7.22	.0087
Quadratic contrast	1	1.86	8.55	.0044
60 min				
Block	7	9.47	3.62	.0018
Trt	3	4.32	3.86	.0018
Error	85	31.75		
Total	95	45.54		
Linear contrast	1	1.34	3.58	.0618
Quadratic contrast	1	2.97	7.96	.0060
120 min				
Block	7	18.46	3.98	.0008
Trt	3	7.16	3.61	.0166
Error	85	56.33		
Total	95	81.95		
Linear contrast	1	2.45	3.69	.0580
Quadratic contrast	1	4.69	7.08	.0093
180 min				
Block	7	31.74	4.54	.0002
Trt	3	9.56	3.19	.0276
Error	85	84.86		
Total	95	126.16		
Linear contrast	1	1.64	1.64	.2039
Quadratic contrast	1	7.93	7.94	.0060
240 min				
Block	7	44.18	5.19	.0001
Trt	3	11.12	3.05	.0330
Error	85	103.38		
Total	95	158.68		
Linear contrast	1	0.92	0.76	.3873
Quadratic contrast	1	10.04	8.26	.0051
300 min				
Block	7	55.80	4.61	.0002
Trt	3	12.93	2.49	.0656
Error	85	147.01		
Total	95	215.74		
Linear contrast	1	0.59	0.34	.5621
Quadratic contrast	1	12.19	7.05	.0095

Appendix C Table 2. Analysis of variance for the effect of intramuscular injection of naloxone hydrochloride on water intake in male Japanese quail (Chapter 3).

Source of variation	df	Sum of squares F	value	P
30 min				
Block	7	23.32	1.99	.0656
Trt	3	0.92	0.15	.9290
Error	85	172.67		
Total	95	201.91		
Linear contrast	1	0.44	0.22	.6426
Quadratic contrast	1	0.04	0.02	.8899
60 min				
Block	7	65.00	2.96	.0079
Trt	3	2.95	0.31	.8152
Error	85	266.49		
Total	95	334.44		
Linear contrast	1	1.16	0.37	.5440
Quadratic contrast	1	1.16	0.37	.5442
120 min				
Block	7	80.43	1.64	.1351
Trt	3	1.09	0.05	.9843
Error	85	595.40		
Total	95	676.92		
Linear contrast	1	0.81	0.12	.7352
Quadratic contrast	1	0.25	0.04	.8517
180 min				
Block	7	133.70	1.55	.1603
Trt	3	0.15	0	.9996
Error	85	1044.50		
Total	95	1178.35		
Linear contrast	1	0.02	0	.9700
Quadratic contrast	1	0.12	0.01	.9210
240 min				
Block	7	206.68	1.80	.0982
Trt	3	0.64	0.01	.9980
Error	85	1396.01		
Total	95	1603.33		
Linear contrast	1	0.43	0.03	.8725
Quadratic contrast	1	0.18	0.01	.9165
300 min				
Block	7	200.79	1.20	.3115
Trt	3	4.05	0.06	.9822
Error	85	2031.08		
Total	95	2235.92		
Linear contrast	1	0.65	0.03	.8697
Quadratic contrast	1	1.02	0.04	.8366

Appendix D Table 1. Analysis of variance for the effect of intramuscular injections of naloxone hydrochloride or naloxone methobromide on food intake in Rock-Cornish cockerels (Chapter 4).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	2	9.92	0.76	.4724
Bird (Rep)	12	159.28	2.04	.0428
Day (Rep)	12	169.68	2.17	.0306
Trt	4	327.07	12.58	.0001
Error	44	286.05		
Total	74	952.00		
Linear contrast	1	320.19	49.25	.0001
Quadratic contrast	1	1.41	0.22	.6441
30 min				
Rep	2	40.51	1.89	.1628
Bird (Rep)	12	186.16	1.45	.1806
Day (Rep)	12	251.36	1.96	.0529
Trt	4	445.73	10.41	.0001
Error	44	470.91		
Total	74	1394.67		
Linear contrast	1	423.45	39.57	.0001
Quadratic contrast	1	3.99	0.37	.5446
45 min				
Rep	2	176.99	10.25	.0002
Bird (Rep)	12	231.76	2.24	.0262
Day (Rep)	12	353.76	3.41	.0014
Trt	4	417.41	12.08	.0001
Error	44	380.03		
Total	74	1559.95		
Linear contrast	1	399.55	46.26	.0001
Quadratic contrast	1	7.54	0.87	.3551
60 min				
Rep	2	163.28	7.91	.0012
Bird (Rep)	12	332.24	2.68	.0085
Day (Rep)	12	425.44	3.44	.0013
Trt	4	457.39	11.08	.0001
Error	44	453.97		
Total	74	1832.32		
Linear contrast	1	437.94	42.45	.0001
Quadratic contrast	1	10.27	1.00	.3240
90 min				
Rep	2	270.11	9.49	.0004
Bird (Rep)	12	599.76	3.51	.0011
Day (Rep)	12	395.36	2.32	.0214
Trt	4	513.47	9.02	.0001
Error	44	625.97		
Total	74	2404.67		
Linear contrast	1	478.10	33.61	.0001
Quadratic contrast	1	30.86	2.17	.1479
120 min				
Rep	2	124.91	4.20	.0214
Bird (Rep)	12	656.08	3.68	.0007
Day (Rep)	12	442.08	2.48	.0143
Trt	4	561.39	9.43	.0001
Error	44	654.53		
Total	74	2438.99		
Linear contrast	1	514.06	34.56	.0001
Quadratic contrast	1	41.87	2.81	.1005

150 min				
Rep	2	251.31	6.84	.0026
Bird (Rep)	12	721.76	3.27	.0020
Day (Rep)	12	443.36	2.01	.0462
Trt	4	738.13	10.05	.0001
Error	44	808.11		
Total	74	2962.67		
Linear contrast	1	670.94	36.53	.0001
Quadratic contrast	1	48.00	2.61	.1131
180 min				
Rep	2	316.03	7.05	.0022
Bird (Rep)	12	968.32	3.60	.0009
Day (Rep)	12	687.92	2.56	.0117
Trt	4	867.01	9.67	.0001
Error	44	986.67		
Total	74	3825.95		
Linear contrast	1	744.05	33.18	.0001
Quadratic contrast	1	102.67	4.58	.0380
240 min				
Rep	2	570.56	10.95	.0001
Bird (Rep)	12	1190.16	3.81	.0005
Day (Rep)	12	942.56	3.01	.0037
Trt	4	971.52	9.32	.0001
Error	44	1146.32		
Total	74	4821.12		
Linear contrast	1	916.08	35.16	.0001
Quadratic contrast	1	53.33	2.05	.1596
300 min				
Rep	2	622.83	10.40	.0002
Bird (Rep)	12	1069.36	2.98	.0041
Day (Rep)	12	1278.56	3.56	.0010
Trt	4	955.92	7.98	.0001
Error	44	1317.52		
Total	74	5244.19		
Linear contrast	1	822.19	27.46	.0001
Quadratic contrast	1	131.43	4.39	.0420
1440 min				
Rep	2	1075.39	3.12	.0543
Bird (Rep)	12	8817.20	4.26	.0002
Day (Rep)	12	25635.20	12.38	.0001
Trt	4	600.45	0.87	.4897
Error	44	7594.75		
Total	74	43722.99		
Linear contrast	1	234.67	1.36	.2499
Quadratic contrast	1	94.41	0.55	.4635

Appendix D Table 2. Analysis of variance for the effect of intramuscular injections of naloxone hydrochloride or naloxone methobromide on food intake in Rock-Cornish cockerels (Chapter 4).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	2	50.00	3.80	.0300
Bird (Rep)	12	182.00	2.31	.0220
Day (Rep)	12	282.00	3.57	.0009
Trt	4	28.67	1.09	.3733
Error	44	289.33		
Total	74	832.00		
Linear contrast	1	0.05	0.01	.9326
Quadratic contrast	1	28.15	4.28	.0445
30 min				
Rep	2	258.00	5.50	.0236
Bird (Rep)	12	364.00	1.43	.1893
Day (Rep)	12	424.00	1.66	.1087
Trt	4	162.00	1.91	.1260
Error	44	934.00		
Total	74	2142.00		
Linear contrast	1	116.68	5.50	.0236
Quadratic contrast	1	12.51	0.59	.4468
45 min				
Rep	2	744.67	10.59	.0002
Bird (Rep)	12	594.00	1.41	.1988
Day (Rep)	12	474.00	1.12	.3667
Trt	4	688.67	4.90	.0024
Error	44	1547.33		
Total	74	4048.67		
Linear contrast	1	597.33	16.99	.0002
Quadratic contrast	1	13.26	0.38	.5424
60 min				
Rep	2	722.00	7.72	.0013
Bird (Rep)	12	1080.00	1.92	.0574
Day (Rep)	12	590.00	1.05	.4223
Trt	4	1572.00	8.40	.0001
Error	44	2058.00		
Total	74	6022.00		
Linear contrast	1	1092.96	23.37	.0001
Quadratic contrast	1	93.60	2.00	.1642
90 min				
Rep	2	816.67	9.52	.0004
Bird (Rep)	12	1690.00	3.28	.0019
Day (Rep)	12	800.00	1.55	.1413
Trt	4	3023.33	17.63	.0001
Error	44	1886.67		
Total	74	8216.67		
Linear contrast	1	2060.19	48.05	.0001
Quadratic contrast	1	370.40	8.64	.0052
120 min				
Rep	2	896.00	6.67	.0029
Bird (Rep)	12	2542.00	3.16	.0026
Day (Rep)	12	1152.00	1.43	.1888
Trt	4	3534.67	13.17	.0001
Error	44	2953.33		
Total	74	11078.00		
Linear contrast	1	2486.30	37.04	.0001
Quadratic contrast	1	519.06	7.73	.0080

150 min				
Rep	2	1474.67	8.00	.0008
Bird (Rep)	12	4264.00	4.05	.0003
Day (Rep)	12	1644.00	1.56	.1391
Trt	4	4335.33	12.35	.0001
Error	44	3860.67		
Total	74	15578.67		
Linear contrast	1	3432.96	39.13	.0001
Quadratic contrast	1	602.77	6.87	.0120
180 min				
Rep	2	2592.00	9.97	.0003
Bird (Rep)	12	5338.00	3.42	.0014
Day (Rep)	12	1358.00	0.87	.5813
Trt	4	4893.33	9.41	.0001
Error	44	5718.67		
Total	74	19900.00		
Linear contrast	1	4214.58	32.43	.0001
Quadratic contrast	1	358.48	2.76	.1039
240 min				
Rep	2	3570.67	8.91	.0006
Bird (Rep)	12	8208.00	3.41	.0014
Day (Rep)	12	2938.00	1.22	.2982
Trt	4	5738.67	7.16	.0002
Error	44	8813.33		
Total	74	29268.67		
Linear contrast	1	5014.30	25.03	.0001
Quadratic contrast	1	676.41	3.38	.0729
300 min				
Rep	2	5469.00	9.97	.0003
Bird (Rep)	12	11992.00	3.62	.0008
Day (Rep)	12	5092.00	1.54	.1467
Trt	4	6724.67	6.10	.0005
Error	44	12133.33		
Total	74	41438.00		
Linear contrast	1	6035.05	21.89	.0001
Quadratic contrast	1	639.06	2.32	.1351
1440 min				
Rep	2	28238.00	14.62	.0001
Bird (Rep)	12	68502.00	5.91	.0001
Day (Rep)	12	98072.00	8.46	.0001
Trt	4	1543.33	0.40	.8079
Error	44	42494.67		
Total	74	238850.00		
Linear contrast	1	198.11	0.21	.6528
Quadratic contrast	1	0.17	0	.9894

Appendix D Table 3. Analysis of variance for the effect of intracerebroventricular injection of naloxone hydrochloride on food intake in Rock-Cornish cockerels (Chapter 4).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	1	107.64	10.78	.0020
Day	7	351.23	5.02	.0003
Bird (Rep)	6	294.34	4.91	.0006
Trt	3	21.55	0.72	.5456
Error	46	459.35		
Total	63	1234.11		
Linear contrast	1	7.87	0.79	.3791
Quadratic contrast	1	0.14	0.01	.9076
30 min				
Rep	1	105.06	10.03	.0027
Day	7	646.50	8.82	.0001
Bird (Rep)	6	387.19	6.16	.0001
Trt	3	30.62	0.97	.4127
Error	46	481.63		
Total	63	1651.00		
Linear contrast	1	18.29	1.75	.1929
Quadratic contrast	1	1.20	0.11	.7364
45 min				
Rep	1	172.27	11.23	.0016
Day	7	996.36	9.28	.0001
Bird (Rep)	6	498.09	5.41	.0003
Trt	3	28.30	0.62	.6088
Error	46	705.46		
Total	63	2400.48		
Linear contrast	1	18.86	1.23	.2732
Quadratic contrast	1	0.06	0	.9495
60 min				
Rep	1	110.25	5.66	.0216
Day	7	1216.44	8.92	.0001
Bird (Rep)	6	440.44	3.77	.0039
Trt	3	32.81	0.56	.6431
Error	46	896.00		
Total	63	2695.94		
Linear contrast	1	18.86	0.97	.3302
Quadratic contrast	1	2.72	0.14	.7101
90 min				
Rep	1	264.06	11.46	.0015
Day	7	1262.75	7.83	.0001
Bird (Rep)	6	500.94	3.62	.0050
Trt	3	22.25	0.32	.8095
Error	46	1059.75		
Total	63	3109.75		
Linear contrast	1	7.87	0.34	.5616
Quadratic contrast	1	7.05	0.31	.5829
120 min				
Rep	1	293.27	12.04	.0011
Day	7	1641.86	9.63	.0001
Bird (Rep)	6	617.59	4.23	.0018
Trt	3	22.67	0.31	.8178
Error	46	1120.34		
Total	63	3695.73		
Linear contrast	1	0.07	0	.9570
Quadratic contrast	1	7.68	0.32	.5772

150 min				
Rep	1	462.25	15.44	.0003
Day	7	2428.00	11.59	.0001
Bird (Rep)	6	763.75	4.25	.0017
Trt	3	74.62	0.83	.4837
Error	46	1377.13		
Total	63	5105.75		
Linear contrast	1	7.14	0.24	.6275
Quadratic contrast	1	30.63	1.02	.3171
180 min				
Rep	1	540.56	15.20	.0003
Day	7	1882.94	7.57	.0001
Bird (Rep)	6	760.37	3.56	.0055
Trt	3	29.69	0.28	.8407
Error	46	1635.38		
Total	63	4848.94		
Linear contrast	1	4.02	0.11	.7383
Quadratic contrast	1	18.06	0.51	.4797
240 min				
Rep	1	370.56	9.06	.0042
Day	7	3547.19	12.39	.0001
Bird (Rep)	6	817.87	3.33	.0083
Trt	3	17.81	0.15	.9323
Error	46	1882.01		
Total 63 6635.44				
Linear contrast	1	1.29	0.03	.8598
Quadratic contrast	1	12.48	0.31	.5834
300 min				
Rep	1	805.14	18.91	.0001
Day	7	4044.86	13.57	.0001
Bird (Rep)	6	931.72	3.65	.0048
Trt	3	19.92	0.16	.9253
Error	46	1958.09		
Total	63	7759.73		
Linear contrast	1	1.00	0.02	.8786
Quadratic contrast	1	2.00	0.05	.8292
1440 min				
Rep	1	16512.25	93.21	.0001
Day	7	42257.00	34.08	.0001
Bird (Rep)	6	2209.75	2.08	.0741
Trt	3	247.87	0.47	.7071
Error	46	8149.13		
Total	63	69376.00		
Linear contrast	1	42.00	0.24	.6286
Quadratic contrast	1	200.09	1.13	.2934

Appendix D Table 4. Analysis of variance for the effect of intracerebroventricular injection of naloxone hydrochloride on water intake in Rock-Cornish cockerels (Chapter 4).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	1	56.25	1.19	.2807
Day	7	1168.75	3.54	.0040
Bird (Rep)	6	1018.75	3.60	.0052
Trt	3	278.12	1.96	.1327
Error	46	2171.88		
Total	63	4693.75		
Linear contrast	1	246.54	5.22	.0270
Quadratic contrast	1	0.40	0.01	.9271
30 min				
Rep	1	156.25	1.56	.2187
Day	7	3398.44	4.83	.0004
Bird (Rep)	6	2117.19	3.51	.0061
Trt	3	804.69	2.67	.0586
Error	46	4621.87		
Total	63	11098.44		
Linear contrast	1	714.29	7.11	.0105
Quadratic contrast	1	0.90	0.01	.9251
45 min				
Rep	1	3.52	0.03	.8594
Day	7	6758.98	8.72	.0001
Bird (Rep)	6	2805.47	4.22	.0018
Trt	3	616.80	1.86	.1504
Error	46	5096.09		
Total	63	15280.86		
Linear contrast	1	501.00	4.52	.0389
Quadratic contrast	1	46.17	0.42	.5217
60 min				
Rep	1	87.89	0.65	.4251
Day	7	8233.98	8.67	.0001
Bird (Rep)	6	3496.09	4.29	.0016
Trt	3	957.42	2.35	.0846
Error	46	6242.98		
Total	63	19018.36		
Linear contrast	1	884.04	6.51	.0141
Quadratic contrast	1	62.15	0.46	.5020
90 min				
Rep	1	47.27	0.32	.5773
Day	7	8787.11	8.37	.0001
Bird (Rep)	6	5389.84	5.99	.0001
Trt	3	829.30	1.84	.1526
Error	46	6899.21		
Total	63	21952.73		
Linear contrast	1	562.61	3.75	.0589
Quadratic contrast	1	140.47	0.94	.3382
120 min				
Rep	1	56.25	0.26	.6132
Day	7	11898.44	7.83	.0001
Bird (Rep)	6	5679.69	4.36	.0015
Trt	3	1101.56	1.69	.1820
Error	46	9987.50		
Total	63	28723.44		
Linear contrast	1	661.72	3.05	.0875
Quadratic contrast	1	304.62	1.40	.2423

150 min				
Rep	1	6.25	0.03	.8697
Day	7	11268.75	7.00	.0001
Bird (Rep)	6	6556.25	4.75	.0008
Trt	3	1096.87	1.59	.2044
Error	46	10571.88		
Total	63	29500.00		
Linear contrast	1	531.36	2.31	.1352
Quadratic contrast	1	560.23	2.44	.1253
180 min				
Rep	1	76.56	0.25	.6217
Day	7	18056.25	8.31	.0001
Bird (Rep)	6	7867.19	4.23	.0018
Trt	3	671.87	0.72	.5441
Error	46	14271.88		
Total	63	40943.75		
Linear contrast	1	290.29	0.94	.3385
Quadratic contrast	1	381.23	1.23	.2734
240 min				
Rep	1	425.39	0.76	.3881
Day	7	20749.61	5.29	.0002
Bird (Rep)	6	7424.22	2.21	.0591
Trt	3	1319.92	0.79	.5084
Error	46	25777.34		
Total	63	55696.48		
Linear contrast	1	0.45	0	.9776
Quadratic contrast	1	231.75	0.41	.5234
300 min				
Rep	1	3.52	0.01	.9317
Day	7	42633.98	12.86	.0001
Bird (Rep)	6	10342.97	3.64	.0049
Trt	3	566.80	0.40	.7544
Error	46	21783.59		
Total	63	75330.86		
Linear contrast	1	75.45	0.16	.6916
Quadratic contrast	1	427.11	0.90	.3472
1440 min				
Rep	1	5166.02	1.66	.2041
Day	7	346987.11	15.92	.0001
Bird (Rep)	6	127089.84	6.80	.0001
Trt	3	3444.92	0.37	.7758
Error	46	143221.09		
Total	63	625908.98		
Linear contrast	1	9.04	0	.9573
Quadratic contrast	1	652.69	0.21	.6492

Appendix D Table 5. Analysis of variance for the effect of intramuscular injection of naloxone hydrochloride at levels effective in attenuating water intake when administered intracerebroventricularly on food intake in Rock-Cornish cockerels (Chapter 4).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	1	1.53	0.19	.6662
Day	3	24.84	1.04	.3987
Bird (Rep)	6	131.44	2.75	.0446
Trt	3	23.59	0.99	.4207
Error	18	143.32		
Total	31	324.72		
Linear contrast	1	16.51	2.07	.1670
Quadratic contrast	1	0.98	0.12	.7302
30 min				
Rep	1	52.53	6.55	.0197
Day	3	47.34	1.97	.1549
Bird (Rep)	6	212.94	4.43	.0064
Trt	3	37.09	1.54	.2379
Error	18	144.32		
Total	31	494.22		
Linear contrast	1	27.01	3.37	.0830
Quadratic contrast	1	8.44	1.05	.3185
45 min				
Rep	1	55.12	5.74	.0276
Day	3	64.62	2.24	.1180
Bird (Rep)	6	235.75	4.09	.0092
Trt	3	50.62	1.76	.1911
Error	18	172.76		
Total	31	578.87		
Linear contrast	1	32.14	3.35	.0838
Quadratic contrast	1	17.32	1.80	.1958
60 min				
Rep	1	34.03	3.31	.0853
Day	3	40.84	1.33	.2969
Bird (Rep)	6	244.94	3.98	.0104
Trt	3	64.09	2.08	.1386
Error	18	184.82		
Total	31	568.72		
Linear contrast	1	30.04	2.93	.1044
Quadratic contrast	1	34.05	3.32	.0852
90 min				
Rep	1	22.78	1.16	.2949
Day	3	169.34	2.88	.0643
Bird (Rep)	6	222.69	1.90	.1367
Trt	3	68.59	1.17	.3494
Error	18	352.32		
Total	31	835.72		
Linear contrast	1	17.29	0.88	.3598
Quadratic contrast	1	50.22	2.57	.1266
120 min				
Rep	1	1.53	0.07	.7968
Day	3	74.34	1.11	.3728
Bird (Rep)	6	170.69	1.27	.3199
Trt	3	55.34	0.82	.4982
Error	18	403.57		
Total	31	705.47		
Linear contrast	1	20.57	0.92	.3508
Quadratic contrast	1	32.34	1.44	.2453

150 min				
Rep	1	12.50	0.65	.4314
Day	3	106.62	1.84	.1757
Bird (Rep)	6	79.87	0.69	.6605
Trt	3	61.62	1.06	.3887
Error	18	347.26		
Total	31	607.87		
Linear contrast	1	19.72	1.02	.3254
Quadratic contrast	1	38.79	2.10	.1733
180 min				
Rep	1	19.53	0.90	.3544
Day	3	233.59	3.60	.0338
Bird (Rep)	6	146.69	1.13	.3841
Trt	3	101.09	1.56	.2339
Error	18	389.07		
Total	31	889.97		
Linear contrast	1	27.01	1.25	.2783
Quadratic contrast	1	72.88	3.37	.0829
240 min				
Rep	1	10.12	0.53	.4772
Day	3	302.62	5.52	.0089
Bird (Rep)	6	176.75	1.53	.2237
Trt	3	39.62	0.69	.5713
Error	18	345.76		
Total	31	874.87		
Linear contrast	1	1.51	0.08	.7825
Quadratic contrast	1	36.83	1.92	.1831
300 min				
Rep	1	1.53	0.06	.8053
Day	3	96.59	1.32	.3002
Bird (Rep)	6	254.94	1.74	.1699
Trt	3	24.09	0.33	.8050
Error	18	440.57		
Total	31	817.72		
Linear contrast	1	0.01	0	.9850
Quadratic contrast	1	10.13	0.41	.5280
1440 min				
Rep	1	38.28	0.27	.6092
Day	3	4024.59	9.49	.0006
Bird (Rep)	6	3796.19	4.47	.0061
Trt	3	381.59	0.90	.4607
Error	18	2545.57		
Total	31	10786.22		
Linear contrast	1	4.32	0.03	.8632
Quadratic contrast	1	121.89	0.86	.3655

Appendix D Table 6. Analysis of variance for the effect of intramuscular injection of naloxone hydrochloride at levels effective in attenuating water intake when administered intracerebroventricularly on water intake in Rock-Cornish cockerels (Chapter 4).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	1	12.50	0.11	.7420
Day	3	43.75	0.13	.9407
Bird (Rep)	6	337.50	0.50	.7979
Trt	3	81.25	0.24	.8657
Error	18	2012.50		
Total	31	2487.50		
Linear contrast	1	14.29	0.13	.7249
Quadratic contrast	1	24.66	0.22	.6442
30 min				
Rep	1	153.12	1.27	.2737
Day	3	53.12	0.15	.9300
Bird (Rep)	6	718.75	1.00	.4569
Trt	3	434.37	1.21	.3363
Error	18	2162.51		
Total	31	3521.87		
Linear contrast	1	0.89	0.01	.9323
Quadratic contrast	1	67.15	0.56	.4643
45 min				
Rep	1	528.12	3.79	.0672
Day	3	68.75	0.16	.9188
Bird (Rep)	6	621.87	0.74	.6214
Trt	3	262.50	0.63	.6060
Error	18	2506.26		
Total	31	3987.50		
Linear contrast	1	57.14	0.41	.5298
Quadratic contrast	1	104.67	0.75	.3973
60 min				
Rep	1	1378.12	12.17	.0026
Day	3	253.12	0.75	.5390
Bird (Rep)	6	793.75	1.17	.3655
Trt	3	234.37	0.69	.5698
Error	18	2037.51		
Total	31	4696.87		
Linear contrast	1	0	0	1.0
Quadratic contrast	1	101.64	0.90	.3559
90 min				
Rep	1	1012.50	5.06	.0372
Day	3	1640.62	2.73	.0739
Bird (Rep)	6	1834.37	1.53	.2252
Trt	3	684.37	1.14	.3594
Error	18	3600.01		
Total	31	8771.87		
Linear contrast	1	8.03	0.04	.8434
Quadratic contrast	1	9.18	0.05	.8328
120 min				
Rep	1	1012.50	3.80	.0671
Day	3	1309.37	1.64	.2161
Bird (Rep)	6	2571.87	1.61	.2023
Trt	3	703.12	0.88	.4705
Error	18	4800.01		
Total	31	10396.87		
Linear contrast	1	8.03	0.03	.8641
Quadratic contrast	1	335.83	1.26	.2765

150 min				
Rep	1	2194.53	7.60	.0130
Day	3	2077.34	2.40	1016
Bird (Rep)	6	4829.69	2.79	.0425
Trt	3	1133.59	1.31	.3021
Error	18	5195.32		
Total	31	15430.47		
Linear contrast	1	3.57	0.01	.9127
Quadratic contrast	1	189.93	0.66	.4278
180 min				
Rep	1	4278.12	13.45	.0018
Day	3	2218.75	2.33	.1091
Bird (Rep)	6	6696.87	3.51	.0178
Trt	3	1631.25	1.71	.2008
Error	18	5725.01		
Total	31	20550.00		
Linear contrast	1	2.01	0.01	.9375
Quadratic contrast	1	185.17	0.58	.4553
240 min				
Rep	1	6612.50	16.79	.0006
Day	3	3106.25	2.66	.0795
Bird (Rep)	6	13512.50	5.78	.0017
Trt	3	2056.25	1.76	.1909
Error	18	7012.50		
Total	31	32300.00		
Linear contrast	1	289.29	0.74	.4002
Quadratic contrast	1	110.85	0.28	.6003
300 min				
Rep	1	6469.53	8.05	.0109
Day	3	1358.59	0.56	.6460
Bird (Rep)	6	20717.19	4.30	.0074
Trt	3	608.59	0.25	.8585
Error	18	14464.07		
Total	31	43617.97		
Linear contrast	1	150.89	0.19	.6699
Quadratic contrast	1	1.04	0	.9717
1440 min				
Rep	1	49219.53	40.15	.0001
Day	3	33002.34	8.97	.0007
Bird (Rep)	6	161173.44	21.91	.0001
Trt	3	3539.84	0.96	.4318
Error	18	22064.07		
Total	31	268999.22		
Linear contrast	1	1032.14	0.84	.3709
Quadratic contrast	1	955.77	0.78	.3889

Appendix E Table 1. Analysis of variance for the effect of intracerebroventricular injection of morphiceptin on food intake in Rock-Cornish cockerels (Chapter 5).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	1	90.25	12.73	.0006
Day	9	84.65	1.33	.2372
Bird (Rep)	8	1207.20	21.29	.0001
Trt	4	30.80	1.09	.3693
Error	77	545.85		
Total	99	1958.75		
Linear contrast	1	7.03	0.99	.3224
Quadratic contrast	1	1.02	0.14	.7049
30 min				
Rep	1	353.44	31.09	.0001
Day	9	140.36	1.37	.2156
Bird (Rep)	8	2609.52	28.69	.0001
Trt	4	20.46	0.45	.7721
Error	77	875.38		
Total	99	3999.16		
Linear contrast	1	1.71	0.15	.6991
Quadratic contrast	1	8.38	0.74	.3933
45 min				
Rep	1	524.41	25.55	.0001
Day	9	326.85	1.77	.0879
Bird (Rep)	8	2861.04	17.42	.0001
Trt	4	59.80	0.73	.5754
Error	77	1580.65		
Total	99	5352.75		
Linear contrast	1	3.64	0.18	.6746
Quadratic contrast	1	29.79	1.45	.2320
60 min				
Rep	1	665.64	23.28	.0001
Day	9	390.80	1.52	.1564
Bird (Rep)	8	2881.96	12.60	.0001
Trt	4	37.80	0.33	.8567
Error	77	2201.80		
Total	99	6178.00		
Linear contrast	1	1.12	0.04	.8433
Quadratic contrast	1	20.08	0.70	.4046
90 min				
Rep	1	635.04	17.44	.0001
Day	9	637.04	1.94	.0578
Bird (Rep)	8	3162.60	10.86	.0001
Trt	4	76.24	0.52	.7187
Error	77	2803.12		
Total	99	7314.04		
Linear contrast	1	1.44	0.04	.8426
Quadratic contrast	1	49.47	1.36	.2473
120 min				
Rep	1	457.96	10.01	.0022
Day	9	698.44	1.70	.1041
Bird (Rep)	8	3541.28	9.68	.0001
Trt	4	65.14	0.36	.8390
Error	77	3521.02		
Total	99	8283.84		
Linear contrast	1	11.28	0.25	.6208
Quadratic contrast	1	41.46	0.91	.3440

150 min				
Rep	1	745.29	15.90	.0002
Day	9	683.65	1.62	.1240
Bird (Rep)	8	3816.56	10.18	.0001
Trt	4	147.10	0.78	.5358
Error	77	3608.15		
Total	99	9000.75		
Linear contrast	1	0.40	0.01	.9262
Quadratic contrast	1	130.61	2.79	.0991
180 min				
Rep	1	676.00	13.42	.0005
Day	9	517.76	1.14	.3443
Bird (Rep)	8	3626.36	9.00	.0001
Trt	4	111.66	0.55	.6966
Error	77	3878.98		
Total	99	8810.76		
Linear contrast	1	3.51	0.07	.7925
Quadratic contrast	1	73.36	1.46	.2312
240 min				
Rep	1	470.89	7.47	.0078
Day	9	636.69	1.12	.3580
Bird (Rep)	8	3363.40	6.67	.0001
Trt	4	127.14	0.50	.7329
Error	77	4856.47		
Total	99	9454.59		
Linear contrast	1	8.40	0.13	.7161
Quadratic contrast	1	69.57	1.10	.2969
300 min				
Rep	1	453.69	5.47	.0219
Day	9	626.41	0.84	.5826
Bird (Rep)	8	4186.72	6.31	.0001
Trt	4	114.86	0.35	.8459
Error	77	6386.83		
Total	99	11768.51		
Linear contrast	1	16.82	0.20	.6537
Quadratic contrast	1	44.82	0.54	.4645
1440 min				
Rep	1	1089.00	2.85	.0956
Day	9	5166.76	1.50	.1627
Bird (Rep)	8	5856.36	1.91	.0697
Trt	4	1658.16	1.08	.3705
Error	77	29452.08		
Total	99	43222.36		
Linear contrast	1	486.72	1.27	.2628
Quadratic contrast	1	549.41	1.44	.2344

Appendix E Table 2. Analysis of variance for the effect of intracerebroventricular injection of morphiceptin on water intake in Rock-Cornish cockerels (Chapter 5).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	1	30.25	0.78	.3797
Day	9	911.25	2.61	.0109
Bird (Rep)	8	1096.00	3.54	.0016
Trt	4	297.50	1.92	.1157
Error	77	2983.75		
Total	99	5318.75		
Linear contrast	1	276.12	7.13	.0093
Quadratic contrast	1	6.96	0.18	.6728
30 min				
Rep	1	169.00	1.81	.1819
Day	9	5114.00	6.10	.0001
Bird (Rep)	8	7145.00	9.59	.0001
Trt	4	1124.00	3.02	.0229
Error	77	7172.00		
Total	99	20724.00		
Linear contrast	1	675.28	7.25	.0087
Quadratic contrast	1	200.43	2.15	.1465
45 min				
Rep	1	1296.00	8.97	.0037
Day	9	6421.00	4.94	.0001
Bird (Rep)	8	9750.00	8.44	.0001
Trt	4	1789.50	3.11	.0199
Error	77	11125.50		
Total	99	30391.00		
Linear contrast	1	1250.00	8.65	.0043
Quadratic contrast	1	339.68	2.35	.1293
60 min				
Rep	1	2550.25	15.11	.0002
Day	9	5901.25	3.88	.0004
Bird (Rep)	8	11821.00	8.75	.0001
Trt	4	1497.50	2.22	.0748
Error	77	12998.75		
Total	99	34768.75		
Linear contrast	1	630.12	3.73	.0570
Quadratic contrast	1	665.36	3.94	.0507
90 min				
Rep	1	4900.00	20.71	.0001
Day	9	7395.00	3.47	.0012
Bird (Rep)	8	24615.00	13.00	.0001
Trt	4	1917.50	2.03	.0991
Error	77	18222.50		
Total	99	57050.00		
Linear contrast	1	790.03	3.34	.0716
Quadratic contrast	1	1108.67	4.68	.0335
120 min				
Rep	1	8836.00	26.16	.0001
Day	9	8580.00	2.82	.0064
Bird (Rep)	8	33519.00	12.40	.0001
Trt	4	2427.50	1.80	.1381
Error	77	26012.50		
Total	99	79375.00		
Linear contrast	1	1104.50	3.27	.0745
Quadratic contrast	1	1273.01	3.77	.0559

150 min				
Rep	1	13456.00	35.11	.0001
Day	9	9960.00	2.89	.0054
Bird (Rep)	8	47634.00	15.54	.0001
Trt	4	3615.00	2.36	.0608
Error	77	29510.00		
Total	99	104175.00		
Linear contrast	1	2261.28	5.90	.0175
Quadratic contrast	1	1180.61	3.08	.0832
180 min				
Rep	1	15876.00	33.20	.0001
Day	9	9361.00	2.18	.0328
Bird (Rep)	8	56970.00	14.89	.0001
Trt	4	3893.50	2.04	.0976
Error	77	36815.50		
Total	99	122916.00		
Linear contrast	1	1250.00	2.61	.1100
Quadratic contrast	1	2400.02	5.02	.0279
240 min				
Rep	1	21025.00	37.27	.0001
Day	9	11925.00	2.35	.0212
Bird (Rep)	8	74665.00	16.55	.0001
Trt	4	2652.50	1.18	.3282
Error	77	43432.50		
Total	99	153700.00		
Linear contrast	1	1262.53	2.24	.1387
Quadratic contrast	1	1080.01	1.91	.1784
300 min				
Rep	1	30625.00	44.02	.0001
Day	9	14484.00	2.31	.0232
Bird (Rep)	8	88999.00	15.99	.0001
Trt	4	3791.50	1.36	.2548
Error	77	53569.50		
Total	99	191469.00		
Linear contrast	1	2112.50	3.04	.0854
Quadratic contrast	1	1364.52	1.96	.1654
1440 min				
Rep	1	258064.00	48.28	.0001
Day	9	117596.00	2.44	.0167
Bird (Rep)	8	651182.00	15.23	.0001
Trt	4	16316.00	0.76	.5525
Error	77	411618.00		
Total	99	1454776.00		
Linear contrast	1	457.53	0.09	.7706
Quadratic contrast	1	11259.82	2.11	.1508

Appendix E Table 3. Analysis of variance for the effect of intramuscular injection of morphiceptin on food intake in Rock-Cornish cockerels (Chapter 5).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	2	14.54	2.42	.1062
Day	3	68.08	7.55	.0007
Bird (Rep)	9	56.87	2.10	.0615
Trt	3	16.25	1.80	.1681
Error	30	90.18		
Total	47	245.92		
Linear contrast	1	8.04	2.67	.1125
Quadratic contrast	1	7.10	2.36	.1348
30 min				
Rep	2	21.79	3.07	.0610
Day	3	42.42	3.99	.0167
Bird (Rep)	9	93.12	2.92	.0131
Trt	3	28.25	2.66	.0663
Error	30	106.34		
Total	47	291.92		
Linear contrast	1	12.00	3.39	.0756
Quadratic contrast	1	13.82	3.90	.0576
45 min				
Rep	2	25.17	4.24	.0239
Day	3	84.33	9.48	.0001
Bird (Rep)	9	92.00	3.45	.0050
Trt	3	37.17	4.18	.0139
Error	30	89.00		
Total	47	327.67		
Linear contrast	1	6.04	2.03	.1641
Quadratic contrast	1	26.68	8.99	.0054
60 min				
Rep	2	27.87	3.76	.0350
Day	3	177.50	15.94	.0001
Bird (Rep)	9	79.12	2.37	.0369
Trt	3	48.17	4.33	.0120
Error	30	111.34		
Total	47	444.00		
Linear contrast	1	3.81	1.03	.3188
Quadratic contrast	1	40.31	10.86	.0025
90 min				
Rep	2	13.54	0.77	.4732
Day	3	283.73	10.72	.0001
Bird (Rep)	9	157.69	1.99	.0771
Trt	3	57.73	2.18	.1110
Error	30	264.79		
Total	47	777.48		
Linear contrast	1	0	0	.9916
Quadratic contrast	1	54.55	6.18	.0187
120 min				
Rep	2	16.62	0.61	.5507
Day	3	274.56	6.70	.0014
Bird (Rep)	9	163.94	1.33	.2617
Trt	3	97.40	2.38	.0897
Error	30	409.79		
Total	47	962.31		
Linear contrast	1	0.17	0.01	.9125
Quadratic contrast	1	93.86	6.87	.0136

150 min				
Rep	2	32.37	0.91	.4132
Day	3	323.56	6.07	.0024
Bird (Rep)	9	200.69	1.25	.3014
Trt	3	77.73	1.46	.2460
Error	30	533.46		
Total	47	11167.81		
Linear contrast	1	2.19	0.12	.7280
Quadratic contrast	1	66.29	3.73	.0630
180 min				
Rep	2	11.37	0.36	.6974
Day	3	288.73	6.17	.0021
Bird (Rep)	9	143.19	1.02	.4466
Trt	3	72.73	1.55	.2209
Error	30	467.79		
Total	47	983.81		
Linear contrast	1	5.29	0.34	.5647
Quadratic contrast	1	58.50	3.75	.0622
240 min				
Rep	2	50.54	1.54	.2307
Day	3	330.08	6.71	.0013
Bird (Rep)	9	227.87	1.54	.1781
Trt	3	69.42	1.41	.2589
Error	30	492.01		
Total	47	1169.92		
Linear contrast	1	0	0	1.0
Quadratic contrast	1	64.96	3.96	.0557
300 min				
Rep	2	47.54	0.99	.3818
Day	3	326.73	4.56	.0096
Bird (Rep)	9	267.19	1.24	.3078
Trt	3	38.90	0.54	.6570
Error	30	717.12		
Total	47	1397.48		
Linear contrast	1	1.21	0.05	.8231
Quadratic contrast	1	26.85	1.12	.2977
1440 min				
Rep	2	1335.54	11.92	.0002
Day	3	6053.40	36.03	.0001
Bird (Rep)	9	3285.19	6.52	.0001
Trt	3	158.73	0.94	.4314
Error	30	1680.12		
Total	47	12512.98		
Linear contrast	1	77.22	1.38	.2495
Quadratic contrast	1	57.50	1.03	.3190

Appendix E Table 4. Analysis of variance for the effect of intramuscular injection of morphiceptin on water intake in Rock-Cornish cockerels (Chapter 5).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	2	162.50	3.29	.0510
Day	3	147.40	1.99	.1367
Trt	3	30.73	0.41	.7435
Error	30	740.62		
Total	47	1382.81		
Linear contrast	1	0.02	0	.9749
Quadratic contrast	1	1.52	0.06	.8056
30 min				
Rep	2	326.04	4.95	.0138
Day	3	110.42	1.12	.3573
Bird (Rep)	9	384.37	1.30	.2791
Trt	3	39.58	0.40	.7534
Error	30	987.51		
Total	47	1847.92		
Linear contrast	1	0.10	0	.9566
Quadratic contrast	1	6.09	0.18	.6702
45 min				
Rep	2	204.17	2.67	.0860
Day	3	155.73	1.36	.2753
Bird (Rep)	9	514.06	1.49	.1963
Trt	3	1.56	0.01	.9978
Error	30	1148.96		
Total	47	2024.48		
Linear contrast	1	0.62	0.02	.8996
Quadratic contrast	1	0.55	0.01	.9056
60 min				
Rep	2	269.79	3.46	.0445
Day	3	318.23	2.72	.0620
Bird (Rep)	9	410.94	1.17	.3480
Trt	3	18.23	0.16	.9251
Error	30	1169.79		
Total	47	2186.98		
Linear contrast	1	5.58	0.14	.7079
Quadratic contrast	1	12.51	0.32	.5754
90 min				
Rep	2	450.00	4.25	.0237
Day	3	156.25	0.98	.4134
Bird (Rep)	9	831.25	1.75	.1218
Trt	3	106.25	0.67	.5775
Error	30	1587.50		
Total	47	3131.25		
Linear contrast	1	28.67	0.54	.4674
Quadratic contrast	1	76.00	1.44	.2401
120 min				
Rep	2	413.54	3.21	.0546
Day	3	243.75	1.26	.3055
Bird (Rep)	9	1321.87	2.28	.0439
Trt	3	85.42	0.44	.7248
Error	30	1933.34		
Total	47	3997.92		
Linear contrast	1	72.32	1.12	.2979
Quadratic contrast	1	13.10	0.20	.6554

150 min				
Rep	2	537.50	2.36	.1117
Day	3	251.56	0.74	.5385
Bird (Rep)	9	1526.56	1.49	.1969
Trt	3	89.06	0.26	.8531
Error	30	3415.63		
Total	47	5820.31		
Linear contrast	1	20.86	0.18	.6717
Quadratic contrast	1	46.60	0.41	.5272
180 min				
Rep	2	707.29	3.03	.0634
Day	3	589.06	1.68	.1920
Bird (Rep)	9	1685.94	1.60	.1590
Trt	3	226.56	0.65	.5911
Error	30	3503.13		
Total	47	6711.98		
Linear contrast	1	86.33	0.74	.3967
Quadratic contrast	1	102.33	0.88	.3567
240 min				
Rep	2	346.87	1.31	.2854
Day	3	1530.73	3.85	.0192
Bird (Rep)	9	1792.19	1.50	.1926
Trt	3	234.90	0.59	.6260
Error	30	3978.12		
Total	47	7882.81		
Linear contrast	1	205.38	1.55	.2229
Quadratic contrast	1	16.24	0.12	.7288
300 min				
Rep	2	1082.29	3.05	.0623
Day	3	1952.08	3.67	.0231
Bird (Rep)	9	2953.12	1.85	.1001
Trt	3	435.42	0.82	.4943
Error	30	5325.01		
Total	47	11747.92		
Linear contrast	1	238.19	1.34	.2558
Quadratic contrast	1	132.58	0.75	.3943
1440 min				
Rep	2	482.29	0.16	.8514
Day	3	59955.73	13.40	.0001
Bird (Rep)	9	42085.94	3.14	.0088
Trt	3	4893.23	1.09	.3670
Error	30	44744.79		
Total	47	152161.98		
Linear contrast	1	675.22	0.45	.5062
Quadratic contrast	1	2591.73	1.74	.1974

Appendix E Table 5. Analysis of variance for the effect of intracerebroventricular injection of [Met^5]-enkephalin on food intake in Rock-Cornish cockerels (Chapter 5).

Source of variation	df	Sum of squares	F value	P
15 min				
Day	4	21.84	0.61	.6622
Bird	4	184.24	5.16	.0118
Trt	4	46.64	1.31	.3224
Error	12	107.12		
Total	24	359.84		
Linear contrast	1	2.88	0.32	.5805
Quadratic contrast	1	35.83	4.01	.0682
30 min				
Day	4	113.76	2.21	.1239
Bird	4	355.76	6.91	.0040
Trt	4	80.56	1.56	.2464
Error	12	154.48		
Total	24	704.56		
Linear contrast	1	13.52	1.05	.3257
Quadratic contrast	1	62.53	4.86	.0478
45 min				
Day	4	482.24	13.21	.0002
Bird	4	354.64	9.71	.0010
Trt	4	491.04	13.45	.0002
Error	12	109.52		
Total	24	1437.44		
Linear contrast	1	12.00	1.32	.2738
Quadratic contrast	1	355.83	38.99	.0001
60 min				
Day	4	543.03	7.24	.0033
Bird	4	276.24	3.68	.0353
Trt	4	664.24	8.85	.0014
Error	12	225.13		
Total	24	1708.64		
Linear contrast	1	7.22	0.38	.5466
Quadratic contrast	1	433.01	23.08	.0004
90 min				
Day	4	388.80	3.98	.0279
Bird	4	220.80	2.26	.1232
Trt	4	803.20	8.22	.0020
Error	12	293.20		
Total	24	1706.00		
Linear contrast	1	17.40	0.71	.4152
Quadratic contrast	1	495.08	20.26	.0007
120 min				
Day	4	436.80	2.96	.0648
Bird	4	291.20	1.97	.1629
Trt	4	681.20	4.62	.0173
Error	12	442.80		
Total	24	1852.00		
Linear contrast	1	8.82	0.24	.6337
Quadratic contrast	1	295.36	8.00	.0152

150 min				
Day	4	398.64	2.76	.0776
Bird	4	586.24	4.05	.0263
Trt	4	653.84	4.52	.0186
Error	12	433.92		
Total	24	2072.64		
Linear contrast	1	1.62	0.04	.8359
Quadratic contrast	1	218.79	6.05	.0300
180 min				
Day	4	375.36	2.32	.1160
Bird	4	419.36	2.59	.0899
Trt	4	764.56	4.73	.0160
Error	12	484.88		
Total	24	2044.16		
Linear contrast	1	0.98	0.02	.8788
Quadratic contrast	1	359.11	8.89	.0115
240 min				
Day	4	834.40	5.28	.0109
Bird	4	449.20	2.84	.0718
Trt	4	998.40	6.32	.0056
Error	12	474.00		
Total	24	2756.00		
Linear contrast	1	38.72	0.98	.3417
Quadratic contrast	1	432.36	10.95	.0062
300 min				
Day	4	751.60	3.69	.0351
Bird	4	431.60	2.12	.1412
Trt	4	1059.60	5.20	.0115
Error	12	611.20		
Total	24	2854.00		
Linear contrast	1	85.80	1.68	.2187
Quadratic contrast	1	117.28	2.30	.1550
1440 min				
Day	4	1055.44	3.70	.0347
Bird	4	2469.04	8.09	.0021
Trt	4	387.84	1.36	.3045
Error	12	855.12		
Total	24	4767.44		
Linear contrast	1	32.00	0.45	.5155
Quadratic contrast	1	223.00	3.13	.1023

Appendix E Table 6. Analysis of variance for the effect of intracerebroventricular injection of [Met^5]-enkephalin on water intake in Rock-Cornish cockerels (Chapter 5).

Source of variation	df	Sum of squares	F value	P
15 min				
Day	4	530.00	2.69	.0820
Bird	4	910.00	4.63	.0172
Trt	4	170.00	0.86	.5127
Error	12	590.00		
Total	24	2200.00		
Linear contrast	1	98.00	1.99	.1834
Quadratic contrast	1	64.54	1.31	.2742
30 min				
Day	4	440.00	1.29	.3265
Bird	4	2690.00	7.91	.0023
Trt	4	250.00	0.74	.5854
Error	12	1020.00		
Total	24	4400.00		
Linear contrast	1	171.12	2.01	.1814
Quadratic contrast	1	47.50	0.56	.4692
45 min				
Day	4	946.00	0.64	.6444
Bird	4	2186.00	1.48	.2695
Trt	4	886.00	0.60	.6705
Error	12	4438.00		
Total	24	8456.00		
Linear contrast	1	8.00	0.02	.8855
Quadratic contrast	1	2.48	0.01	.9361
60 min				
Day	4	414.00	0.17	.9500
Bird	4	3784.00	1.55	.2511
Trt	4	1684.00	0.69	.6140
Error	12	7342.00		
Total	24	13224.00		
Linear contrast	1	276.12	0.45	.5144
Quadratic contrast	1	232.27	0.38	.5493
90 min				
Day	4	1854.00	0.82	.5394
Bird	4	1314.00	0.58	.6834
Trt	4	764.00	0.34	.8485
Error	12	6822.00		
Total	24	10754.00		
Linear contrast	1	36.12	0.06	.8052
Quadratic contrast	1	8.72	0.02	.9035
120 min				
Day	4	3094.00	1.24	.3442
Bird	4	2384.00	0.96	.4648
Trt	4	2654.00	1.07	.4147
Error	12	7462.00		
Total	24	15594.00		
Linear contrast	1	21.12	0.03	.8568
Quadratic contrast	1	1139.03	1.83	.2009

150 min				
Day	4	4904.00	1.34	.3117
Bird	4	5314.00	1.45	.2773
Trt	4	2674.00	0.73	.5887
Error	12	10992.00		
Total	24	23884.00		
Linear contrast	1	36.12	0.04	.8459
Quadratic contrast	1	1468.25	1.60	.2295
180 min				
Day	4	7374.00	1.49	.2671
Bird	4	5804.00	1.17	.3721
Trt	4	4514.00	0.91	.4889
Error	12	14882.00		
Total	24	32574.00		
Linear contrast	1	722.00	0.58	.4602
Quadratic contrast	1	1417.39	1.14	.3061
240 min				
Day	4	15454.00	2.80	.0747
Bird	4	11594.00	2.10	.1438
Trt	4	6054.00	1.10	.4020
Error	12	16562.00		
Total	24	49664.00		
Linear contrast	1	2048.00	1.48	.2466
Quadratic contrast	1	514.54	0.37	.5529
300 min				
Day	4	9774.00	1.27	.3350
Bird	4	18914.00	2.46	.1022
Trt	4	11314.00	1.47	.2717
Error	12	23092.00		
Total	24	63094.00		
Linear contrast	1	903.12	0.47	.5063
Quadratic contrast	1	134.96	0.07	.7956
1440 min				
Day	4	71366.00	2.48	.1000
Bird	4	232826.00	8.09	.0021
Trt	4	1506.00	.05	.9942
Error	12	86328.00		
Total	24	392026.00		
Linear contrast	1	231.12	0.03	.8607
Quadratic contrast	1	31.71	0	.9482

Appendix E Table 7. Analysis of variance for the effect of intramuscular injection of [Met^5]-enkephalin on food intake in Rock-Cornish cockerels (Chapter 5).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	1	19.53	11.89	.0029
Day	3	21.84	4.43	.0168
Bird (Rep)	6	111.69	11.33	.0001
Trt	3	13.34	2.71	.0758
Error	18	29.57		
Total	31	195.97		
Linear contrast	1	0	0	.9763
Quadratic contrast	1	11.59	7.06	.0161
30 min				
Rep	1	28.12	11.13	.0037
Day	3	58.75	7.75	.0016
Bird (Rep)	6	84.87	5.60	.0020
Trt	3	20.25	2.67	.0785
Error	18	45.51		
Total	31	237.50		
Linear contrast	1	0.15	0.06	.8110
Quadratic contrast	1	14.04	5.55	.0300
45 min				
Rep	1	12.50	4.48	.0485
Day	3	25.62	3.06	.0548
Bird (Rep)	6	109.37	6.53	.0009
Trt	3	43.12	5.15	.0096
Error	18	50.26		
Total	31	240.87		
Linear contrast	1	0.21	0.08	.7849
Quadratic contrast	1	38.00	13.61	.0017
60 min				
Rep	1	12.50	4.76	.0426
Day	3	40.50	5.14	.0096
Bird (Rep)	6	89.50	5.68	.0018
Trt	3	42.25	5.37	.0081
Error	18	47.25		
Total	31	232.00		
Linear contrast	1	0.05	0.02	.8880
Quadratic contrast	1	40.01	15.24	.0010
90 min				
Rep	1	16.53	4.93	.0394
Day	3	16.59	1.65	.2131
Bird (Rep)	6	58.94	2.93	.0356
Trt	3	37.84	3.76	.0294
Error	18	60.32		
Total	31	190.22		
Linear contrast	1	1.08	0.32	.5764
Quadratic contrast	1	34.84	10.40	.0047
120 min				
Rep	1	22.78	3.99	.0612
Day	3	54.84	3.20	.0482
Bird (Rep)	6	126.94	3.70	.0142
Trt	3	17.59	1.03	.4042
Error	18	102.82		
Total	31	321.97		
Linear contrast	1	0.66	0.11	.7386
Quadratic contrast	1	16.72	2.93	.1042

150 min				
Rep	1	18.00	2.27	.1489
Day	3	153.12	6.45	.0037
Bird (Rep)	6	115.87	2.44	.0665
Trt	3	24.37	1.03	.4044
Error	18	142.51		
Total	31	453.87		
Linear contrast	1	4.34	0.55	.4686
Quadratic contrast	1	20.00	2.53	.1294
180 min				
Rep	1	22.78	3.56	.0756
Day	3	124.84	6.50	.0036
Bird (Rep)	6	161.94	4.21	.0080
Trt	3	20.59	1.07	.3860
Error	18	115.32		
Total	31	445.47		
Linear contrast	1	0.18	0.03	.8687
Quadratic contrast	1	19.82	3.09	.0956
240 min				
Rep	1	12.50	2.33	.1442
Day	3	53.25	3.31	.0437
Bird (Rep)	6	343.00	10.66	.0001
Trt	3	16.75	1.04	.3981
Error	18	96.50		
Total	31	522.00		
Linear contrast	1	3.72	0.69	.4157
Quadratic contrast	1	12.57	2.34	.1431
300 min				
Rep	1	19.53	2.49	.1321
Day	3	92.34	3.92	.0257
Bird (Rep)	6	460.94	9.79	.0001
Trt	3	8.59	0.36	.7792
Error	18	141.32		
Total	31	722.72		
Linear contrast	1	0.33	0.04	.8387
Quadratic contrast	1	7.84	1.00	.3308
1440 min				
Rep	1	325.12	13.08	.0020
Day	3	677.25	9.08	.0007
Bird (Rep)	6	3748.37	25.13	.0001
Trt	3	51.25	0.69	.5715
Error	18	447.51		
Total	31	5249.50		
Linear contrast	1	4.34	0.17	.6810
Quadratic contrast	1	46.36	1.86	.1889

Appendix E Table 8. Analysis of variance for the effect of intramuscular injection of [Met^5]-enkephalin on water intake in Rock-Cornish cockerels (Chapter 5).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	1	0.78	0.15	.7054
Day	3	46.09	2.90	.0633
Bird (Rep)	6	273.44	8.61	.0002
Trt	3	14.84	0.93	.4445
Error	18	95.32		
Total	31	430.47		
Linear contrast	1	10.75	2.03	.1713
Quadratic contrast	1	1.23	0.23	.6360
30 min				
Rep	1	0.78	0.01	.9055
Day	3	39.84	0.25	.8628
Bird (Rep)	6	173.44	0.54	.7738
Trt	3	221.09	1.37	.2846
Error	18	970.32		
Total	31	1405.47		
Linear contrast	1	104.50	1.94	.1808
Quadratic contrast	1	76.80	1.42	.2481
45 min				
Rep	1	38.28	0.68	.4205
Day	3	39.84	0.24	.8703
Bird (Rep)	6	223.44	0.66	.6817
Trt	3	164.84	0.98	.4262
Error	18	1014.07		
Total	31	1480.47		
Linear contrast	1	82.18	1.46	.2428
Quadratic contrast	1	42.87	0.76	.3945
60 min				
Rep	1	0	0	1.0
Day	3	256.25	0.97	.4292
Bird (Rep)	6	650.00	1.23	.3377
Trt	3	93.75	0.35	.7866
Error	18	1587.50		
Total	31	2587.50		
Linear contrast	1	78.72	0.89	3573
Quadratic contrast	1	14.65	0.17	.6884
90 min				
Rep	1	0.78	0.01	.9215
Day	3	558.59	2.38	.1035
Bird (Rep)	6	385.94	0.82	.5672
Trt	3	39.84	0.17	.9154
Error	18	1407.82		
Total	31	2392.97		
Linear contrast	1	4.50	0.06	.8131
Quadratic contrast	1	28.50	0.36	.5536
120 min				
Rep	1	7.03	0.10	.7553
Day	3	483.59	2.30	.1123
Bird (Rep)	6	485.94	1.15	.3730
Trt	3	183.59	0.87	.4741
Error	18	1264.07		
Total	31	2424.22		
Linear contrast	1	157.18	2.24	.1520
Quadratic contrast	1	3.66	0.05	.8219

150 min				
Rep	1	0.78	0.01	.9283
Day	3	721.09	2.56	.0870
Bird (Rep)	6	967.19	1.72	.1741
Trt	3	296.09	1.05	.3939
Error	18	1689.07		
Total	31	3674.22		
Linear contrast	1	167.00	1.78	.1988
Quadratic contrast	1	76.80	0.82	.3776
180 min				
Rep	1	112.50	1.20	.2869
Day	3	550.00	1.96	.1557
Bird (Rep)	6	1337.50	2.39	.0713
Trt	3	206.25	0.74	.5441
Error	18	1681.25		
Total	31	3887.50		
Linear contrast	1	162.05	1.73	.2043
Quadratic contrast	1	36.53	0.39	.5396
240 min				
Rep	1	38.28	0.51	.4864
Day	3	1014.84	4.46	.0164
Bird (Rep)	6	1773.44	3.90	.0114
Trt	3	102.34	0.45	.7203
Error	18	1364.07		
Total	31	4292.97		
Linear contrast	1	68.79	0.91	.3533
Quadratic contrast	1	30.69	0.41	.5325
300 min				
Rep	1	175.78	2.63	.1220
Day	3	1352.34	6.75	.0030
Bird (Rep)	6	3410.94	8.52	.0002
Trt	3	152.34	0.76	.5307
Error	18	1201.57		
Total	31	6292.97		
Linear contrast	1	27.12	0.41	.5319
Quadratic contrast	1	99.44	1.49	.2380
1440 min				
Rep	1	3300.78	7.14	.0155
Day	3	14502.34	10.46	.0003
Bird (Rep)	6	88135.94	31.78	.0001
Trt	3	1821.09	1.31	.3009
Error	18	8320.32		
Total	31	116080.47		
Linear contrast	1	0.04	0	.9929
Quadratic contrast	1	1336.94	2.89	.1062

Appendix F Table 1. Analysis of variance for the effect of intracerebroventricular injection of β-endorphin (.5 to 2.0 ug) on food intake in Rock-Cornish cockerels (Chapter 6).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	1	6.12	0.30	.5919
Day	3	88.00	1.43	.2677
Bird (Rep)	6	110.87	0.90	.5168
Trt	3	9.00	0.15	.9309
Error	18	370.01		
Total	31	584.00		
Linear contrast	1	0.51	0.03	.8761
Quadratic contrast	1	3.54	0.17	.6832
30 min				
Rep	1	1.12	0.04	.8372
Day	3	64.12	0.83	.4966
Bird (Rep)	6	93.75	0.60	.7239
Trt	3	31.12	0.40	.7540
Error	18	465.76		
Total	31	655.87		
Linear contrast	1	3.43	0.13	.7200
Quadratic contrast	1	20.78	0.80	.3820
45 min				
Rep	1	36.12	0.97	.3374
Day	3	112.37	1.01	.4125
Bird (Rep)	6	151.75	0.68	.6678
Trt	3	27.12	0.24	.8651
Error	18	669.51		
Total	31	996.87		
Linear contrast	1	0.03	0	.9769
Quadratic contrast	1	13.09	0.35	.5604
60 min				
Rep	1	55.12	1.19	.2905
Day	3	228.37	1.64	.2158
Bird (Rep)	6	220.25	0.79	.5894
Trt	3	53.62	0.38	.7653
Error	18	836.51		
Total	31	1393.87		
Linear contrast	1	27.03	0.58	.4555
Quadratic contrast	1	19.50	0.42	.5253
90 min				
Rep	1	60.50	1.45	.2437
Day	3	228.12	1.83	.1785
Bird (Rep)	6	330.37	1.32	.2977
Trt	3	79.37	0.64	.6018
Error	18	749.51		
Total	31	1447.87		
Linear contrast	1	55.80	1.34	.2621
Quadratic contrast	1	9.83	0.24	.6330
120 min				
Rep	1	15.12	0.29	.5995
Day	3	291.25	1.83	.1772
Bird (Rep)	6	602.87	1.90	.1365
Trt	3	129.25	0.81	.5029
Error	18	953.01		
Total	31	1991.50		
Linear contrast	1	115.71	2.19	.1566
Quadratic contrast	1	13.51	0.26	.6195

150 min				
Rep	1	26.28	0.41	.5306
Day	3	124.34	0.64	.5962
Bird (Rep)	6	852.19	2.21	.0899
Trt	3	275.09	1.43	.2677
Error	18	1156.82		
Total	31	2434.72		
Linear contrast	1	257.47	4.01	.0606
Quadratic contrast	1	13.94	0.22	.6470
180 min				
Rep	1	40.50	0.64	.4346
Day	3	191.25	1.01	.4132
Bird (Rep)	6	1641.00	4.31	.0072
Trt	3	475.75	2.50	.0922
Error	18	1141.50		
Total	31	3490.00		
Linear contrast	1	365.71	5.77	.0273
Quadratic contrast	1	109.36	1.72	.2056
240 min				
Rep	1	66.12	0.95	.3416
Day	3	189.12	0.91	.4558
Bird (Rep)	6	2535.25	6.10	.0013
Trt	3	754.12	3.63	.0330
Error	18	1247.26		
Total	31	4791.87		
Linear contrast	1	486.29	7.02	.0163
Quadratic contrast	1	250.99	3.62	.0731
300 min				
Rep	1	98.00	0.89	.3573
Day	3	223.00	0.68	.5773
Bird (Rep)	6	3248.00	4.93	.0038
Trt	3	718.75	2.18	.1254
Error	18	1976.25		
Total	31	6264.00		
Linear contrast	1	488.93	4.45	.0491
Quadratic contrast	1	198.94	1.81	.1950
1440 min				
Rep	1	480.50	3.57	.0752
Day	3	3178.12	7.86	.0015
Bird (Rep)	6	11740.87	14.53	.0001
Trt	3	1188.62	2.94	.0610
Error	18	2424.71		
Total	31	19012.87		
Linear contrast	1	14.17	0.11	.7494
Quadratic contrast	1	17.55	0.13	.7223

Appendix F Table 2. Analysis of variance for the effect of intracerebroventricular injection of β-endorphin (.5 to 2.0 ug) on water intake in Rock-Cornish cockerels (Chapter 6).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	1	63.28	1.03	.3227
Day	3	121.09	0.66	.5875
Bird (Rep)	6	260.94	0.71	.6456
Trt	3	146.09	0.80	.5122
Error	18	1101.57		
Total	31	1692.97		
Linear contrast	1	72.52	1.19	.2907
Quadratic contrast	1	8.11	0.13	.7200
30 min				
Rep	1	488.28	2.61	.1234
Day	3	246.09	0.44	.7279
Bird (Rep)	6	548.44	0.49	.8080
Trt	3	271.09	0.48	.6979
Error	18	3364.97		
Total	31	4917.97		
Linear contrast	1	118.95	0.64	.4354
Quadratic contrast	1	42.92	0.23	.6375
45 min				
Rep	1	1313.28	4.84	.0412
Day	3	208.59	0.26	.8561
Bird (Rep)	6	1160.94	0.71	.6443
Trt	3	421.09	0.52	.6760
Error	18	4889.07		
Total	31	7992.97		
Linear contrast	1	265.20	0.98	.3362
Quadratic contrast	1	108.10	0.40	.5360
60 min				
Rep	1	2907.03	6.68	.0187
Day	3	421.09	0.32	.8090
Bird (Rep)	6	3592.19	1.38	.2770
Trt	3	839.84	0.64	.5971
Error	18	7832.82		
Total	31	15592.97		
Linear contrast	1	508.95	1.17	.2938
Quadratic contrast	1	153.54	0.35	.5599
90 min				
Rep	1	3200.00	4.71	.0436
Day	3	1318.75	0.65	.5948
Bird (Rep)	6	8987.50	2.21	.0904
Trt	3	2818.75	1.38	.2799
Error	18	12225.00		
Total	31	28550.00		
Linear contrast	1	1603.21	2.36	.1418
Quadratic contrast	1	558.98	0.82	.3736
120 min				
Rep	1	2538.28	2.65	.1210
Day	3	2089.84	0.73	.5492
Bird (Rep)	6	15310.94	2.66	.0499
Trt	3	4802.34	1.67	.2089
Error	18	17251.57		
Total	31	41992.97		
Linear contrast	1	4460.02	4.65	.0448
Quadratic contrast	1	146.46	0.15	.7004

150 min				
Rep	1	2194.53	2.03	.1711
Day	3	2246.09	0.69	.5679
Bird (Rep)	6	22642.19	3.50	.0181
Trt	3	4714.84	1.46	.2599
Error	18	19432.82		
Total	31	51230.47		
Linear contrast	1	4661.81	4.32	.0523
Quadratic contrast	1	52.87	0.05	.8274
180 min				
Rep	1	2812.50	2.29	.1474
Day	3	1678.12	0.46	.7164
Bird (Rep)	6	20734.37	2.82	.0411
Trt	3	8209.37	2.23	.1197
Error	18	22087.51		
Total	31	55521.87		
Linear contrast	1	8197.23	6.68	.0187
Quadratic contrast	1	6.61	0.01	.9423
240 min				
Rep	1	5382.03	3.61	.0736
Day	3	1658.59	0.37	.7750
Bird (Rep)	6	23767.19	2.66	.0503
Trt	3	11889.84	2.66	.0794
Error	18	26832.82		
Total	31	69530.47		
Linear contrast	1	11283.95	7.57	.0131
Quadratic contrast	1	57.15	0.04	.8470
300 min				
Rep	1	7969.53	4.25	.0540
Day	3	2777.34	0.49	.6912
Bird (Rep)	6	28867.19	2.56	.0566
Trt	3	11852.34	2.11	.1352
Error	18	33764.07		
Total	31	85230.47		
Linear contrast	1	10596.45	5.65	.0288
Quadratic contrast	1	108.34	0.06	.8128
1440 min				
Rep	1	23925.78	10.20	.0050
Day	3	13714.84	1.95	.1578
Bird (Rep)	6	51842.19	3.68	.0145
Trt	3	13983.59	1.99	.1519
Error	18	42207.82		
Total	31	145674.22		
Linear contrast	1	8170.20	3.48	.0783
Quadratic contrast	1	921.21	0.39	.5387

Appendix F Table 3. Analysis of variance for the effect of intracerebroventricular injection of β-endorphin (.5 to 2.0 ug) on colonic temperature in Rock-Cornish cockerels (Chapter 6).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	1	0.0528	0.75	.3989
Day	3	0.2459	1.16	.3527
Bird (Rep)	6	0.7694	1.81	.1530
Trt	3	0.4534	2.14	.1311
Error	18	1.2732		
Total	31	2.7947		
Linear contrast	1	0.4520	6.39	.0210
Quadratic contrast	1	0.0003	0	.9496
30 min				
Rep	1	0.2112	3.66	.0719
Day	3	0.2050	1.18	.3442
Bird (Rep)	6	0.3737	1.08	.4116
Trt	3	0.8700	5.02	.0106
Error	18	1.0401		
Total	31	2.7000		
Linear contrast	1	0.6223	10.77	.0041
Quadratic contrast	1	0.2237	3.87	.0647
45 min				
Rep	1	0.2278	5.22	.0347
Day	3	0.5259	4.02	.0237
Bird (Rep)	6	0.6419	2.45	.0655
Trt	3	1.2309	9.40	.0006
Error	18	0.7857		
Total	31	3.4122		
Linear contrast	1	0.9547	21.87	.0002
Quadratic contrast	1	0.2237	5.37	.0361
60 min				
Rep	1	0.0800	2.81	.1110
Day	3	0.4162	4.87	.0118
Bird (Rep)	6	0.7137	4.18	.0084
Trt	3	1.6462	19.27	.0001
Error	18	0.5126		
Total	31	3.3687		
Linear contrast	1	1.4143	49.67	.0001
Quadratic contrast	1	0.0764	2.68	.1187
90 min				
Rep	1	0.0012	0.02	.8998
Day	3	0.0175	0.08	.9721
Bird (Rep)	6	0.8937	1.94	.1284
Trt	3	2.1075	9.16	.0007
Error	18	1.3801		
Total	31	4.4000		
Linear contrast	1	1.6663	21.73	.0002
Quadratic contrast	1	0.4258	5.55	.0300
120 min				
Rep	1	0.0112	0.07	.7894
Day	3	0.1575	0.34	.79 45
Bird (Rep)	6	1.3387	1.46	.2479
Trt	3	1.5925	3.47	.0380
Error	18	2.7551		
Total	31	5.8550		
Linear contrast	1	1.2893	8.42	.0095
Quadratic contrast	1	0.2749	1.80	.1969

150 min				
Rep	1	0.0800	0.72	.4073
Day	3	0.6725	2.02	.1475
Bird (Rep)	6	1.3300	2.00	.1197
Trt	3	1.3975	4.19	.0205
Error	18	2.0000		
Total	31	5.4800		
Linear contrast	1	0.9373	8.44	.0095
Quadratic contrast	1	0.3894	3.50	.0775
180 min				
Rep	1	0.0612	0.49	.4941
Day	3	1.3837	3.67	.0319
Bird (Rep)	6	1.6975	2.25	.0852
Trt	3	1.4037	3.72	.0304
Error	18	2.2626		
Total	31	6.8087		
Linear contrast	1	0.9960	7.92	.0115
Quadratic contrast	1	0.4001	3.18	.0913
240 min				
Rep	1	0.0078	0.08	.7798
Day	3	1.2384	4.26	.0194
Bird (Rep)	6	0.8269	1.42	.2606
Trt	3	0.8734	5.88	.0577
Error	18	1.7457		
Total	31	4.6922		
Linear contrast	1	0.2925	3.02	.0995
Quadratic contrast	1	0.5701	5.88	.0261
300 min				
Rep	1	0.0312	0.23	.6343
Day	3	0.8162	2.04	.1445
Bird (Rep)	6	0.7575	0.95	.4875
Trt	3	0.3212	0.80	.5088
Error	18	2.4026		
Total	31	4.3287		
Linear contrast	1	0.1243	0.93	.3473
Quadratic contrast	1	0.0764	0.57	.4591

Appendix F Table 4. Analysis of variance for the effect of intracerebroventricular injection of β-endorphin (1.5 to 6.0 ug) on food intake in Rock-Cornish cockerels (Chapter 6).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	3	208.50	4.60	.0085
Day (Rep)	12	207.25	1.14	.3607
Bird (Rep)	12	293.25	1.62	.1341
Trt	3	84.62	1.87	.1541
Error	33	498.13		
Total	63	1291.75		
Linear contrast	1	71.43	4.73	.0369
Quadratic contrast	1	12.28	0.81	.3736
30 min				
Rep	3	258.05	3.96	.0163
Day (Rep)	12	440.69	1.69	.1149
Bird (Rep)	12	630.69	2.42	.0223
Trt	3	126.05	1.93	.1435
Error	33	717.50		
Total	63	2172.98		
Linear contrast	1	63.45	2.92	.0979
Quadratic contrast	1	58.29	2.68	.1111
45 min				
Rep	3	286.30	3.87	.0177
Day (Rep)	12	601.56	2.03	.0530
Bird (Rep)	12	965.06	3.26	.0035
Trt	3	219.92	2.97	.0457
Error	33	813.27		
Total	63	2886.11		
Linear contrast	1	88.40	3.59	.0670
Quadratic contrast	1	101.45	4.12	.0506
60 min				
Rep	3	383.81	4.36	.0108
Day (Rep)	12	888.62	2.52	.0175
Bird (Rep)	12	1117.63	3.18	.0042
Trt	3	332.56	3.78	.0195
Error	33	967.81		
Total	63	3690.43		
Linear contrast	1	82.54	2.81	.1029
Quadratic contrast	1	197.15	6.72	.0141
90 min				
Rep	3	401.81	3.30	.0322
Day (Rep)	12	1284.63	2.64	.0135
Bird (Rep)	12	1489.63	3.06	.0054
Trt	3	476.56	3.92	.0169
Error	33	1337.81		
Total	63	4990.44		
Linear contrast	1	116.12	2.86	.1000
Quadratic contrast	1	296.66	7.32	.0107
120 min				
Rep	3	842.17	4.68	.0079
Day (Rep)	12	1576.44	2.19	.0373
Bird (Rep)	12	2112.94	2.93	.0071
Trt	3	694.30	3.86	.0180
Error	33	1980.01		
Total	63	7205.86		
Linear contrast	1	122.11	2.04	.1631
Quadratic contrast	1	425.53	7.09	.0119

150 min				
Rep	3	965.06	4.43	.0101
Day (Rep)	12	2148.87	2.46	.0200
Bird (Rep)	12	2589.37	2.97	.0066
Trt	3	596.19	2.74	.0592
Error	33	2397.45		
Total	63	8696.94		
Linear contrast	1	84.09	1.16	.2898
Quadratic contrast	1	423.87	5.83	.0214
180 min				
Rep	3	1327.06	5.56	.0033
Day (Rep)	12	2112.88	2.21	.0353
Bird (Rep)	12	2506.88	2.63	.0140
Trt	3	540.06	2.26	.0994
Error	33	2625.06		
Total	64	9111.94		
Linear contrast	1	53.44	0.67	.4183
Quadratic contrast	1	392.95	4.94	.0332
240 min				
Rep	3	1578.81	5.18	.0048
Day (Rep)	12	3225.63	2.65	.0133
Bird (Rep)	12	3135.63	2.57	.0157
Trt	3	769.31	2.53	.0745
Error	33	3351.06		
Total	63	12060.44		
Linear contrast	1	0.14	0	.9701
Quadratic contrast	1	511.84	5.04	.0316
300 min				
Rep	3	3872.81	12.98	.0001
Day (Rep)	12	3125.63	2.62	.0142
Bird (Rep)	12	3179.63	2.66	.0129
Trt	3	615.56	2.06	.1242
Error	33	3282.81		
Total	63	14076.44		
Linear contrast	1	22.00	0.22	.6412
Quadratic contrast	1	297.38	2.99	.0932
1440 min				
Rep	3	11001.12	10.70	.0001
Day (Rep)	12	17774.63	4.32	.0004
Bird (Rep)	12	32447.63	7.89	.0001
Trt	3	1271.62	1.24	.3120
Error	33	11310.75		
Total	63	73805.75		
Linear contrast	1	0.18	0	.9819
Quadratic contrast	1	826.46	2.41	.1300

Appendix F Table 5. Analysis of variance for the effect of intracerebroventricular injection of β-endorphin (1.5 to 6.0 ug) on water intake in Rock-Cornish cockerels (Chapter 6).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	3	0		
Day (Rep)	12	0		
Bird (Rep)	12	0		
Trt	3	0		
Error	33	0		
Total	63	0		
Linear contrast	1	0		
Quadratic contrast	1	0		
30 min				
Rep	3	76.17	3.62	.0231
Day (Rep)	12	151.56	1.80	.0899
Bird (Rep)	12	191.56	1.21	.3200
Trt	3	48.05	2.28	.0974
Error	33	231.64		
Total	63	608.98		
Linear contrast	1	16.98	2.42	.1295
Quadratic contrast	1	21.47	3.06	.0896
45 min				
Rep	3	425.00	2.56	.0719
Day (Rep)	12	756.25	1.14	.3653
Bird (Rep)	12	518.75	0.78	.6660
Trt	3	415.62	2.50	.0765
Error	33	1828.13		
Total	63	3943.75		
Linear contrast	1	140.00	2.53	.1214
Quadratic contrast	1	219.28	3.96	.0550
60 min				
Rep	3	326.17	1.20	.3247
Day (Rep)	12	1364.06	1.26	.2898
Bird (Rep)	12	1401.56	1.29	.2702
Trt	3	1129.30	4.16	.0133
Error	33	2987.89		
Total	63	7208.98		
Linear contrast	1	616.35	6.81	.0135
Quadratic contrast	1	495.64	5.47	.0255
90 min				
Rep	3	646.87	1.41	.2568
Day (Rep)	12	2653.13	1.45	.1945
Bird (Rep)	12	2703.13	1.47	.1835
Trt	3	1156.25	2.52	.0747
Error	33	5040.62		
Total	63	12200.00		
Linear contrast	1	754.46	4.94	.0332
Quadratic contrast	1	366.37	2.40	.1310
120 min				
Rep	3	1248.05	1.57	.2157
Day (Rep)	12	4626.56	1.45	.1922
Bird (Rep)	12	4539.06	1.43	.2038
Trt	3	1098.05	1.38	.2662
Error	33	8756.64		
Total	63	20268.36		
Linear contrast	1	680.90	2.57	.1187
Quadratic contrast	1	343.58	1.29	.2634

150 min				
Rep	3	3796.88	2.62	.0668
Day (Rep)	12	9271.88	1.60	.1392
Bird (Rep)	12	6296.87	1.09	.4011
Trt	3	962.50	0.67	.5794
Error	33	15915.62		
Total	63	36243.75		
Linear contrast	1	446.43	0.93	.3430
Quadratic contrast	1	105.59	0.22	.6429
180 min				
Rep	3	6941.79	4.11	.0139
Day (Rep)	12	10029.69	1.49	.1791
Bird (Rep)	12	11829.69	1.75	.0998
Trt	3	1238.67	0.73	.5392
Error	33	18562.89		
Total	64	48602.73		
Linear contrast	1	59.47	0.11	.7471
Quadratic contrast	1	1.01	0	.9664
240 min				
Rep	3	17431.25	8.40	.0003
Day (Rep)	12	19912.50	2.40	.0233
Bird (Rep)	12	22000.00	2.65	.0133
Trt	3	1865.62		
Error	33	22834.38		
Total	63	84043.75		
Linear contrast	1	0.18	0	.9873
Quadratic contrast	1	1374.07	1.99	.1681
300 min				
Rep	3	28251.17	12.22	.0001
Day (Rep)	12	20435.94	2.21	.0357
Bird (Rep)	12	32473.44	3.51	.0021
Trt	3	4304.30	1.86	.1554
Error	33	25441.01		
Total	63	110905.86		
Linear contrast	1	997.78	1.29	.2635
Quadratic contrast	1	907.31	1.18	.2859
1440 min				
Rep	3	72184.38	6.65	.0012
Day (Rep)	12	43534.38	1.00	.4683
Bird (Rep)	12	180546.87	4.16	.0006
Trt	3	17390.62	1.60	.2077
Error	33	119437.50		
Total	63	433093.75		
Linear contrast	1	2745.71	0.76	.3901
Quadratic contrast	1	4405.34	1.22	.2779

Appendix F Table 6. Analysis of variance for the effect of intracerebroventricular injection of β-endorphin (1.5 to 6.0 ug) on colonic temperature in Rock-Cornish cockerels (Chapter 6).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	3	1.2892	7.39	.0006
Day (Rep)	12	0.5806	0.83	.6186
Bird (Rep)	12	1.5656	2.24	.0331
Trt	3	0.3567	2.04	.1286
Error	33	1.9202		
Total	63	5.7123		
Linear contrast	1	0.1305	2.24	.1437
Quadratic contrast	1	0.1000	1.72	.1988
30 min				
Rep	3	3.3955	16.94	.0001
Day (Rep)	12	0.8456	1.05	.4256
Bird (Rep)	12	2.8306	3.53	.0020
Trt	3	1.1417	5.70	.0029
Error	33	2.2052		
Total	63	10.4186		
Linear contrast	1	0.6412	9.60	.0040
Quadratic contrast	1	0.3533	5.29	.0280
45 min				
Rep	3	3.8006	11.80	.0001
Day (Rep)	12	1.7638	1.37	.2295
Bird (Rep)	12	2.7588	2.14	.0416
Trt	3	1.6531	5.13	.0050
Error	33	3.5431		
Total	63	13.5194		
Linear contrast	1	0.7072	6.59	.0150
Quadratic contrast	1	0.7367	6.72	.0141
60 min				
Rep	3	5.0580	16.00	.0001
Day (Rep)	12	1.1831	0.94	.5246
Bird (Rep)	12	3.0681	2.43	.0218
Trt	3	2.0330	8.84	.0055
Error	33	3.4764		
Total	63	14.8186		
Linear contrast	1	0.9653	9.16	.0048
Quadratic contrast	1	0.9313	8.84	.0055
90 min				
Rep	3	5.7412	16.00	.0001
Day (Rep)	12	1.6163	1.13	.3735
Bird (Rep)	12	2.0813	1.45	.1935
Trt	3	1.7712	4.94	.0061
Error	33	3.9475		
Total	63	15.1575		
Linear contrast	1	1.5018	12.55	.0012
Quadratic contrast	1	0.2417	2.02	.1645
120 min				
Rep	3	5.5842	14.58	.0001
Day (Rep)	12	1.7419	1.14	.3656
Bird (Rep)	12	2.1019	1.37	.2280
Trt	3	2.2980	6.00	.0022
Error	33	4.2126		
Total	63	15.9386		
Linear contrast	1	1.6469	12.89	.0011
Quadratic contrast	1	0.5528	4.33	.0453

150 min				
Rep	3	5.8292	13.82	.0001
Day (Rep)	12	2.0481	1.21	.3149
Bird (Rep)	12	1.8681	1.11	.3870
Trt	3	2.1692	5.14	.0050
Error	33	4.6402		
Total	63	16.5548		
Linear contrast	1	1.7888	12.72	.0011
Quadratic contrast	1	0.3093	2.20	.1475
180 min				
Rep	3	5.2931	10.61	.0001
Day (Rep)	12	2.2012	1.10	.3898
Bird (Rep)	12	1.5912	0.80	.6501
Trt	3	2.4269	4.87	.0065
Error	33	5.4870		
Total	63	16.9994		
Linear contrast	1	2.2000	13.23	.0009
Quadratic contrast	1	0.0666	0.40	.5313
240 min				
Rep	3	8.2862	16.40	.0001
Day (Rep)	12	4.6988	2.33	.0274
Bird (Rep)	12	2.1188	1.05	.4313
Trt	3	1.8287	3.62	.0231
Error	33	5.5575		
Total	63	22.4900		
Linear contrast	1	1.7383	10.32	.0029
Quadratic contrast	1	0.0003	0	.9687
300 min				
Rep	3	10.9875	27.46	.0001
Day (Rep)	12	4.2200	2.64	.0136
Bird (Rep)	12	1.7850	1.12	.3811
Trt	3	0.2037	0.51	.6787
Error	33	4.4013		
Total	63	21.5975		
Linear contrast	1	0.1931	1.45	.2374
Quadratic contrast	1	0.0090	0.07	.7970

Appendix F Table 7. Analysis of variance for the effect of intracerebroventricular injection of β-endorphin (.5 to 2.0 ug) on food intake in Single-Comb White Leghorn cockerels (Chapter 6).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	1	0.12	0.04	.8474
Day	3	16.37	1.67	.2100
Bird (Rep)	6	9.75	0.50	.8032
Trt	3	14.62	1.49	.2517
Error	18	59.01		
Total	31	99.87		
Linear contrast	1	9.29	2.83	.1095
Quadratic contrast	1	0.23	0.07	.7922
30 min				
Rep	1	0	0	1.0000
Day	3	10.75	0.92	.4520
Bird (Rep)	6	12.00	0.51	.7912
Trt	3	27.00	2.31	.1112
Error	18	70.25		
Total	31	120.00		
Linear contrast	1	8.23	2.11	.1637
Quadratic contrast	1	0.73	0.19	.6705
45 min				
Rep	1	0.78	0.25	.6256
Day	3	26.09	2.74	.0733
Bird (Rep)	6	22.44	1.18	.3603
Trt	3	17.59	1.85	.1743
Error	18	57.07		
Total	31	123.97		
Linear contrast	1	2.14	0.68	.4216
Quadratic contrast	1	1.95	0.61	.4432
60 min				
Rep	1	1.12	0.29	.5940
Day	3	26.12	2.28	.1140
Bird (Rep)	6	20.75	0.91	.5127
Trt	3	19.12	1.67	.2092
Error	18	68.77		
Total	31	135.87		
Linear contrast	1	3.89	1.02	.3263
Quadratic contrast	1	1.23	0.32	.5767
90 min				
Rep	1	2.53	0.54	.4737
Day	3	20.59	1.45	.2608
Bird (Rep)	6	72.44	2.55	.0573
Trt	3	26.09	1.84	.1760
Error	18	85.07		
Total	31	206.72		
Linear contrast	1	2.90	0.61	.4435
Quadratic contrast	1	7.64	1.62	.2198
120 min				
Rep	1	16.53	2.58	.1256
Day	3	25.09	1.31	.3032
Bird (Rep)	6	84.44	2.20	.0915
Trt	3	31.84	1.66	.2118
Error	18	115.32		
Total	31	273.22		
Linear contrast	1	1.81	0.28	.6017
Quadratic contrast	1	12.28	1.92	.1831

150 min				
Rep	1	9.03	1.28	.2724
Day	3	10.59	0.50	.6862
Bird (Rep)	6	116.19	2.75	.0448
Trt	3	45.34	2.15	.1301
Error	18	126.82		
Total	31	307.97		
Linear contrast	1	0.02	0	.9557
Quadratic contrast	1	18.02	2.56	.1271
180 min				
Rep	1	8.00	0.83	.3750
Day	3	30.75	1.06	.3904
Bird (Rep)	6	194.50	3.35	.0213
Trt	3	102.75	3.54	.0356
Error	18	174.00		
Total	31	510.00		
Linear contrast	1	1.73	0.18	.6774
Quadratic contrast	1	67.80	7.01	.0163
240 min				
Rep	1	21.12	1.16	.2953
Day	3	63.25	1.16	.3524
Bird (Rep)	6	223.37	2.05	.1115
Trt	3	156.50	2.87	.0652
Error	18	327.26		
Total	31	791.50		
Linear contrast	1	26.41	1.45	.2437
Quadratic contrast	1	101.73	5.60	.0294
300 min				
Rep	1	98.00	3.28	.0870
Day	3	333.75	3.72	.0305
Bird (Rep)	6	188.00	1.05	.4284
Trt	3	173.25	1.93	.1609
Error	18	538.50		
Total	31	1331.50		
Linear contrast	1	60.36	2.02	.1726
Quadratic contrast	1	92.20	3.08	.0962
1440 min				
Rep	1	210.12	7.06	.0160
Day	3	548.12	6.14	.0046
Bird (Rep)	6	710.75	3.98	.0104
Trt	3	118.12	1.32	.2979
Error	18	535.76		
Total	31	2122.87		
Linear contrast	1	24.60	0.83	.3753
Quadratic contrast	1	79.53	2.67	.1195

Appendix F Table 8. Analysis of variance for the effect of intracerebroventricular injection of β-endorphin (.5 to 2.0 ug) on water intake in Single-Comb White Leghorn cockerels (Chapter 6).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	1	0.78	0.36	.5560
Day	3	2.34	0.36	.7826
Bird (Rep)	6	23.44	1.80	.1558
Trt	3	2.34	0.36	.7826
Error	18	39.07		
Total	31	67.97		
Linear contrast	1	1.81	0.83	.3734
Quadratic contrast	1	0.51	0.23	.6346
30 min				
Rep	1	0.78	0.20	.6601
Day	3	21.09	1.80	.1833
Bird (Rep)	6	29.69	1.27	.3208
Trt	3	14.84	1.27	.3156
Error	18	70.32		
Total	31	136.72		
Linear contrast	1	8.23	2.11	.1637
Quadratic contrast	1	0.73	0.19	.6705
45 min				
Rep	1	0.78	0.07	.7915
Day	3	39.84	1.22	.3298
Bird (Rep)	6	104.69	1.61	.2021
Trt	3	58.59	1.80	.1833
Error	18	195.32		
Total	31	399.22		
Linear contrast	1	53.59	4.94	.0393
Quadratic contrast	1	3.98	0.37	.5523
60 min				
Rep	1	7.03	0.74	.4000
Day	3	58.59	2.06	.1409
Bird (Rep)	6	179.69	3.17	.0267
Trt	3	77.34	2.72	.0746
Error	18	170.32		
Total	31	492.97		
Linear contrast	1	72.52	7.66	.0127
Quadratic contrast	1	0.02	0	.9634
90 min				
Rep	1	3.12	0.07	.7992
Day	3	184.37	1.31	.3015
Bird (Rep)	6	456.25	1.62	.1983
Trt	3	459.37	3.27	.0454
Error	18	843.76		
Total	31	1946.87		
Linear contrast	1	354.37	7.56	.01322
Quadratic contrast	1	15.90	0.34	.5675
120 min				
Rep	1	0.78	0.01	.9150
Day	3	627.34	3.13	.0512
Bird (Rep)	6	379.69	0.95	.4862
Trt	3	402.34	3.02	.0994
Error	18	1201.57		
Total	31	2611.72		
.sp 2mm				
Linear contrast	1	1.81	0.28	.6017
Quadratic contrast	1	12.28	1.92	.1831

150 min				
Rep	1	12.50	0.19	.6669
Day	3	1003.12	5.12	.0098
Bird (Rep)	6	346.87	0.89	.5253
Trt	3	609.37	3.11	.0522
Error	18	1175.01		
Total	31	3146.87		
Linear contrast	1	109.37	1.68	.2119
Quadratic contrast	1	397.70	6.09	.0238
180 min				
Rep	1	50.00	0.76	.3942
Day	3	1237.50	6.29	.0042
Bird (Rep)	6	362.50	0.92	.5031
Trt	3	818.75	4.16	.0211
Error	18	1181.25		
Total	31	3650.00		
Linear contrast	1	157.50	2.40	.1387
Quadratic contrast	1	525.96	8.01	.0111
240 min				
Rep	1	0.78	0.01	.9120
Day	3	321.09	1.72	.1987
Bird (Rep)	6	817.19	2.19	.0925
Trt	3	289.84	1.55	.2355
Error	18	1120.32		
Total	31	2549.22		
Linear contrast	1	0.56	0.01	.9256
Quadratic contrast	1	187.02	3.00	.1001
300 min				
Rep	1	7.03	0.12	.7357
Day	3	2564.84	14.29	.0001
Bird (Rep)	6	1004.69	2.80	.0420
Trt	3	277.34	1.55	.2371
Error	18	1076.57		
Total	31	4930.47		
Linear contrast	1	53.59	0.90	.3564
Quadratic contrast	1	223.74	3.74	.0690
1440 min				
Rep	1	282.03	0.55	.4699
Day	3	1402.34	0.90	.4589
Bird (Rep)	6	4229.69	1.36	.2821
Trt	3	2089.84	1.35	.2908
Error	18	9314.07		
Total	31	17317.97		
Linear contrast	1	1170.56	2.26	.1499
Quadratic contrast	1	763.86	1.48	.2401

Appendix F Table 9. Analysis of variance for the effect of intracerebroventricular injection of β-endorphin (.5 to 2.0 ug) on colonic temperature in Single-Comb White Leghorn cockerels (Chapter 6).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	1	0.0450	0.50	.4899
Day	3	0.6275	2.31	.1108
Bird (Rep)	6	0.4800	0.88	.5268
Trt	3	0.5175	1.90	.1650
Error	18	1.6300		
Total	31	3.3000		
Linear contrast	1	0.3716	4.10	.0579
Quadratic contrast	1	0.1037	1.14	.2987
30 min				
Rep	1	0.0012	0.02	.8971
Day	3	0.4112	1.89	.1679
Bird (Rep)	6	0.4575	1.05	.4271
Trt	3	1.2012	5.51	.0073
Error	18	1.3076		
Total	31	3.3787		
Linear contrast	1	0.6900	9.50	.0064
Quadratic contrast	1	0.3280	4.52	.0477
45 min				
Rep	1	0.0153	0.27	.6118
Day	3	0.3934	2.28	.1135
Bird (Rep)	6	0.5844	1.70	.1791
Trt	3	0.9409	5.46	.0075
Error	18	1.0332		
Total	31	2.9672		
Linear contrast	1	0.5536	9.64	.0061
Quadratic contrast	1	0.2210	3.85	.0654
60 min				
Rep	1	0.0153	0.31	.5854
Day	3	0.2009	1.35	.2897
Bird (Rep)	6	0.6769	2.27	.0826
Trt	3	1.0534	7.08	.0024
Error	18	0.8932		
Total	31	2.8397		
Linear contrast	1	0.5625	11.34	.0034
Quadratic contrast	1	0.2870	5.78	.0272
90 min				
Rep	1	0.0528	1.56	.2281
Day	3	0.1984	1.95	.1578
Bird (Rep)	6	1.0769	5.29	.0027
Trt	3	1.3984	13.74	.0001
Error	18	0.6107		
Total	31	3.3372		
Linear contrast	1	0.8747	25.79	.0001
Quadratic contras	1	0.1876	5.53	.0303
120 min				
Rep	1	0.0003	0.01	.9139
Day	3	0.1884	2.42	.1001
Bird (Rep)	6	0.7069	4.53	.0057
Trt	3	1.1259	14.43	.0001
Error	18	0.4682		
Total	31	2.4897		
Linear contrast	1	0.6654	25.59	.0001
Quadratic contrast	1	0.1436	5.52	.0304

150 min				
Rep	1	0.0050	0.09	.7645
Day	3	0.1362	0.84	.4893
Bird (Rep)	6	0.6987	2.16	.0966
Trt	3	1.2362	7.63	.0017
Error	18	0.9726		
Total	31	3.0487		
Linear contrast	1	0.5580	10.33	.0048
Quadratic contrast	1	0.2237	4.14	.0568
180 min				
Rep	1	0.0050	0.17	.6879
Day	3	0.0575	0.64	.5997
Bird (Rep)	6	0.8800	4.89	.0040
Trt	3	0.7925	8.81	.0008
Error	18	0.5400		
Total	31	2.2750		
Linear contrast	1	0.2286	7.62	.0129
Quadratic contrast	1	0.0387	1.29	.2711
240 min				
Rep	1	0.0312	0.55	.4689
Day	3	0.0025	0.01	.9975
Bird (Rep)	6	0.6037	1.76	.1638
Trt	3	0.3900	2.28	.1143
Error	18	1.0276		
Total	31	2.0550		
Linear contrast	1	0.0966	1.69	.2098
Quadratic contrast	1	0.1130	1.98	.1764
300 min				
Rep	1	0.0003	0	.9491
Day	3	0.1684	0.75	.5352
Bird (Rep)	6	0.5469	1.22	.3408
Trt	3	0.3959	1.77	.1891
Error	18	1.3432		
Total	31	2.4547		
Linear contrast	1	0.0165	0.22	.6437
Quadratic contrast	1	0.1169	1.57	.2267

Appendix F Table 10. Analysis of variance for the effect of intracerebroventricular injection of β-endorphin (1.5 to 6.0 ug) on food intake in Single-Comb White Leghorn cockerels (Chapter 6).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	1	22.78	27.22	.0001
Day	3	12.34	4.92	.0114
Bird (Rep)	6	2.69	0.54	.7745
Trt	3	6.84	2.73	.0745
Error	18	15.07		
Total	31	59.72		
Linear contrast	1	0.02	0.03	.8721
Quadratic contrast	1	0.04	0.51	.4830
30 min				
Rep	1	47.53	37.50	.0001
Day	3	5.34	1.41	.2737
Bird (Rep)	6	5.94	0.78	.5957
Trt	3	5.59	1.47	.2558
Error	18	22.82		
Total	31	87.22		
Linear contrast	1	1.22	0.96	.3391
Quadratic contrast	1	0.55	0.43	.5190
45 min				
Rep	1	52.53	31.72	.0001
Day	3	15.84	3.19	.0487
Bird (Rep)	6	15.69	1.58	.2104
Trt	3	8.59	1.73	.1968
Error	18	29.82		
Total	31	122.47		
Linear contrast	1	2.90	1.75	.2023
Quadratic contrast	1	1.87	1.13	.3021
60 min				
Rep	1	52.53	38.89	.0001
Day	3	21.09	5.21	.0092
Bird (Rep)	6	16.19	2.00	.1193
Trt	3	6.84	1.69	.2050
Error	18	24.32		
Total	31	120.97		
Linear contrast	1	2.32	1.72	.2063
Quadratic contrast	1	1.79	1.33	.2644
90 min				
Rep	1	98.00	40.55	.0001
Day	3	50.75	7.00	.0026
Bird (Rep)	6	24.50	1.69	.1809
Trt	3	21.25	2.93	.0616
Error	18	43.50		
Total	31	238.00		
Linear contrast	1	3.66	1.51	.2345
Quadratic contrast	1	15.68	6.49	.0202
120 min				
Rep	1	101.53	43.19	.0001
Day	3	64.09	9.09	.0007
Bird (Rep)	6	36.44	2.58	.0552
Trt	3	20.34	2.88	.0643
Error	18	42.32		
Total	31	264.72		
Linear contrast	1	2.32	0.99	.3334
Quadratic contrast	1	17.06	7.26	.0148

150 min				
Rep	1	166.53	30.72	.0001
Day	3	100.34	6.17	.0045
Bird (Rep)	6	32.94	1.01	.4478
Trt	3	30.34	1.87	.1715
Error	18	97.57		
Total	31	427.72		
Linear contrast	1	2.14	0.40	.5373
Quadratic contrast	1	26.89	4.96	.0389
180 min				
Rep	1	413.28	6.57	.0196
Day	3	2.34	0.01	.9980
Bird (Rep)	6	1617.19	4.28	.0075
Trt	3	296.09	1.57	.2317
Error	18	1132.82		
Total	31	3461.72		
Linear contrast	1	3.77	0.06	.8094
Quadratic contrast	1	292.20	4.64	.0450
240 min				
Rep	1	153.12	1.87	.1885
Day	3	643.75	2.62	.0824
Bird (Rep)	6	1196.87	2.43	.0670
Trt	3	731.25	2.97	.0592
Error	18	1475.01		
Total	31	4200.00		
Linear contrast	1	0	0	1.0
Quadratic contrast	1	322.18	3.93	.0629
300 min				
Rep	1	94.53	1.72	.2061
Day	3	308.59	1.87	.1705
Bird (Rep)	6	1117.19	3.39	.0205
Trt	3	858.59	5.21	.0091
Error	18	989.07		
Total	31	3367.97		
Linear contrast	1	161.27	2.94	.1038
Quadratic contrast	1	679.63	12.37	.0025
1440 min				
Rep	1	850.78	2.34	.1437
Day	3	283.59	0.26	.8534
Bird (Rep)	6	11085.94	5.08	.0033
Trt	3	683.59	0.63	.6074
Error	18	6551.57		
Total	31	19455.47		
Linear contrast	1	550.20	1.51	.2347
Quadratic contrast	1	133.09	0.37	.5529

Appendix F Table 11. Analysis of variance for the effect of intracerebroventricular injection of β-endorphin (1.5 to 6.0 ug) on water intake in Single-Comb White Leghorn cockerels (Chapter 6).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	1	3.12	1.00	.3306
Day	3	37.50	4.00	.0240
Bird (Rep)	6	21.87	1.17	.3665
Trt	3	18.75	2.00	.1501
Error	18	56.26		
Total	33	137.50		
Linear contrast	1	12.87	4.11	.0576
Quadratic contrast	1	5.87	1.88	.1875
30 min				
Rep	1	0	0	1.0
Day	3	106.25	6.37	.0039
Bird (Rep)	6	37.50	1.12	.3872
Trt	3	56.25	3.37	.0412
Error	18	100.00		
Total	33	300.00		
Linear contrast	1	43.21	7.78	.0121
Quadratic contrast	1	10.74	1.93	.1814
45 min				
Rep	1	0.78	0.07	.7977
Day	3	164.84	4.76	.0130
Bird (Rep)	6	73.44	1.06	.4213
Trt	3	133.59	3.86	.0217
Error	18	207.82		
Total	33	580.47		
Linear contrast	1	94.31	8.17	.0104
Quadratic contrast	1	39.30	3.40	.0816
60 min				
Rep	1	0.78	0.07	.7977
Day	3	164.84	4.76	.0130
Bird (Rep)	6	73.44	1.06	.4213
Trt	3	133.59	3.86	.0271
Error	18	207.82		
Total	33	580.47		
Linear contrast	1	94.31	8.17	.0104
Quadratic contrast	1	39.30	3.40	.0816
90 min				
Rep	1	175.78	3.74	.0689
Day	3	246.09	1.75	.1934
Bird (Rep)	6	173.44	0.62	.7153
Trt	3	214.84	1.52	.2422
Error	18	845.32		
Total	33	1655.47		
Linear contrast	1	201.45	4.29	.0530
Quadratic contrast	1	12.69	0.27	.6095
120 min				
Rep	1	282.03	3.94	.0627
Day	3	252.34	1.17	.3471
Bird (Rep)	6	573.44	1.33	.2929
Trt	3	139.84	0.65	.5926
Error	18	1289.07		
Total	33	2536.72		
Linear contrast	1	125.56	1.75	.2020
Quadratic contrast	1	2.92	0.04	.8421

150 min				
Rep	1	378.12	4.67	.0443
Day	3	90.62	0.37	.7732
Bird (Rep)	6	1206.25	2.48	.0627
Trt	3	15.62	0.06	.9780
Error	18	1456.26		
Total	33	3146.87		
Linear contrast	1	0.80	0.01	.9217
Quadratic contrast	1	14.79	0.18	.6740
180 min				
Rep	1	413.28	6.57	.0196
Day	3	2.34	0.01	.9980
Bird (Rep)	6	1617.19	4.28	.0075
Trt	3	296.09	1.57	.2317
Error	18	1132.82		
Total	33	3461.72		
Linear contrast	1	3.77	0.06	.8094
Quadratic contrast	1	292.20	4.64	.0450
240 min				
Rep	1	153.12	1.87	.1885
Day	3	643.75	2.62	.0824
Bird (Rep)	6	1196.87	2.43	.0670
Trt	3	731.25	2.97	.0592
Error	18	1475.01		
Total	33	4200.00		
Linear contrast	1	0	0	1.0
Quadratic contrast	1	322.18	3.93	.0629
300 min				
Rep	1	94.53	1.72	.2061
Day	3	308.59	1.87	.1705
Bird (Rep)	6	1117.19	3.39	.0205
Trt	3	858.59	5.21	.0091
Error	18	989.07		
Total	33	3367.97		
Linear contrast	1	161.27	2.94	.1038
Quadratic contrast	1	679.63	12.37	.0025
1440 min				
Rep	1	850.78	2.34	.1437
Day	3	283.59	0.26	.8534
Bird (Rep)	6	11085.94	5.08	.0033
Trt	3	683.59	0.63	.6074
Error	18	6551.57		
Total	33	19455.47		
Linear contrast	1	550.20	1.51	.2347
Quadratic contrast	1	133.09	0.37	.5529

Appendix F Table 12. Analysis of variance for the effect of intracerebroventricular injection of β-endorphin (1.5 to 6.0 ug) on colonic temperature in Single-Comb White Leghorn cockerels (Chapter 6).

Source of variation	df	Sum of squares	F value	P
15 min				
Rep	1	0.0112	0.34	.5685
Day	3	0.0662	0.66	.5858
Bird (Rep)	6	0.0775	0.39	.8774
Trt	3	0.2337	2.34	.1079
Error	18	0.6001		
Total	31	0.9887		
Linear contrast	1	0.1603	4.81	.0417
Quadratic contrast	1	0.0494	1.48	.2392
30 min				
Rep	1	0.2812	4.07	.0589
Day	3	0.2425	1.17	.3492
Bird (Rep)	6	0.5487	1.32	.2977
Trt	3	1.0375	5.00	.0107
Error	18	1.2451		
Total	31	3.3550		
Linear contrast	1	0.9606	13.89	.0015
Quadratic contrast	1	0.0764	1.10	.3071
45 min				
Rep	1	0.1012	2.05	.1690
Day	3	0.4112	2.78	.0708
Bird (Rep)	6	0.4475	1.51	.2301
Trt	3	1.5312	10.35	.0003
Error	18	0.8876		
Total	31	3.3787		
Linear contrast	1	1.3580	27.54	.0001
Quadratic contrast	1	0.1707	3.46	.0792
60 min				
Rep	1	0.0378	1.22	.2840
Day	3	0.2784	2.99	.0582
Bird (Rep)	6	0.4069	2.19	.0927
Trt	3	1.9809	21.30	.0001
Error	18	0.5582		
Total	31	3.2622		
Linear contrast	1	1.7681	57.02	.0001
Quadratic contrast	1	0.2027	6.54	.0198
90 min				
Rep	1	0.0050	0.10	.7535
Day	3	0.7037	4.77	.0128
Bird (Rep)	6	1.0537	3.57	.0165
Trt	3	1.9112	12.96	.0001
Error	18	0.8851		
Total	31	4.5587		
Linear contrast	1	1.3029	26.50	.0001
Quadratic contrast	1	0.5321	10.82	.0041
120 min				
Rep	1	0.0528	0.82	.3779
Day	3	0.4584	2.36	.1050
Bird (Rep)	6	1.3319	3.44	.0194
Trt	3	1.5109	7.79	.0015
Error	18	1.1632		
Total	31	4.5172		
Linear contrast	1	1.1126	17.22	.0006
Quadratic contrast	1	0.2779	4.30	.0527

150 min				
Rep	1	0.0078	0.12	.7324
Day	3	0.2384	1.23	.3287
Bird (Rep)	6	1.5894	4.09	.0092
Trt	3	0.8584	4.42	.0170
Error	18	1.1657		
Total	31	3.8597		
Linear contrast	1	0.7151	11.04	.0038
Quadratic contrast	1	0.0828	1.28	.2729
180 min				
Rep	1	0.0200	0.17	.6853
Day	3	0.3562	1.01	.4125
Bird (Rep)	6	1.4187	2.01	.1181
Trt	3	0.8112	2.29	.1126
Error	18	2.1226		
Total	31	4.7287		
Linear contrast	1	0.7717	6.54	.0198
Quadratic contrast	1	0.0064	0.05	.8188
240 min				
Rep	1	0.1128	1.22	.2848
Day	3	0.3234	1.16	.3517
Bird (Rep)	6	0.9719	1.75	.1678
Trt	3	0.4784	1.72	.1990
Error	18	1.6707		
Total	31	3.5572		
Linear contrast	1	0.3679	3.96	.0619
Quadratic contrast	1	0.0895	0.96	.3391
300 min				
Rep	1	0.0253	0.49	.4919
Day	3	0.4059	2.63	.0815
Bird (Rep)	6	0.3569	1.16	.3714
Trt	3	0.1059	0.69	.5718
Error	18	0.9257		
Total	31	1.8197		
Linear contrast	1	0.0984	1.91	.1834
Quadratic contrast	1	0.0063	0.12	.7292

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