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Opioid receptors in the chicken brain

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For my beloved grandmother, grandfather and mother

Dedicated to all experimental chickens

1 Introduction

Study of brain opioid receptors is a useful way of examination of opioidergic control of brain functions or central nervous (CNS) control of endocrine system. Opioids are (neuro)peptides, which are synthesized in the CNS in the whole animal kingdom (**HÖKFELT** et al. 2000). They play a role both as neurotransmitter and neuromodulator and exert their actions via specific receptors. Opioid peptides and their receptors are implicated in the modulation of a number of behavioral and physiological functions in birds (e.g., reproduction, endocrinology, water balance, social behavior and painful stimulus) in a manner similar to that described for mammals (**AKIL** et al. 1984; **MARTIN** 1984; **STANSFIELD** and **CUNNINGHAM** 1988; **OLSON** et al. 1991, 1992, 1993). Nowadays, more attention is being paid to the effect of the opioid system on the water balance in birds. Opioid drugs were also introduced in veterinary medicine for pain relieving and anesthesia of birds (**CONCANNON** et al. 1995; **NOLAN** 2003).

Endogenous opioid peptides (EOP) influence various hormones such as Luteinizing hormone (LH), Arginine Vasotocin (AVT), Growth hormone (GH) and adrenal and sexual steroids (**SAITO** et al. 1999; **SASAKI** et al. 2000). There is evidence that EOP suppress the secretion of AVT during osmotic stress. Conversely, Naloxone (NAL) provokes the increase of plasma levels of AVT (**SAITO** et al. 1999).

In general, receptor binding properties are known to be regulated by a variety of factors such as physiological, pathological and pharmacological ones. Numerous studies of the impact of physiological modulation on opioid binding sites have been performed in order to elucidate the nature of this effects (**WILKINSON** et al. 1985; **BECKMAN** et al. 1986; **HNATOWICH** et al. 1986; **BRADY** and **HERKENHAM** 1987; **CICERO** et al. 1987; **CRAIN** et al. 1987; **MILLAN** et al. 1987). Most of these studies have been conducted in mammals using binding assay or autoradiographic methods. Nevertheless, there is very little data available on opioid binding sites in different physiological condition, especially in the chicken. Thus the present work was undertaken to investigate the regulatory effect of age, sex, and castration on the opioid receptors. Furthermore, the effect of dehydration on the chicken opioid binding sites has been determined.

2 Literature review

2.1 Nomenclature

The term "**opioid**" generally refers to any substance, endogenous or synthetic peptide or non-peptide, which directly acts through an interaction with any of the four major types of opioid receptors present in the cell membrane and its effects can be stereospecifically blocked by pure narcotic antagonists like naloxone (**PRADHAN** and **DUTTA** 1986; **HÖLLT** 2004). To be precise, the term "**opiate**" refers to an opioid which its structure and biological properties are similar to morphine (non-peptidic structure) (**HÖLLT** 2004). The old term 'opiates' has been now replaced by the term 'opioids'. The term **endogenous opioids** refer to any material occurring in the brain or other organs. These opioids have pharmacological properties similar to the opiate substance morphine and serve as natural ligands of the opioid receptors. Endogenous opioids are mostly peptides, although reports of non-peptide endogenous opioid are also known (**FREDERICKSON** 1984). Unlike opiates, opioid peptides are rapidly degraded after being released, and they do not accumulate in large sufficient amounts to induce tolerance (**PRADHAN** and **DUTTA** 1986).

2.2 Historical perspective of opioid peptides and receptors

In 1954 **BECKETT** and **CASY** initiated for the first time the concept of pharmacologically relevant receptors for opiates, based on activities of stereoisomers. Later **PORTOGHESE** (1965) introduced the concept of different modes of interaction of morphine and other analgesics with opioid receptors and observed the possible existence of separate opioid receptors. The stereospecific requirements of these receptors were then subsequently confirmed by both behavioral (**MARTIN** 1967) and biochemical studies (**GOLDSTEIN** et al. 1971; **PERT** and **SNYDER** 1973; **TERENIUS** 1973). **GOLDSTEIN** et al. (1971) subsequently proposed that radiolabeled compounds might be used to demonstrate the existence of these receptors and to characterize them. The characterization of biological receptors in mammalian brain tissue with high selectivity led to the search for an endogenous opioid. As soon as radioligands with high specific activities were available, three different groups showed that there are stereospecific opioid binding sites in the mammalian brain (**PERT** and **SNYDER** 1973; **SIMON** et al. 1973; **TERENIUS** 1973). Later on, two groups reported the detection of substances in brain extracts that exhibited opiatelike (opioid)

activities (**HUGHES** et al.1975; **TERENIUS** and **WALSTROM** 1975). **HUGHES** et al. (1975) succeeded in isolating and characterizing the first endogenous molecules with opioid activity and high affinity for opioid receptors in aqueous extracts of pig brain. These peptides differed only in the C-terminal amino acid and were named Methionine enkephalin (Met-ENK, Tyr-Gly-Gly-Phe-Met) and Leucine enkephalin (Leu-ENK, Tyr-Gly-Gly-Phe-Leu) ([*enkephalin* meaning "in the head"]). This initial discovery led to the identification of opioid peptide and its receptors, which have been now characterized in a variety of species.

2.3 Opioid receptors

The rigid structural and stereochemical requirements for opiate activity led to the theory that they exert their effects by interacting with a specific receptor. The specificity of brain receptors towards opiate alkaloids was first demonstrated with two pentapeptides, Met–ENK and Leu-ENK (**HUGHES** et al. 1975). A further breakthrough came when **MARTIN** et al. (1976) discovered that opiates with different chemical structures exhibited different pharmacological effects in dogs with long-term spinal transsection. He found out that morphine, ketazocine and N-allyinormetazocine (SKF-10047) had different effects on respiration, heart rate and locomotor activity. Furthermore, these compounds were unable to substitute for each other in the prevention of withdrawal symptoms in dogs chronically treated with one of these compounds. On the basis of pharmacological experiments, Martin classified opioid receptors into three subtypes: μ- (morphine-like), κ- (ketazocine-like), and σ- receptors (N-allylnormetazocine-like). The existence of the additional δ-receptors was proposed to explain the in vitro activity of enkephalins and the relative potency of the non-selective opioid antagonist naloxone (**LORD** et al. 1977). Further studies have shown that the σ syndrome associated with SKF-10047 is not blocked by naloxone, thus the σ receptor was no longer considered part of the opioid system (**ZUKIN** and **ZUKIN** 1981; **QUIRION** et al. 1987). Indeed, opioid receptors are present not only in the CNS but also in the periphery. This fact has been exploited to provide functional models of the opioid action. Following the discovery of enkephalins, some scientists discovered that electrically evoked contractions of the isolated guinea pig ileum were much more sensitive to inhibition by morphine and related opioid alkaloids than by enkephalins, whereas the opposite was observed in the mouse vas deferens preparation. Moreover, the effects of the opioid peptides on the vas deferens were relatively insensitive to naloxone. Based on these observations, **LORD** et al. (1977) proposed that the

fourth type of opioid receptor, δ (deferens), was present in mouse vas deferens. There are thus three main types of opioid receptors: μ-, δ- and κ. These three major types of opioid receptors have been distinguished by radioligand binding assays, in vitro pharmacological assays on isolated smooth muscle preparations, electrophysiological and neurochemical assays, and behavioral models (**CHANG** 1984; **REES** and **HUNTER** 1990). Moreover, they have been confirmed by cloning of their three corresponding gene and subsequent confirmation that they show more than 60% homology (**MCNALLY** and **AKIL 2**002). In addition to the three major classes of opioid receptor (μ, δ, κ), an additional number of subtypes have been proposed, usually based on bioassays carried out in different species. There is some evidence to suggest that the epsilon (ε) (**SCHULZ** et al. 1979), iota (ι) (**OKA** 1980), zeta (ζ) (**ZAGON** et al. 1991), and a high affinity binding site lambda (λ) receptor (**GREVEL** and **SADÉE** 1983) may also be parts of the opioid receptor system. Finally, the latest opioid receptor has been identified by its high homology to the other opioid receptor subtypes. It has been termed opioid receptor like (ORL1), because none of the endogenous opioid or opiate drugs show a high affinity for it.

The action of opioid agonist, antagonists and mixed agonist-antagonists could be explained at best by actions on multiple opioid receptors. Several subtypes of the opioid receptors (viz. μ_1, μ_2; δ_1, δ_2; κ_1, κ_2, κ_3) have been postulated on the basis of pharmacological studies. However, attempts to identify subtypes of opioid receptors have not been successful (**KOCH** et al. 1998; **UHL** et al. 1999; **ABBADIE** et al. 2000). With regard to the nomenclature of the well defined opioid receptors, the situation is rather confusing due to the fact that although the use of Greek letters is generally accepted by pharmacologists, molecular biologists prefer to name the μ-, δ- and κ receptors MOR, DOR, and KOR respectively. Similarly, the nomenclature proposed by the molecular biologists is not satisfactory because it derives directly from the Greek letters. Based on the guidelines defined by the International Union of Pharmacology Committee (IUPHAR) for Receptor Nomenclature and Drug Classification (**DHAWAN** et al. 1996), receptors should be named after their endogenous ligands and identified by a numerical subscript corresponding to the chronological order of the formal demonstration of their existence by cloning and sequencing. Thus, the generic designation of these receptors on which all opioids act as agonists should be OP. As the mouse δ receptor was the first one cloned (**EVANS** et al. 1992; **KIEFFER** et al. 1992),

it should be renamed OP_1. As this initial cloning facilitated the rapid cloning of the rest of receptors, the κ- (**CHEN** et al. 1993b; **LI** et al. 1993; **MENG** et al. 1993) and μ- (**CHEN** 1993a; **FUKUDA** et al. 1993) receptors have been renamed to OP_2, and OP_3 receptors, respectively.

Table 1. Rational (IUPHAR recommendation) and current nomenclature of opioid receptors

Preferential Endogenous Opioid Ligands	Opioid receptors					
	IUPHAR recommendation	Pharmacology nomenclature	Molecular biology nomenclature	Rank order of potency	Selective agonist	Selective antagonist
Enkephalin	OP_1	δ	DOR	β-END = leu = met > DYN A	DPDPE DSBULET [D-Ala^2]Deltorphin	Naltrindole NNDT TIPP
Dynorphin	OP_2	κ	KOR	DYN A >> β-END > leu > met	U69593 C1977 ICI197067	Nor-binaltorphine
β-Endorphin	OP_3	μ	MOR	β-END > DYN A > met = leu	Endomorphin-1 Endomorphin-2 DAMGO Sufentanil PL017	CTOP
Nociceptin	OP_4	ORL 1	NOP	N/OFQ >> DYN A	N/OFQ N/OFQ-(1-13)-NH_2 Ro646198	J11397

After modified from **DHAWAN** et al. (1996); **HÖLLT** (2004) and **ALEXANDER** et al. (2004)

2.3.1 Types of opioid receptors

2.3.1.1 δ (OP1) Opioid receptor

Subtypes of delta receptors, term δ_1 and δ_2, have been proposed based on studies with antagonists (Table 2) (**JIANG** et al. 1991; **SOFUOGLU** et al. 1991; **SOFUOGLU** et al. 1993). The δ- opioid receptor ligands are shown in the appendix (app. Table 1). Although their pharmacology has not been completely evaluated, there is evidence, which suggests that δ_2 receptors mediate analgesia in the spinal canal and in the brain, whereas δ_1 receptors elicit analgesia primarily in the brain (**PASTERNAK** 2003). In addition to this, there is no evidence for the existence of splice variants of this receptor (**OFFERMANNS** and **ROSENTHAL** 2004).

2.3.1.2 κ (OP2) Opioid receptor

κ-Receptors (KOR) are characterized by having a high affinity for some benzomorphan drugs such as bremazocine and arylacetamides (U50488H and U69593) and dynorphin (DYN) (**GOLDSTEIN** and **NAIDU** 1989). These receptors mediate many of the actions of these compounds, including food and water intake, gastrointestinal transit, thermoregulation and numerous endocrine effects. There is strong pharmacological evidence for the existence of κ- receptors subtypes (**WOLLEMMANN** et al. 1993). κ- Receptors have been classified into three subtypes, term κ_1, κ_2, and κ_3 (Table 2) (**CHANG** et al. 1984; **CASTANAS** et al. 1985). The κ- opioid receptor ligands are shown in the appendix (app. Table 2). Dynorphin A is the endogenous ligand for the κ_1 receptor. κ_2 Receptor is not fully understood and it may actually be a dimer composed of a κ_1 receptor physically associated with a δ receptor. Furthermore, in mice deficient in μ-, δ- and κ opioid receptors (triple knockouts) no evidence for κ_2 binding sites could be found (**OFFERMANNS** and **ROSENTHAL** 2004). The κ_3 receptor is appeared to be closely related, at the gene level, to the nociceptin receptor (**PASTERNAK** 2003).

2.3.1.3 μ (OP_3) Opioid receptor

The μ-opioid receptor (MOR) is the classical target for morphine and mediates the analgesic and additive effects of opiates. Therefore, in μ-opioid receptor deficient mice

morphine does not exhibit analgesic and positive reinforcing properties (**OFFERMANNS** and **ROSENTHAL** 2004). μ-Receptors are characterized by having a high affinity for morphine-like drugs and for several endogenous opioid peptides, including β-END, DYN A (1-8) and BAM18 (**LESLIE** 1987), endomorphin-1 and -2 (**ZADINA** et al. 1997) and it may be an isoreceptor for the enkephalin. The μ- opioid receptor ligands are shown in the appendix (app. Table 3). These receptors mediate prototypical effects of morphine or heroine (or diacetamorphine is derived from morphine. Heroin made by exposing morphine to acetic acid, causing a change in the chemical in phenolic and alcohol OH group) administration, including analgesia, physical dependence and respiratory depression. Several splice variants of MOR that differ in their amino acid sequence at the C-terminal end have been cloned (including viz. MOP-A, -B, -C, -D, -E, -F) (**PASTERNAK** 2001). These receptor variants differ in the rate of internalization and desensitization upon agonist exposure but have similar binding and coupling properties. μ Receptors have been subclassified into μ_1 and μ_2 subtypes (Table 2), based on radioligand binding and functional studies which showed, that [^{3}H]-labeled μ-, δ- and κ-ligands displayed biphasic binding characteristics (**WOLOZIN** and **PASTERNAK** 1981; **PASTERNAK** and **WOOD** 1986; **PASTERNAK** 1988). μ_1-Receptor is supraspinal and has been implicated in prolactin release, feeding and acetylcholine release and analgesic actions of morphine within the brain. μ_2-Receptor is mostly spinal concerning its localization and it is physiologically implicated in respiratory depression, gastrointestinal transit, brain dopamine turnover, feeding and cardiovascular actions (**PASTERNAK** 2001; **KASCHOW** and **GERACIOTI** 2002).

2.3.1.4 ORL1 or N/OFQ (OP_4)

A novel receptor which bore a high degree of homology with the classical opioid receptor types was identified in three species: the human clone ORL1 (**MOLLEREAU** et al. 1994), the one found in rat brain libraries (ROR-C, oprl, LC132, XOR1, Hyp 8-1 or C3) (**FUKUDA** et al. 1994; **CHEN** et al. 1994; **BUNZOW** et al. 1994; **WANG** et al. 1994; **WICK** et al. 1994; **LACHOWICZ** et al. 1995) and the one from the mouse genomic library (MOR-C) (**NISHI** et al. 1994), with >90% degree of homology among the species variants. Although the putative receptor had received many names, there is some consensus due to the fact that the original designation for the human form (ORL1) is mostly used. The endogenous peptide agonist for ORL1 has been termed orphanin FQ or nociceptin because of its putative

ability to lower pain thresholds. The ORL1 receptor was identified by its high homology to the other opioid receptor subtypes but there is no corresponding pharmacological homology. Even non-selective ligands that exhibit uniformly high affinity towards μ-, κ- and δ-receptors, have very low affinity for the ORL1 receptor. For this reason the receptor was called an orphan opioid receptor or opioid receptor like. However, none, of the endogenous opioid peptides or the opiate drugs show a high affinity for this receptor. J-113397, a drug with potent and selective antagonistic activity at ORL1 receptors, has been characterized (**KAWAMOTO** et al. 1999). The N/OFQ opioid receptor ligands are shown in the appendix (app. Table 4). Splice variants have been found in the human and mouse NOR receptor with no known functional significance. NOR receptors are widely distributed throughout the brain and in the spinal cord. They are also present in immune cells. A functional role for N/OFQ has been proposed in nociception, locomotoric activity, reward, stress and immunomodulation (**OFFERMANNS** and **ROSENTHAL** 2004). Table 2 is presenting the opioid receptor subtypes and their major action.

Table 2. Opioid receptor subtypes and their major actions

Receptor	Major action
Mu (μ)	
Mu_1	Supraspinal and peripheral analgesia
	Prolactin release, feeding
	Acetylcholine release in the hippocampus
Mu_2	Spinal analgesia, respiratory depression
	Bradycardia, euphoria, physical dependence
	Inhibition of gastrointestinal transit
	Dopamine release by nigrostriatal neurons
	Guinea pig ileum bioassay, feeding
Mu_3	Hyperpolarization of peripheral nerves induced
	By inflammation/immune response
Kappa (κ)	
$Kappa_1$	Analgesia, Dysphoria
	Diuresis, Feeding
$Kappa_2$	Unknown
$Kappa_3$	Analgesia
Delta (δ)	Mouse vas deferens bioassay, feeding
	Dopamine turnover in the striatum
$Delta_1$	Supraspinal analgesia
$Delta_2$	Spinal and supraspinal analgesia

Modified from **PASTERNAK** (2003); **WAGNER** (2002)

2.3.2 Biochemical and molecular properties of opioid receptors

Opioid receptors are members of the seven-transmembrane domain G protein-coupled receptor family in the plasma membrane (**REISINE** and **BELL** 1993; **UHL** et al. 1994). From the top view surface, these seven spans are arranged in a donut-like shape, binding the drugs to the cavity in their center (Figure 1).

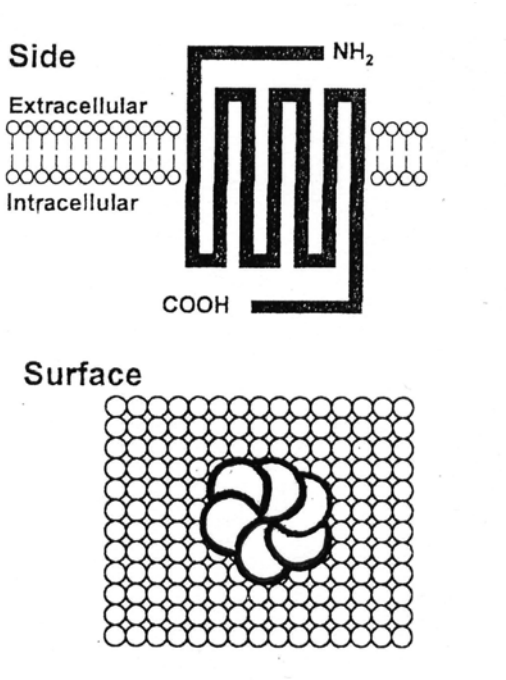

Figure 1. Schematic structure of opioid receptors. G protein-coupled receptors traverse the membrane seven times (side-viewed) and arranged in a donut-like shape (surface-viewed), with a cavity in the center in which the ligand binds.
(After **PASTERNAK** (2003))

All opioid receptors show a composition of amino acids with a 60-67% degree of similarity. The sequences are highly conserved among vertebrates but not in invertebrates. There are single copies of each gene and their chromosomal locations are known. The three opiate receptor genes are located on different chromosomes, as it is the case of the genes for the μ-, κ- and δ- opioid receptors in mice, which are located in chromosomes 10, 1 and 4, respectively (**EVAN** et al. 1992; **YASUDA** et al. 1993; **WANG** et al. 1993; **UHL** et al. 1994). The coding regions of human genes for opioid receptors have been subsequently isolated and chromosomally assigned (**BEFORT** et al. 1994; **WANG** et al. 1994; **YASUDA** et al. 1994). From studies of the cloned receptors it became clear that high affinity interactions between

each of the precursor and receptor families are possible (**MANSOUR** et al. 1995). Selectivity for the different ligands depends on variations within the N- and C-terminal regions, the fourth transmembrane loop, as well as the second and third extracellular loops (**REISINE** et al. 1994). β-Endorphin constituted an excellent example and studies from a number of laboratories imply a discrete receptor for this opioid peptides, termed ε (epsilon).

Opioid binding is inhibited by sulfhydryl reagents, such as *N*-ethylmaleimide and iodoacetate. The role of lipids in opioid binding is less clear. The pH optimum for binding is in the physiological range (pH 7 to 8). Moreover, there is also evidence that sodium ions in the incubation medium increase the affinity of antagonist binding while decreasing that of agonist binding. In general, monovalent cations such as sodium reduce agonist affinity while divalent cations such as magnesium increase agonist affinity (**PERT** and **SNYDER** 1974; **BLUME** 1978; **PFEIFFER** 1982a). Interestingly, the binding of radiolabeled opioid antagonists is not affected by sodium ion or guanine nucleotides (**PFEIFFER** 1982a; **FRANCES** et al. 1985).

2.3.3 Mechanisms of opioid actions on neurons

Although the activation of all opioid receptors leads to an inhibition of the release of neurotransmitters, the precise effect depends on the type of neurotransmitter and the CNS region involved. The three major subtypes of opioid receptors make use of cyclic AMP (cAMP) as their second messenger, while the functions of the other types have not been precisely described. The activation of the opioid receptors results in inhibition of adenyl cyclase, which catalyses the formation of cAMP from ATP (**SIMON** 1991). The inhibitory effects of the opioid receptors are mediated by inhibitory guanine triphosphate (GTP) binding regulatory protein (Gi protein). The μ- and δ- receptor mediated inhibition of adenyl cyclase has been clearly demonstrated while the role of the κ- modulation of the activity of either adenylate cyclase or phospholipase C is still controversial. The opioid receptors also participate in the modulation of ion channels via Go (G other) protein. Studies show that binding to μ- and δ- receptors causes the opening of potassium channels, whereas binding to κ- receptors mediates the closure of calcium channels. **UHL** et al. (1994) reported that μ-system has also been linked to phosphatidyl inositide action. Opioid receptors can be located on either the presynaptic (mainly κ but also μ) or postsynaptic (μ and δ) membranes (**HUNTER** et al. 1994). Opioid drugs can be classified according to their receptor selectivity

and they can be active at one, two or all of the receptors. It cannot be assumed directly that a certain receptor subtype will have an identical function in all species (**NOLAN** 2000).

2.3.4 Distribution of opioid receptors

The distribution of opioid receptors has been described in detail in many animal species such as humans, monkeys, cows, guinea pigs and rats (**PATERSON** et al. 1984). Each receptor is distributed differently throughout the CNS. The complexity of the opioid receptor and peptide interactions is magnified when the differences among species are considered. Anatomically, species differences can be observed within the distributions of each of the receptor types and at several levels of the neuroaxis. Opioid binding sites are found in the brain and in the dorsal horn of the spinal cord, and in a number of peripheral tissues. Opioid receptors found in the periphery mediate opioid effects such as the decrease of gastrointestinal motility (**NOLAN** 2000). Table 3 summarizes the regions containing high levels of opioid receptors.

Table 3. Location of opioid receptors proposed to mediate specific opioid effects

Opioid effect	Location of opioid receptors
ANALGESIA	
Spinal (body)	Laminae I and II of dorsal horn
Trigeminal	Substantia gelatinosa of trigeminal nerve
Supraspinal	Periaqueductal gray matter, medial thalamic nuclei, intralaminar thalamic nuclei, ?striatum
AUTONOMIC REFLEX	
Suppression of cough	Nuclei tractus solitarius, commisuralis, Ambiguous and locus coeruleus
Orthostatic hypotension	
Inhibition of gastric secretion	
Respiratory depression	Nuclei tractus solitarius, parabrachial nuclei
Nausea and vomiting	Area postrema
Meiosis	Superior colliculus, pretectal nuclei
ENDOCRINE EFFECTS	
Inhibition of vasopressin secretion	Posterior pituitary
Effects on other hormonal systems including	Hypothalamic infundibulum, Hypothalamic nuclei, Accessory optic system, Amygdala
EFFECTS ON BEHAVIOR AND MOOD	
	Amygdala, Nucleus stria terminalis, Hippocampus, Cortex, Medial thalamic nucleus, Nucleus accumbens Basal ganglia
MOTOR RIGIDITY	Striatum

After **SIMON** and **HILLER** (1994)

A poor correlation between the anatomical distribution of opioid peptide-containing nerve fibers and terminals and the distribution of the major types of opioid binding sites exists (**HERKENHAM** 1987). For example, in rats the caudate and various cortical areas possess high concentrations of opioid binding sites but low concentrations of opioid peptides. This is in contrast to the situation observed in the globus pallidus, which has a very high concentration of ENK but only low levels of δ-opioid-binding sites (**HERKENHAM** 1987; **REINER** et al.1989).

2.4 Opioid peptides

2.4.1 Structure

The amino acid sequences of the opioid peptides are shown in the appendix (app. Table 5). The classical opioid peptides are:-

(1) The pentapeptides Met-Enkephalin and Leu-Enkephalin, they are derived from the precursor preproenkephalin (PENK) although these sequences are also present in the preproopiomelanocortin (POMC) or PENK B. Their production depends on the selective processing of the precursor by endopeptidase in the cells expressing the PENK A gene. Enkephalin pentapeptides differ in having either Leucine or Methionine in their C termini. The enkephalin bind to both the μ- and δ- receptors.

(2) β-Endorphin, one of the active products of the POMC precursor. β-Lipotropin serves as a prohormone for α-, β-, and γ-endorphin.

(3) The dynorphins are principal products of the prodynorphin and which is sometimes called PENK B. Dynorphin binds more selectively to the κ receptor.

(4) Nociceptins are derived from the pronociceptin.

All of these endogenous opioids possess in the N-terminal sequence Tyr-Gly-Gly-Phe-Met/Leu. The effects of all these classical opioid peptide substances are antagonized by naloxone. However, higher concentrations of naloxone are required to antagonize the effects of ethylketocyclazocine and Leu-ENK as compared to morphine and β-endorphin (**ILLES** and **JACKISCH** 1991). Each endogenous opioid peptide generally has poor selectivity for the different types of opioid receptor although they are potent. Enkephalin prefer μ- or δ-

receptor, while the extended enkephalin, products of the proenkephalin A precursor, have higher affinity for the κ- receptor. Dynorphin shows a preference for the κ- receptor whereas endomorphins has a marked affinity for the μ- receptor.

A novel amidated tetrapeptide with opioid actions have been identified from the bovine and human brain (**ZADINA** et al. 1997; **HACKLER** et al. 1997) and named endormorphin-1 (EM_1) and endormorphin-2 (EM_2). They have a characteristic structure, which differs from the opioid core but they bind with high selectivity to μ-opioid receptors. The precursor and gene of these peptides are unidentified. Endormorphin-2 is found in discrete regions of rat brain, some of which are known to contain high concentrations of μ-receptors (**SCHREFF** et al. 1998) and endormorphin-2 is also present in primary sensory neurons and in the dorsal horn of the spinal cord (**MARTIN-SCHILD** et al. 1997). The endormorphins have both analgesic and gastrointestinal effects. At the cellular level, they activate G proteins and likewise inhibit calcium currents. The discovery of the endomorphins, which do not derive from the three precursor molecules, indicate the existence of additional opioid peptide genes.

2.5 Possible roles of endogenous opioid peptides

The broad distribution of opioid peptides in the brain suggests that they serve general roles as neurotransmitters or neuromodulators or both (**REISINE** and **BELL** 1993). While the pituitary and adrenal medulla may be considered as the major peripheral sources of endogenous opioid peptides, the blood brain barrier remains relatively resistant to penetration by such hydrophobic molecules. Therefore, opioid peptide modulation of CNS events appears to be rather through localized release from projection neurons to a specific area of the brain and spinal cord. Opioid peptides are contained in a widespread network of fibers, being colocalized in many instances with other neurotrasmitters including monoamines, peptides and amino acids, with the potential role of acting as either co-transmitters or neuromodulators. The recognized functions of opioid peptides have been deduced from their observed pharmacological effects, their anatomical distribution in regions known to control various physiological and behavioral functions, as well as from the effects of the administration of the opiate antagonist naloxone. Opioids modulate an array of functions including pain perception, stress mechanisms, respiratory regulation, temperature control, tolerance development, physical dependence, modulation of diuretic and cardiovascular functions. Behavioral patterns which seem to be under the influence of opioid peptides include sexual behavior, feeding and drinking, grooming and locomotor behavior (**IMURA** et al. 1985; **SIMON** and **HILLER** 1994). Like opiates, opioid peptides interact with the endocrine system, modulate the release of GH, ACTH, prolactin (PRL), antidiuretic hormone (ADH), LH and FSH (**CELLA** et al. 1993). Table 4 summarizes the most important known functions of opioids.

Table 4. Possible physiological functions of endogenous opioids and related peptides with related opioid receptor types

Function	Receptor types
1. Defence against noxious stimuli	
Pain inhibition	μ and δ (supraspinal, spinal)
	δ (medullary reticular formation)
	κ (spinal)
2. Modulation of the vegetative nervous system	
Cardiovascular regulation	μ, δ, κ
Respiration	μ and δ: may mediate respiratory depression
Thermoregulation	μ: may mediate hypothermia
	δ: may mediate hypothermia
3. Modulation of neuroendocrine function	
Anterior and posterior pituitary hormones	
Stimulatory effects	
GH	δ
ACTH	δ and κ
PRL	μ and κ
Inhibitory effects	
LH	μ, κ and δ
FSH	μ and κ
Vasopressin	μ and κ
4. Behavior action	
Locomotor behavior	
	μ: increased activity
	κ: sedation
Food and water intake	
Eating behavior	μ, δ, κ
Fluid balance	κ: diuresis
	μ: antidiuresis

2.6 Opioid peptides and opioid receptors in birds

2.6.1 Opioid peptides in birds

The first opioid peptides were isolated from the avian brain more than two decades ago. The peptides are present in several orders of birds such as Struthioniformes, Galliformes, Passeriformes, and Columbiformes. Immunocytochemical methods and radioimmunoassay demonstrated the distribution of the members of the three opioid peptide families (ENK, END, DYN) in the forebrain and midbrain and pituitary gland (**BAYON** et al. 1980; **DE LANEROLLE** et al. 1981; **RYAN** et al. 1981; **ERICHSEN** et al. 1982a,b; **BLÄHSER**, 1983; **MIKAMI** et al. 1983; **WHITE** et al. 1985; **MIKAMI**, 1986; **BALL** et al. 1988; **SHIMIZU** and **KARTEN**, 1990; **GÜNTÜRKÜN** and **KARTEN**, 1991 and **MARTIN** et al. 1992).

Immunoreactive Met-ENK neurons were found in the telencephalon, diencephalons and mesencephalon (**DE LANEROLLE** et al. 1981), ciliary ganglion (**WHITE** et al. 1985), amacrine cells in retina (**TORNQVIST** et al. 1981), neurohypophysis (**MARTIN** et al. 1992) of chickens and hypothalamus area including nucleus accumbens, nucleus of stria terminalis, lateral septal area, ventral part of the paraventricular nucleus (PVN) and infundibular nucleus in Japanese quail. Opioidergic fibers were found in the median forebrain bundle, preoptic area and medio-ventral hypothalamus and median eminence (**MIKAMI** et al. 1983, **MIKAMI** 1986). In pigeons; Enkephalin-like-immunoreactivity neurons were found in visual wulst (**SHIMIZU** and **KARTEN**, 1990), lateral geniculate complex (**GÜNTÜRKÜN** and **KARTEN**, 1991), brain stem, pituitary stalk, organum vasculosum hypothalami (**BAYON** et al. 1980) and ciliary ganglion, a ganglion of the cranial component of the parasympathetic nervous system (**ERICHSEN** et al. 1982a, **WHITE** et al. 1985). Passerine bird such as zebra finches, European starling and song sparrow have been shown to have series of nuclei containing enkephalin–like immunoreactivity in vocal control regions including the caudal nucleus of hyperstriatum ventrale, the robust nucleus of the archistriatum, the magnocellular nucleus of the neostriatum, area X of the lobus parolfactorius, nucleus interface, intercollicular nucleus and the tracheaosyringeal portion of the hypoglossal motor nucleus (**RYAN** et al. 1981; **BALL** et al. 1988).

Dynorphin-immunoreactive neurons were present in striatonigral projections of pigeons (**ANDERSON** and **REINER** 1991) the hypothalamus of songbirds (**BALL** et al. 1986) and in auditory nuclei brain stem (**CODE** 1996). However, the neurons were not found in the neurohypophysis of chickens (**MARTIN** et al. 1992).

β-endorphin-like-immunoreactive cells were observed in the preoptic area and anterior hypothalamus of chickens and quails (**VAN GILS** et al. 1994).

2.6.2 Opioid receptors in birds

In birds, there is relatively little information about the identification of opioid receptor subtypes; however, **STANSFIELD** and **CUNNINGHAM** (1987a,b) reported that in cockerels, μ- and δ-receptor subtypes may be involved in the regulation of LHRH release. Additionally, autoradiographic studies using highly specific agonists demonstrated that three subtypes of opioid receptor (μ-, δ-, κ-receptors) localize in different regions of the chicken brains (**REINER** et. al. 1989; **CSILLAG** et. al. 1990; **DEVICHE** et al. 1993). There are no data investigating the ORL1 receptor in birds.

In general, distribution of opioid receptor types is conserved across animal species in brainstem and spinal cord but varies significantly in the forebrain (**PAUL-MURPHY** and **LUDDERS** 2001). All three classical types of opioid receptors are widely distributed throughout the forebrain (telencephalon and diencephalons), midbrain (mesencephalon) in pigeons and chickens but low or absent in the medulla and cerebellum of chickens. Within the telencephalon of the pigeons, all three receptors type were abundant in the hyperstriatrum ventrale and striatum. Within the midbrain, all three receptor types were abundant in the tectum. When compare pigeons and chickens in the forebrain and midbrain areas, δ- or κ-binding levels are higher than μ- binding level in pigeons. In contrast to chickens, pigeons have less δ- binding sites than μ- and κ- binding sites in the forebrain. Both chickens and pigeons have only low binding levels of ligands in the diencephalons (**REINER**, et. al. 1989; **CSILLAG** et. al. 1990; **DEVICHE** et al. 1993). The variations between chicken and pigeons in distributions of the opioid receptor subclasses may also be a reflection of species-specific differences. Like mammals, both of the studies in chickens and pigeons showed that receptor and peptide are mismatched. The distribution of μ-, δ-, κ- receptors do not completely match the distribution of opioid peptides (**REINER** et al. 1989). The relative proportions of the

opioid receptor types in pigeon forebrain tissue were 14, 10 and 76% (fmol per mg tissue) μ-, δ-, κ- opioid receptor binding, respectively (**MANSOUR** 1988). The chicken opioid receptors have general pharmacological properties resembling those of mammalian receptors (**CSILLAG** et. al. 1990).

2.7 Ontogeny of opioid system in the chickens

Immunoreactive enkephalin peptides are visible in domestic fowl brain few days before hatching (**BLÄHSER**, 1983).

Table 5. Ontogeny of opioid receptors in the chickens

Age of chicken	Location	Ligand	Author
10,12-day-old chicken embryo	Whole brain	[^{3}H]Naloxone	HENDRICKSON and LIN (1980)
4,6,7,10,15,17,20-day of incubation and 3 day post hatch	Whole brain	[^{3}H]Etorphine	GIBSON and VERNADAKIS (1982)
1, 7, 14, 21 and 28 post hatch days	Forebrain, Midbrain	[^{3}H]Naloxone	BARDO et al. (1982)
18-day-old chicken embryo, 1and 30 post hatch days and adult	Supraopticus nucleus and Paraventricular nucleus	[^{3}H]Diprenorphine	ÖRDING et al. (1994)

As shown in table 5, only few binding studies have been employed to study opioid receptor development in the CNS of embryonal and posthatched chickens. Two of these studies have assayed whole brain homogenates. **GIBSON** and **VERNADAKIS** (1982) detected etorphine binding in chick embryo by day 4 of incubation. The first synapses in the lumbar spinal cord of the chicken embryo are detected in the ventrolateral marginal zone at day 4; the initial synapses are all axodendritic and contain spherical synaptic vesicles. Met-Enkephalin like immunoreactivity appears in the lumbar spinal cord at least as early as chick embryo day 4.5, the lumbar spinal cord begins the synthesis of opioid peptides at or before the initiation of synaptogenesis (**MADERDRUT** et al. 1986). Enkephalin-like immunoreactivity

has been reported as early as chick embryo day 5 in the telencephalic vesicles of the chick embryo (**DAVIS** et al. 1980) and both Leu- and Met-ENK have been detected in chick embryo and gut at 5 days of incubation (**EPSTEIN** et al. 1981). These data may indicate opiate receptors precede their corresponding endogenous binding ligands (**GIBSON** and **VERNADAKIS** 1980, 1982). By day 10 of incubation binding activity was confined to the neural tissue. Scatchard analysis showed the increase in binding to be due to increases in the receptor concentration and not changes in receptor affinity. Moreover, the result of one study showed that naloxone binding in chick brain neuronal cells occurred at 10 – 12 –day-old chicken embryo (**HENDRICKSON** and **LIN** 1980). **BARDO** et al. (1982) followed post-hatch development (1, 7, 14, 21 or 28 posthatching days of age) of naloxone binding in the midbrain and forebrain of the chick and found numbers of binding sites age-related decreased in both areas but to a greater extent in the midbrain. Likewise, [^{3}H]Diprenorphine binding has been detected in chick embryo day 18, 1, 30-day after hatching and adult in the PVN and supraopticus nucleus. This result indicated that there was also a concomitant age-related decrease in the binding site in the PVN (**ÖRDING** et al. 1994).

When the ontogeny of opioid receptor in chicks is compared to that in the rat, binding of naloxone in the rat's wholebrain can be detected as early as day 15 of the embryonic stage (**COYLE** and **PERT** 1976) and in the spinal cord, binding of diprenorphine (DPN) can be show at day 16, the chick opioid receptor was appeared earlier than the rat. Neuropeptidergic systems appear to develop in general earlier in the chick than in the rat, for example, substance P plexuses were seen in the median eminence of chick at embryonic Day 11 whereas in the rat they were detected only at postnatal day 1 (**HO** and **DEPALATIS** 1980; **ANDERSON** and **REINER** 1991).

2.8 Opioid receptor functions in the chickens

Opioid peptides influence various physiological and behavioral responses in birds including embryonic motility (**MADERBRUT** et al. 1985), body temperature (**MCCORMACK** and **DENBOW** 1988; **KAVALIERS** 1991), pain sensitivity (**SUFKA** et al. 1991; **CONCANNON** et al. 1995; **AZNARTE** et al. 2000, **BENSON** 2002), dendritic growth and spine formation (**HAUSER** et al. 1989), initiation of migrating myoelectrical intestinal activity (**JIMÉNEZ** et al. 1992), aggressive and sexual behavior (**FURUKAWA** et al. 1995), vocalization (**PANKSEPP** et al. 1978; **BALL** et al. 1988), ingestive behaviors

(**DEVICHE** and **SCHEPERS** 1984a,b; **UEMURA** et al. 1984; **MCCORMACK** and **DENBOW** 1987a,c), and endocrine regulation (**STANSFIELD** and **CUNNINGHAM** 1987a,b; **CONTIJOCH** et al. 1993; **FAN** et al. 1996).

Opiate receptors have been postulated in eliciting ingestive behavior in mammals (**TAKAI** et al. 1989). In mammals, several researches reported that EOP are involved in regulating the release of neurohypophysial peptides in relation to osmoregulation (**WADE** 1985; **YAMADA** et al. 1988). Several studies demonstrated that the administration of opioid peptide or naloxone affects water intake in birds (**UEMURA** et al. 1984; **MCCORMACK** and **DENBOW** 1987b; **FIRMAN** and **VOLMERT** 1991), suggesting a physiological action of opioid peptides in the regulation of osmoregulation in birds. In the pigeon, morphine and levorphanol, produced an initial suppression of both feeding and drinking, followed by a delayed hyperdipsia (**COOPER** and **TURKISH** 1981; **DEVICHE** and **SCHEPERS** 1984a). In the Japanese quail, Leu-ENK injected into the lateral ventricle inhibited natural and angiotensin II-induced drinking (**UEMURA** et al.1984). Naloxone inhibited feeding but not drinking in water-replete pigeons (**COOPER** and **TURKISH** 1981; **DEVICHE** and **WOHLAND** 1984) and did not inhibit drinking in 24 hours water-deprived pigeons. Naloxone injected intraperitoneally induced copious drinking in the Japanese quail (**UEMURA** et al. 1984). Since the dipsogenic effect of naloxone is modified by the sodium content of quail diet, the effect of the opioid peptides and their antagonists should be interpreted with caution (**TAKAI** et al. 1989).

2.8.1 Endogenous opioid and dehydration

Opioids have a modulatory role in the regulation of fluid balance in mammals (**FORSLING** 1985) and birds (**UEMURA** et al. 1984; **MCCORMACK** and **DENBOW** 1987b; **MCCORMACK** and **DENBOW** 1989; **FIRMAN** and **VOLMERT** 1991). In chickens, EOP also inhibits the release of AVT induced by osmotic stimulation (**SAITO** et al. 1999; **SASAKI** et al. 2000) like in mammals (**WADE** 1985).

Dehydration of both cellular and extracellular compartments (absolute dehydration) is inducible in terrestrial animals by water deprivation. Water deprivation has been shown to be an effective stimulus for vasopressin (ADH) or AVT from the posterior pituitary gland of mammal and avian, respectively. The main action of AVT is the stimulation of renal water

reabsorption at the distal convoluted tubules and the collecting ducts in the kidney (**STALLONE** and **BRAUN** 1986).

Opioid peptides are intrinsic to the neurohypophysis since PENK and PDYN are confined to the neural lobe of pituitary gland. Dynorphin A is co-localized and co-released with vasopressin whereas Met-ENK co-localized with oxytocin (**WATSON** et al. 1982). Dynorphin peptides are at high concentration in the neural lobe compared with the most other brain areas. Moreover, opioid binding sites of the κ- subtype are present in the neurohypophysis and around magnocellualr neurons (**LIGHTMAN** et al. 1983; **MANSOUR** et al. 1988). Opioid peptides have marked effects on the regulation of the release of posterior pituitary hormones in mammals. The early reports described antidiuretic actions of morphine and stimulation of vasopressin release by opioids (**BISSET** et al. 1978). Later studies reported the opposite effect of opioids on vasopressin (**AZIZ** et al. 1981). Naloxone did not affected to plasma vasopressin level elevated by dehydration (**SUMMY-LONG** et al. 1984). This may reflect the differential actions of opioids at the neurohypophysis. Effects certainly also depends on the dose used, experiment conditions, time course, route of administration and different animal species used in the experiments.

SLIZGI and **LUDENS** (1982) and **LEANDER** (1983) reported that κ-opioid receptor agonist such as bremazocine and ethylketocyclazocine produce dose-dependent increase of urinary output which can be antagonized by naloxone and MR226, κ-receptor antagonist. Other studies reported that dynorphin and β-endorphin are potent inhibitors of vasopressin and oxytocin release but enkephalin were without any effect (**TEN HAAF** et al. 1986, 1987). Later, **VAN DE HEIJNING** et al. (1991) presented evidence for an involvement of κ- and μ-opioid receptors, and not δ-opioid receptors, in the control of the vasopressin and oxytocin release from the rat neural lobes.

Several studies in birds demonstrated that the administration of opioid peptide and antagonist affects water intake (**DUKE** 1986). In the pigeon, an opioid agonist, morphine and levorphanol, produced an initial suppression of both feeding and drinking, followed by a delayed hyperdipsia (**COOPER** and **TURKISH** 1981; **DEVICHE** and **SCHEPERS** 1984a). In the Japanese quail, Leu-ENK injected into the lateral ventricle inhibited drinking (**UEMURA** et al.1984). Naloxone inhibited feeding but not drinking in water-replete pigeons (**COOPER** and **TURKISH** 1981; **DEVICHE** and **WOHLAND** 1984; **TAKAI** et al. 1989).

In another report, naloxone injected intraperitoneally induced copious drinking in the Japanese quail (**UEMURA** et al. 1984). Thus, the dipsogenic effect of naloxone is modified by opioids modulation of AVT secretion in chickens. In chickens, EOPs also inhibit the release of AVT induced by osmotic stimulation (**SAITO** et al. 1999; **SASAKI** et al. 2000). The neurohypophyseal hormones in birds are AVT and mesotocin (MT) (**ACHER** et al. 1970; **ACHER** 1993). AVT is the antidiuretic hormone in birds, and increases in plasma levels of the hormone are observed following dehydration or hypertonic stimulation (**KOIKE** et al. 1979; **STALLONE** and **BRAUN** 1986). **SASAKI** et al. (2000) demonstrated that opioid systems are involved in regulating AVT release in response to injection of hypertonic saline. In this study, morphine suppressed the increase in plasma levels of AVT induced by the injection of hypertonic saline. This inhibitory effect on AVT release was dose dependent and was diminished by simultaneous treatment with naloxone. The injection of naloxone alone potentiated AVT secretion after i.v. injection of hypertonic saline. Opioid peptides are also intrinsic to the avian neurohypophysial system (**KOTEGAWA** et al. 1995) and high concentrations of Met-ENK, unlike dynorphin, are found in the avian neurohypophysis (**MARTIN** et al. 1992). The fowl neurohypophysis also has opioid peptide binding sites, although the receptor subtypes have not been determined (**KAWASHIMA** et al. 1995). **SASAKI** et al. (2000) demonstrated that μ- and κ-opioid receptor subtypes are involved in the inhibition of AVT release by hyperosmotic and angiotensin II in chicks.

Deprivation of water for 5 days, or administration of 2% saline for 5 days, was reported to decrease the binding of ^{3}H-bremazocine in the posterior pituitary in rats by 35-50% (**BRADY** and **HERKENHAM** 1987). Other workers have observed a 65% increase in δ-, but not μ- binding in the nucleus tractus solitarius and spinal trigeminal nucleus following water deprivation for 5 days (**HWANG** et al. 1986). A single day of water deprivation is reported to increase total opioid binding (labeled by [^{3}H]DPN) in the claustrum, lateral hypothalamus and ventral tegmental area, while decreasing binding in the cortex, lateral septum and periaqueductal grey matter (**BLAKE** et al. 1987).

2.9 Steroid hormone and opioid receptors

Steroid modulation of EOP receptor activity has frequently been inferred but never proven unequivocally. Back in 1979, **HAHN** and **FISHMAN** reported that castration produced an increase in the Bmax of opiate binding sites in the whole brain of male rats.

These effects were readily reversible by administration of testosterone. Other research groups (**DIEZ** and **ROBERTS** 1982; **CICERO** et al. 1983) have been not able to replicate the result of this work. In the female rat, long-term ovariectomy increases the Bmax of μ- opioid binding (naloxone) in homogenates of anterior hypothalamus, while estrogen treatment decreases the Bmax (**WILKINSON** et al. 1985). **WEILAND** and **WISE** (1990) found that estrogen decreased naloxone binding and estrogen plus progesterone caused a reduction of the binding site in a region of the hypothalamus of ovarectomized rats. **WILKINSON** et al. (1983, 1985) and **VERTES** (1986) have demonstrated that both long term ovariectomy and acute administration of estradiol influence both the Kd and the Bmax of opioid receptors in the hypothalamus, but not the whole brain of female rats. It is suggested that the influence of steroid on opioid system may be confined to some area in the brain. The autoradiographic study by **HAMMER** (1985) showed a significant increase in opioid receptor content (^{3}H-Naloxone as ligand) in the sexually dimorphic nucleus of female rats from birth to 6 days but no such a change in opioid receptors occurred in males during this time. Interestingly, castrated male rats showed patterns of opioid receptor labeling in medial preoptic area identical to those observed in the female and testosterone administration to female resulted in a male pattern of opioid receptors. We could conclude from these data that regulation of opioid receptors may be sex hormone dependent.

In female animals, the ovarian cycles is characterized by cyclical variations in the secretion of estrogen and progesterone. In contrast to LH, FSH seems to be weakly influenced by opioids (**ALMEIDA** 1993). The maximal LH responses to naloxone are found in the luteal phase of a variety of species (**BROOK** et al. 1986). In contrast, opioidergic inhibition seemed reduced during the follicular phase, relying on the extent of naloxone-induced increased in LH secretion. However, **KAHLE** and **PARVIZI** (1993) reported that there are no influences of cyclicity in brain opioid binding site in the pig. This work is quite similar to the result in the sheep (**YANG** et al. 1989; **WEESNER** et al. 1989). The effect of endogenous opioid peptides suppress GnRH are mediated by opioid receptors since they are naloxone reversible. Most opioid effects upon GnRH secretion appear to be mediated by μ-receptors, as shown by **KAHLE** and **PARVIZI** (1993) using a variety of receptor-selective drug.

3 Aims and objectives

As the literature review indicates, the studies reported in this dissertation were undertaken to further elucidate opioid receptors and their regulation. To this end we evaluated the receptor binding profiles - the dissociation constant (Kd) and maximum binding capacity (Bmax) - in various brain regions. Following questions were addressed using the chicken as model.

- Are opioid binding profiles gender-, and/or age-dependent in the chicken brain?

- Are opioid binding profiles affected by dehydration in the chicken brain?

- Are opioid binding profiles testosterone-dependent?

- Are opioid binding profiles affected by opioid antagonists?

4 Materials and methods

4.1 Materials

4.1.1 Animals and experimental design

For all experiments Lohman-Selected Leghorn (LSL: *Gallus gallus domesticus*) chickens were bought from a commercial hatchery (Horstmann, Stolzenau and Lohman Tierzucht GmbH, Cuxhaven). Chickens were obtained at 1 day of age and raised in container with deep litter in an environmentally controlled lighting and temperature-regulated (temperature: 18-20°C; relative humidity: 50-60%) pen. Continuous light was provided during the first week of age and then from the second week onward a photo-schedule of 16 hours of light and 8 hours of darkness was used. Animals had free access to water and feed. Fully-grown fowl were 28-36 weeks of age. Adult chickens were housed under conditions of 12 hours of light and 12 hours of darkness with a layer feed and tap water provided for ad libitum consumption. Animal maintenance and research were conducted in accordance with the relevant laws and regulations that govern the treatment of experimental animals (AZ: 5096 - 4250213). All studies were performed at the Institut für Tierzucht der Bundesforschungsanstalt fur Landwirstschaft.

4.2 Methods

4.2.1 Receptor binding assay

Receptor binding assay using radiolabeled opioid is one of *in vitro* techniques that has been used to define and characterize opioid receptors (**KUHAR** et al. 1973; **HENDRICKSON** and **LIN** 1980; **BARDO** et al. 1982). This procedure permits the evaluation of compounds for their direct interaction with cell surface recognition sites or receptors (**LIMBIRD** 1996). There are two basic types of binding assay that utilize radioligands. Firstly, the direct binding assay measures the direct interaction of a radioligand with a receptor. Secondly, the indirect binding assay measures the inhibition of the binding of a radioligand by an unlabeled ligand to assume indirectly the affinity of receptors for the unlabeled ligand (**MCGONIGLE** and **MOLINOFF** 1994). Receptor binding assays require only a small amount of compounds and animal tissues and are rapid to perform. However, these assays have certain drawbacks. They measure potency rather than efficacy. Since binding assays do not distinguish between the agonist or the antagonist nature of the ligand-

receptor interaction, it is necessary to correlate the results obtained in binding assays with those obtained in bioassays.

4.2.2 Basic principles

The principle of the receptor binding assay is based on the small amount of radioactive ligand bound to membrane receptors (**ENNA** 1978). Tracer is a radioactive isotope, which ideally does not interfere with the binding properties of the protein or the ligand. With tracer labeled ligand competes then with a non-labeled ligand for the membrane receptor. Thus, at a given radioligand concentration the amount of specifically bound isotope will depend on the amount and affinity of unlabeled substance present in the incubation medium. When the reaction is allowed to continue, an equilibrium will occur. After equilibrium, the reaction is terminated by separating the membrane-bound ligand from the isotope in samples. The membranes are then rinsed free of excess radioligand and the bound radioactivity can be quantified (**ENNA** 1982, **HRDINA** 1986). A ligand in the assay can be an antagonist or an agonist that binds to a receptor. The use of antagonists as radioligands seems preferable, because antagonists have usually a high affinity to the receptor. The radioligand should have a high specific activity so that small quantity of bound ligand can be accurately measured (**OTTO** 1993). There are basic requirements that should be met in a ligand of the receptor binding assay. These include: purity and stability of the radioligand, biological activity, sufficiently high specific activity and specificity for the receptor sites (**ENNA** 1984; **HUCHO** 1986).

The total binding (TB) is the amount of radioactivity bound to the tissue preparation and includes the ligand that is specifically bound to receptors as well as non-specific binding (NSB) of ligand. The non-specific binding is determined by including a large amount of unlabelled hormone (100-1,000 times excess) to saturate all the specific receptor sites. The specific binding (SB) is determined as the difference between the non-specific and the total binding. The relationship among total, specific and non-specific binding is illustrated in Figure 4.

4.2.3 Scatchard plot

Due to the nonlinearity of receptor binding saturation curves, both the apparent Bmax and Kd values can be only approximated from this plot and it has become commonplace to

transform and plot saturation data in linear form as a Scatchard plot (**SCATCHARD** 1949). This plot where B/F (y-axis) is plotted as a function of B (x-axis) where B is the amount of ligand specifically bound and F represents the amount of free ligand. Using this procedure, the Bmax value can be obtained from the intercept on the abscissa and the Kd values is obtained from the negative reciprocal of the slope (Figure 5). The Bmax value is the amount of binding observed at saturating concentrations of radioligand. The Kd value, which is indicative of the affinity of the receptor for the radioligand, is that concentration of ligand at which 50% of the total number of receptors can be labeled. An advantage of using Scatchard analysis is that it provides the interaction between ligand and receptor. In situation where the radioligand binds to a single site, a linear Scatchard plot is generated and reasonable estimates of Bmax and Kd values can be obtained. However, non-linear Scatchard plots can also be obtained when a nonselective radioligand binds to more than one site. Such plots indicate cooperativity in the binding and imply multiple affinities (**WEILAND** and **MOLINOFF** 1981).

4.2.4 Treatments and collection of samples

Experiment I: Opioid receptor in normo-hydrated female and male chicken

Male and female 10-day-old, 10-week-old and adult chickens (28-36-week-old) (n = 4-6 in all groups) were a forceful blow on the back of the head and were killed by decapitation. The brains were quickly removed and transferred to dry ice and then stored at – 80 °C until receptor assay. Frontal cortex (FC), Lateral septum (LS), Hypothalamus (HYP), Amygdala (AMY), Hippocampus (HPC) and Striatum (ST) were punched out of serial sections (See 4.2.7). Opioid receptor binding sites were determined using radioreceptor binding assay (See 4.2.8).

Experiment II: Opioid receptor in dehydrated female and male chicken

Male and female chickens (n = 4-7 in all groups) at age of 10-day-old, 10-week-old and adult were deprived of water for 1 (10-day-old) or 2 days. Animals were scarified, blood samples for determination of plasma AVT and osmolality were collected and brains were processed as in the experiment I.

Experiment III: Opioid receptor in castrated and castrated Testosterone-substituted male chicken

Male chicks at 3-4 weeks of age were bilaterally castrated and sham-operated under anesthesia. The anesthesia was conducted by intravenous injection of 8-10 mg/kg body weight Pentobarbital sodium (Narcoren^{R}, Merial, Hallbergmoos). Cockerels were fasted for 12 hours before the operation. The testis was exposed by a lateral approach between the last two ribs. The tunica was slit and both testes were removed by forceps through this incision. At the age of 8 months, the castrated chickens were divided into 3 groups.

Group 1 (n = 6-7): received no treatment

Group 2 (n = 7-8): received intramuscularly testosterone (10mg/kg, Fein Biochemica, Heidelberg) injections every three days for 10 days

Group 3 (n = 10): received testosterone as group 2, in addition this group was treated with naltrexone (0.1mg/kg, Sigma, Steinheim) in 8 hours intervals for a period of 7 days beginning on day 3 after start of testosterone treatment (Figure 2).

Each animal was provided with an indwelling catheter in the brachial vein to inject naltrexone intravenously three times a day and to collect blood samples. Animals were immediately killed after collection of blood samples on day 10. Brains were processed, as in the experiment I. Chickens were autopsied. Cocks with residual testicular tissue were removed from the study.

Quiet conditions were maintained in the room during all experimentations to avoid excitation of animals. Blood samples for blood gas analysis were withdrawn from the brachial vein into heparinized syringes prior to the decapitation. A measurement of blood gas was determined using the Blood Gas Electrolyte Analysis (CCD Blutgas-System 800, Ciba Corning). Blood samples were collected into heparinized tubes and centrifuged at 3,000 rpm for 30 minutes at 4°C. Samples were stored either at 4°C (for osmolality) or at –20°C (for RIA analysis of AVT and testosterone) until required for assay. Plasma concentrations of AVT, osmolality and testosterone (only in the experiment III) were measured. Plasma osmolalities were determined using a vapour pressure osmometer (Model 5100B, Wescor, Utah). All osmolality measurements were made on fresh samples.

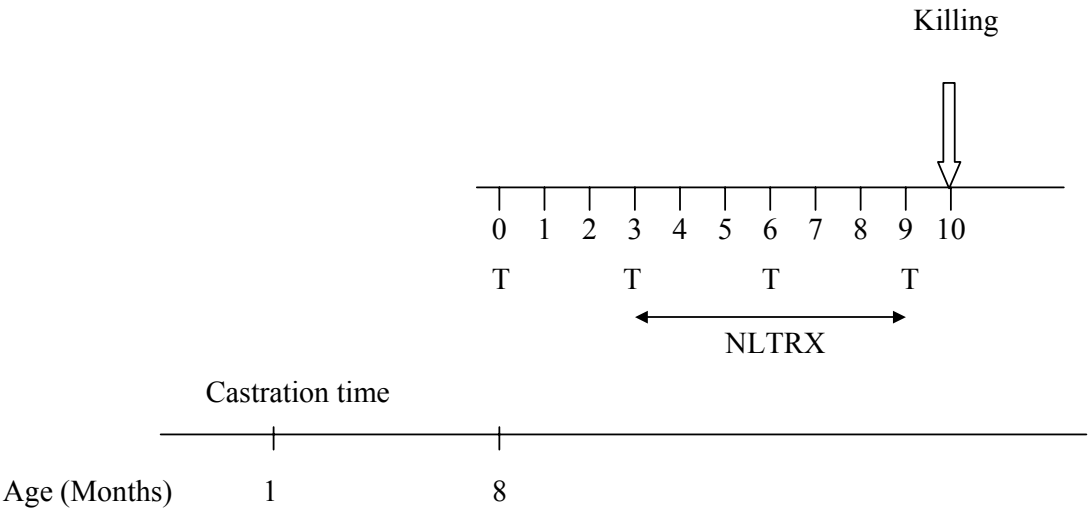

Figure 2. Schematic representation of the animal model used. Male chickens have been castrated for long term and injected at eight months after castration. Control chickens received only testosterone, while the treatment group received NLTRX. (T = testosterone; NLTRX = naltrexone)

4.2.5 Enzyme immonoassay of testosterone measurement

Testosterone enzyme immunoassay was performed according to **MÜNSTER** (1989). The diluent used throughout was testosterone assay buffer (pH 7.2). Testosterone hormone concentrations were determined in 20 µl plasma chickens diluted 1: 20 without prior extraction. Testosterone antiserum had been raised in rabbit using testosterone-3-CMO coupled to BSA as antigen, as described by **ELSAESSER** (1980). It could be used at a final dilution of 1:1,900,000. Horse-Radish-Peroxidase (Sigma, Steinheim) coupled to testosterone (HRP-T; 1:10000) was used as a tracer. Buffer solution (pH 4.05) with tetramethylbenzidine (Sigma, Steinheim) was used as a substrate. Aliquots of tracer (50µl/well) and of plasma samples or testosterone standard solution (20 µl/well) were pipetted into the wells coated with goat antibody against rabbit IgG and saturation of plastic surface with casein. The specific antiserum was added 50 µl/well to all samples, mixed and incubated overnight at 4°C. The supernatant were decanted and then washed 4-time with PBS+Tween-20 (Merck, Darmstadt). Amount of 150 µl substrate solution was added into each well. The plate was incubated for 40 minutes in the dark at 27°C. The reaction was stopped by addition of 50 µl of 2M H_2SO_4 and then optical density was read by spectrophotometer at 450 nm (SLT Spectra, Tecan, Crailsheim). Results were calculated by a computer program Easy-Win Fitting Version 6.0a

(Tecan, Crailsheim). The interassay coefficients of variation for the standard curve containing 50 and 300 pg/ml were 6.7 and 12.3 %, respectively. The detection limit of the assay is about 200 pg/ml.

4.2.6 Radioimmunoassay of AVT measurement

Arginine vasotocin radioimmunoassay was performed according to **XU** (1991). The chicken plasma samples (150 µl) were extracted with 400 µl cold acetone (-20°C) and centrifuged at 4,000 rpm for 10 minutes at 4°C. The supernatant was decanted and mixed with 800 µl petroleum benzene and shaken 30 seconds and then left at room temperature for 30 minutes. The ether phase was discarded and the aqueous layer extracted once again with petroleum benzene. After discarding the ether phase, the aqueous phase was dried under vacuum in a SpeedVac (Savant, New York). The dried extract was dissolved in assay buffer (0.1M Tris-HCl, pH 7.4) and was stored at –20°C until assayed. The RIA was performed in duplicate using synthetic AVT (Sigma, Steinheim) as a standard. The AVT antiserum was kindly supplied by Dr. Gray, Max-Planck-Institute for Physiological and Clinical Research, Bad Nauheim (**GRAY** and **SIMON** 1983).

The unlabeled peptide and antiserum was incubated 48 hours at 4°C before the addition of labeled tracer [^{125}I] (Specific activity 109 mCi/ml, Amersham, Buckinghamshire). Standard curves were obtained by adding 200 µl of doubling dilutions of standard AVT and 200 µl of the antiserum working dilution giving a final dilution of 1:200,000. The dried extracts were redissolved in 200 µl of 0.1M Tris-HCl, pH 7.4 and 200 µl were transferred to assay tubes in duplicate in place of standard. Control tubes containing either tracer alone (200 µl of 0.1M Tris-HCl, pH 7.4 and 200 µl AVT antiserum) or buffer (400 µl of 0.1M Tris-HCl, pH 7.4) in place of sample were put in every assay. The 50 µl (3,000 cpm) of labeled tracer was added and the incubation continued at 4°C for a further 24 hours. The separation of bound and free tracer was done by the rapid addition of 800 µl absolute ethanol followed by mixing and centrifugation at 3,500 rpm for 20 minutes at 4°C. The supernatant were removed by aspiration and radioactivity in the pellets was read by using the gamma counter spectrometry (1277 Gamma Master, LKB Wallac). The results were analyzed with RiaCalc Program (Pharmacia, LKB Wallac). The detection limit of the assay is about 1.5 pg/ml.

4.2.7 Dissection and preparation of brain tissues

Chicken brains were taken from –80 °C and brought to –20 °C. Each brain was dissected to yield the following separate parts: FC, LS, HYP, AMY, HPC and ST. These brain regions were dissected according to the anatomical landmarks of the stereotaxic atlas of the brain of the chicken (**KUENZEL** and **MASSON** 1988; **KUENZEL** 2000). The brains were dissected with a Cryostat blade as shown in Figure 3. Cut 1 was made just at the rhinencephalon. The cortex-surrounding slab of this brain part was referred to as frontal cortex (Figure 3A). The striatum included the remaining tissue lateral to the ventricle. Cut 2 was made parallel to cut 1 and anterior to the hypothalamus. The lateral septum tissue fragment was taken from this cut at the border (Figure 3B). Cut 3 was made at the position of hypothalamus and used the optic nerve (CN II) to keep it parallel. The hypothalamus was located ventral to the anterior commissure, dorsal to the optic chiasma. The amygdala sample included the remaining tissue lateral to the HYP. The amygdala and hippocampus were collected as shown in Figure 3. The punched brain regions were put directly in ice-cold 1 ml Tris-HCl buffer (pH 7.8); sonicated for 30-60 seconds with a Homogenizer (model cell disruptor B15, setting 3, 50 volts) on ice. Homogenated tissues were transferred to glass-tubes and suspended with ice-cold Tris-HCl buffer (pH 7.8). The protein concentration was determined using the Coomassie Blue Procedure (**BRADFORD** 1976). The final protein concentration of the brain suspensions was set at approximately 1.5-2.0 mg protein/ml in a volume of 1 ml.

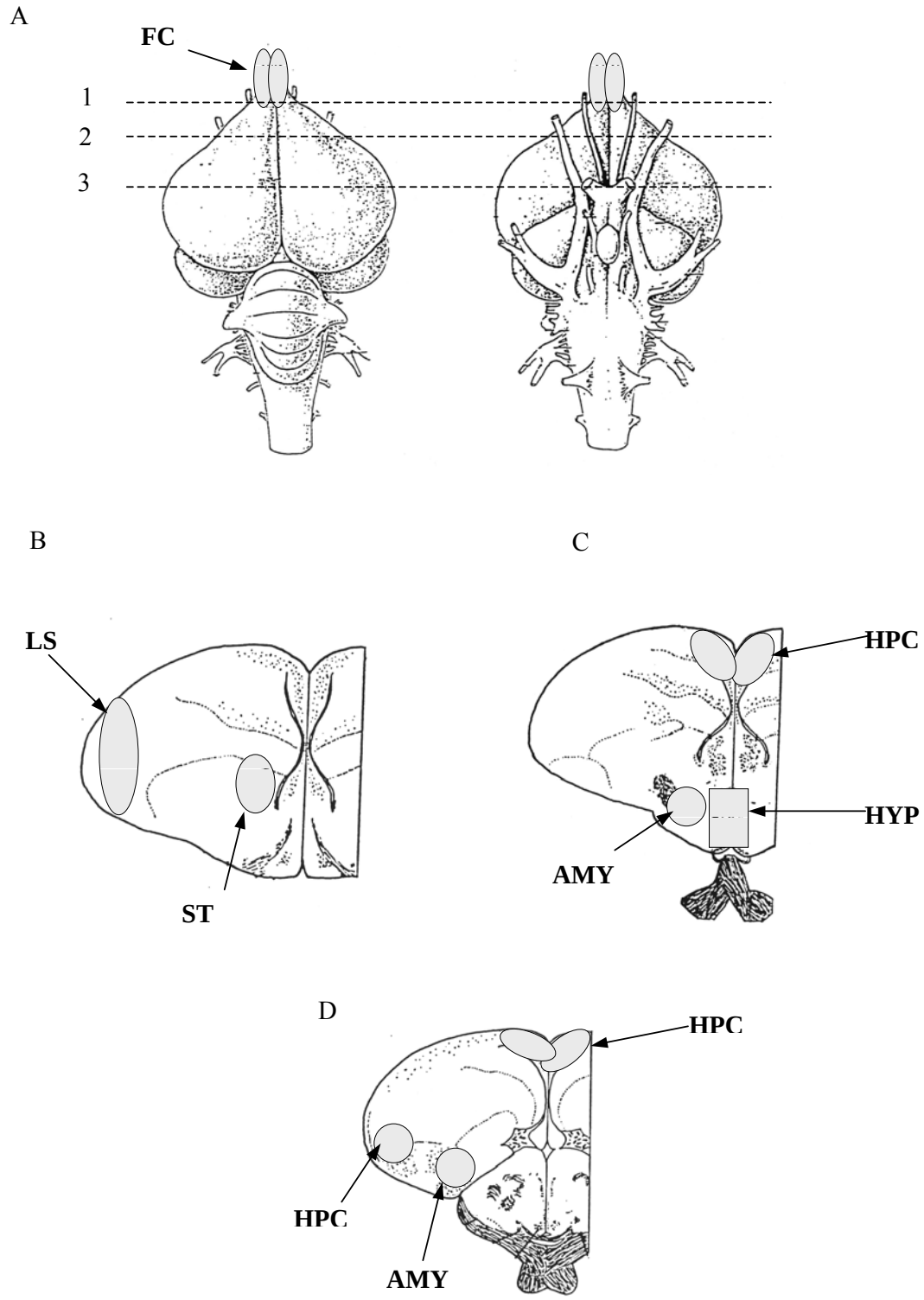

Figure 3. Diagrammatic representation of dissection procedure for chicken brain, Picture A. dorsal and ventral view of a chicken brain. The cut 1, 2 and 3 were made with a Cryostat blade at the positions indicated with dotted line. Shade area was FC. Picture B. shows cut 2 level which collected LS and ST. Picture C. shows HPC, AMY and HYP regions and picture D HPC and AMY regions. After **KUENZEL** (2000); **KÖNIG** and **LIEBICH** (2001)

4.2.8 Saturation binding assays

Opioid binding receptor assay was conducted by using [^{3}H]diprenorphine (DPN) (Specific activity 90.9 mCi/mg, Amersham, Buckinghamshire). Diprenorphine (C_{26} $H_{36}CINO_4$) was found to bind with high affinity to μ-, δ-, κ-site and hence can be considered as a universal opioid receptor ligand (**SADÉE** et al. 1982).

Radioreceptor assay was performed according to **KAHLE** (1993). Portions of 50μl homogenated brain tissues obtained as described above were incubated for 10 minutes at room temperature with 10 μM unlabeled naloxone. The naloxone was initially dissolved in Tris-HCl Buffer (pH 7.8) and a stock solution was made to give a concentration of 100 μl/ml. Aliquots of this stock solution were stored at -20 °C, on each day of assay a working concentration was made up in assay buffer by diluting 100 μl of stock naloxone to 900 μl Tris-HCl buffer (pH 7.8). Saturation curves of specific [^{3}H]DPN binding were generated by incubating aliquots of brain homogenates with increasing concentrations of the labeled ligand [^{3}H]DPN (0.2, 0.4, 0.6, 1.0, 1.5 and 2.5 nM) in the presence or absence of naloxone. The final incubation volume was 0.1 ml. After 60 minutes incubation at 23°C, six times washing with 200 μl ice-cold Tris-HCl buffer (pH 7.8) and filtration with vacuum terminated the binding reactions. Labeled ligand was added to the first row of 96-well plate for measurement of total values. The plate was dried in a microwave at 80 Watt about 30 minutes. The scintillation cocktails (Betaplate Scint^R, Perkin Elmer,Wallac Oy) 25 μl were added to the sample rows. The standard rows were filled with scintillation cocktails (Optiphase Supermix^R, Perkin Elmer, Wallac Oy) 100 μl and then the plate was left at room temperature for 30 minutes before counting in a liquid scintillation spectrometra (1450 Microbeta Trilux, Perkin Elmer, Wallac Oy) with a counting efficiency of 39 %. The specific binding was calculated as the radioactivity obtained in the absence of naloxone (total binding) minus the radioactivity obtained in the presence of naloxone (nonspecific binding). Receptor binding properties were analyzed with a MultiCalc^R program (Wallac Oy, Turku); this analysis utilized nonlinear regression analysis of the saturation isotherm. Saturation curve and Scatchard plot were computed directly using this program. The equilibrium dissociation constant (Kd) and the maximum binding capacity (Bmax) of opioid receptors were determined by the method of **SCATCHARD** (1949).

4.2.9 Processing and analysis of data

The success of dehydration and castration of chicken was based on the analysis of plasma AVT and T values and external appearance such as comb including post mortem inspection. In all instances, data were presented and depicted as mean ± SEM. All statistical data were performed using a software package ('SigmaStat, Jandel Scientific Corporation, 1995). The significance level in all tests was preset at $p \leq 0.05$. In those experiments which comparisons were being made between two groups of animals, data were evaluated by means of Student's unpaired t-test. In the situation which more than two groups were being compared, data were analyzed by one-way analysis of variance (ANOVA) or repeated measure ANOVA followed by Turkey's test to identify group differences (multiple pairwise comparisons).

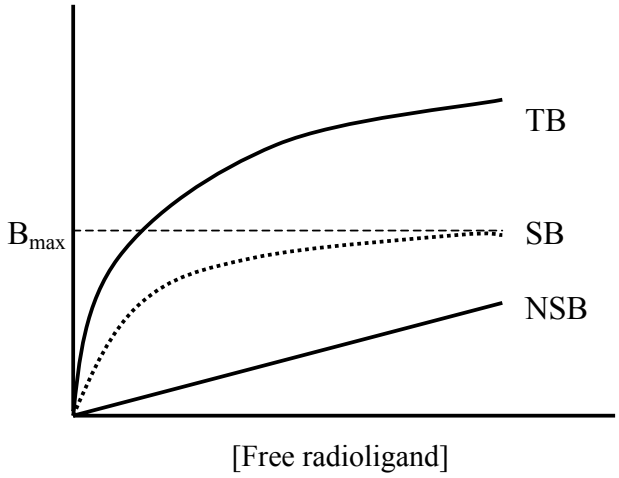

Figure 4. Typical curves generated from a direct binding assay displayed total (TB), specific (SB) and non-specific binding (NSB). The solid lines represent theoretical levels of total binding and non-specific binding and the dashed line is specific binding curve, given by the different between total and non-specific binding.

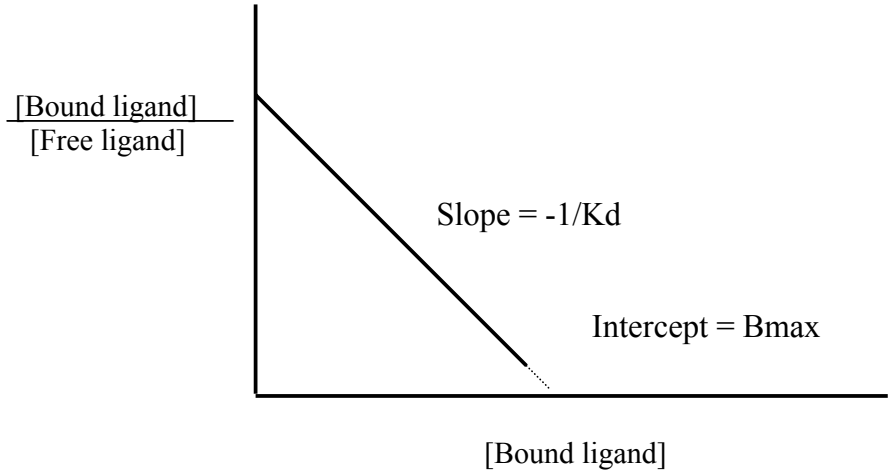

Figure 5. A typical Scatchard plot for a single class of binding sites. The amount of ligand bound (x-axis) is calculated per milligram of sample protein. A linear Scatchard plot is obtained if there is only single class of binding site present and if there is no cooperativity of binding at the sites on one receptor complex. The intercept on the x-axis is the value of Bmax, that is, the number of the binding sites per milligram of protein present. The slope of a linear Scatchard plot is the negative of the affinity, which is the reciprocal of Kd, the equilibrium binding (dissociation) constant for the ligand-receptor site interaction.

5 Results

5.1. Characteristics of [^{3}H]diprenorphine binding in homogenates of chicken brain

The presence of opioid binding sites in homogenated chicken brain samples was detected using the nonselective opioid ligand ([^{3}H]DPN). Saturation binding experiments were carried out with radioligand concentration increasing from 0.2 to 2.5 nM. The Bmax and Kd values were calculated by linear regression analysis of the saturation binding curves from Scatchard plots. Bmax and Kd values were expressed as picomoles (pmol/mg) radioligand bound per mg protein and nM, respectively. Scatchard plots of the saturable binding generated a linear plot in all cases indicating the radioligand was bound to a single class of binding sites (Figure 6). This single straight line from Scatchard plot for each experiment had correlation coefficients ranging from 0.88 to 0.97. The calculated Hill coefficients were close to one. An incubation period of one hour was sufficient to allow the equilibrium. The binding of [^{3}H]DPN to brain tissues was a saturable process as depicted in Figure 6 which shows the results from a representative sample of hypothalamic region. The results of all saturation experiments are summarized in Tables 7-13. Specific binding of [^{3}H]DPN in selected regions of chicken brain were saturable at a reasonably low nM concentration of radioligand and were in proportion to the concentration of tissue in the incubation mixture.

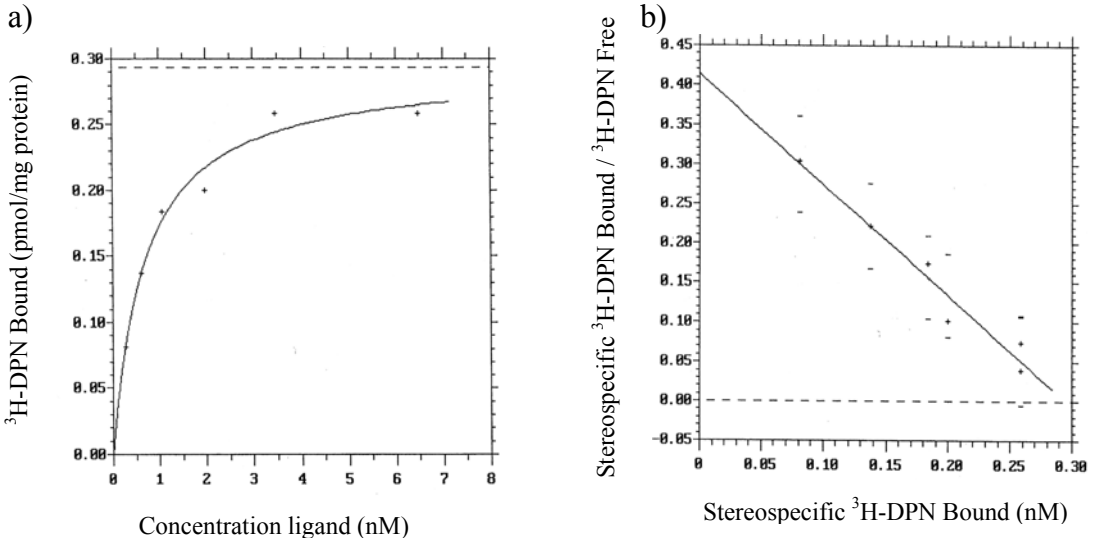

Figure 6. Saturation binding curve (a) and Scatchard plot (b) were performed by incubating increasing concentration of [^{3}H]DPN with the homogenates of hypothalamus. Naloxone was used to define non-specific binding.

5.2 Characteristics of [^{3}H]diprenorphine binding in selected brain areas of male and female chicken

Specific binding of [^{3}H]DPN measured in homogenated brain samples were studied in both sexes of 10-day-old, 10-week-old and adult (28 to 36-week-old) chickens. The regional distribution of [^{3}H]DPN binding profiles in normal chickens as revealed from saturation experiments are given in Tables 7-9. The Bmax of [^{3}H]DPN binding sites in female chickens showed the highest value in the FC (0.48 ± 0.06 pmol/mg) in 10-week-old and lowest in the HYP (0.19 ± 0.06 pmol/mg) in adult chickens. Interestingly, also in males the highest Bmax value was measured in the FC (0.41 ± 0.03 pmol/mg) in 10-week-old chickens and the lowest in the HYP (0.17 ± 0.05 pmol/mg) in adult chickens. Thus, the Bmax of [^{3}H]DPN binding sites in the FC was about 2.5 and 2.4 times greater than that in the HYP in 10-week-old and adult female and male chickens, respectively. The highest levels for the affinity of [^{3}H]DPN binding sites in female chickens were measured in the HYP (0.28 ± 0.02 nM) in 10-week-old chickens and the lowest in the ST (1.80 ± 0.95 nM) in 10-day-old chickens. Conversely, HYP (1.01 ± 0.52 nM) had the lowest affinity for [^{3}H]DPN binding in male 10-day-old chickens. The highest affinity levels were measured in the HPC (0.26 ± 0.09 nM) of adult male chickens. This means that Kd values were different about 6.4 and 3.8 times between HYP and ST in female and HYP and HPC in male chickens, respectively. Sexual dimorphism of the Bmax was only observed in the 10-day-old chickens. The females displayed a higher density of opioid binding sites than males in the AMY ($p = 0.012$) and HPC ($p = 0.013$). The density of opioid bindings was in both of these two regions 1.43 times higher in females than in males. However, a significant ($p = 0.016$) sexual dimorphism of the Kd was evident in the HYP in 10-week-old chickens, with males having approximately a 2 times higher Kd than the females.

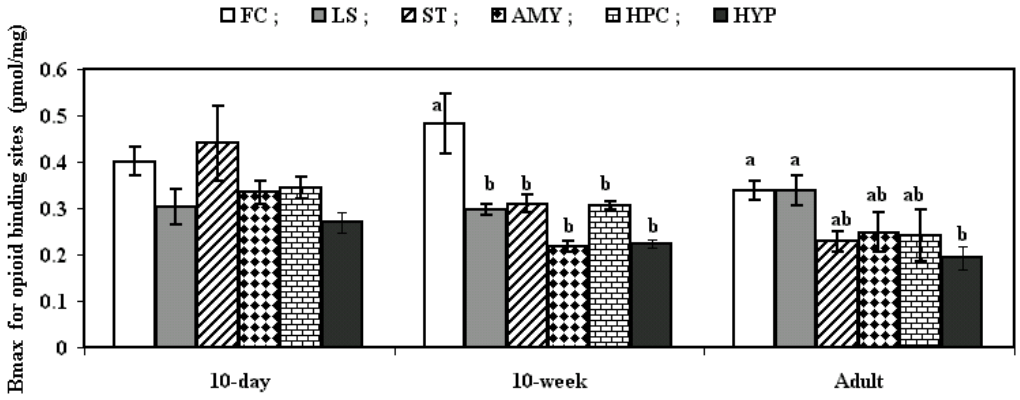

Figure 7. [^{3}H]DPN binding capacity detected in brain homogenates collected from 10-day-old, 10-week-old and adult control female chicken. Values are means ± SEM; Number of animals: 10-day-old = 5-6; 10-week-old = 4-6; adult = 4-6. Differences between regions within each age group are indicated by different lowercase letters above the bars, p at least ≤ 0.05.

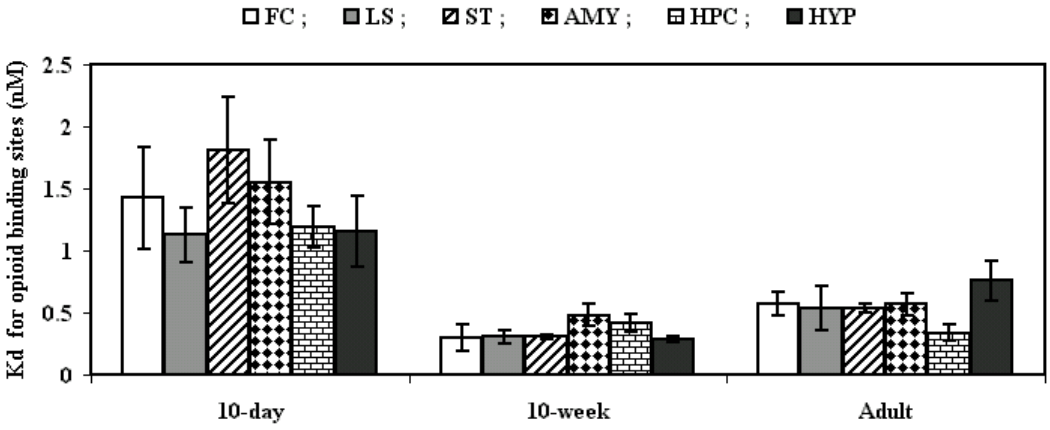

Figure 8. [^{3}H]DPN binding affinity detected in brain homogenates collected from 10-day-old, 10-week-old and adult control female chicken. Values are means ± SEM; Number of animals: 10-day-old = 5-6; 10-week-old = 4-7; adult = 4-6.

The concentration of opioid binding sites were detected to be in the following descending orders: ST > FC > HPC > AMY > LS > HYP; FC > ST > HPC > LS > HYP > AMY; LS > FC > AMY > HPC > ST > HYP and FC > ST > LS > HYP > HPC > AMY; FC > HPC > ST > LS > AMY > HYP and HPC > FC > LS > ST > AMY > HYP in 10-day-old, 10-week-old and adult of female and male chicken, respectively.

As shown in Figures 7 and 9, mean DPN Bmax values in the six investigated regions exhibited significant differences in 10-day-old males ($p=0.003$), both sexes of 10-week-old ($p\leq0.001$), and adult female ($p\leq0.001$) and males ($p=0.004$). In contrast, only Kd values obtained in the 10-week-old ($p=0.008$) and adult male ($p=0.034$) chickens showed significant difference among the six regions examined. However, the Kd value of [^{3}H]DPN binding did not change significantly with age in any of the brain regions in female chickens (Figure 8).

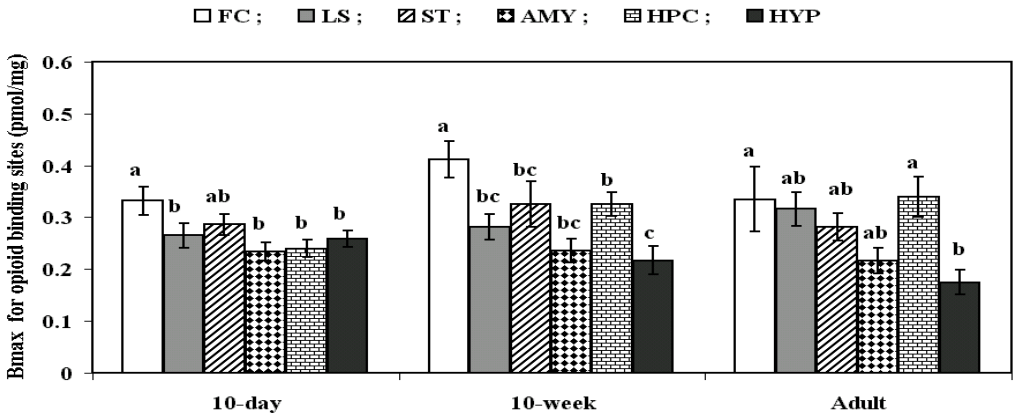

Figure 9. [^{3}H]DPN binding capacity detected in brain homogenates collected from 10-day-old, 10-week-old and adult control male chicken. Values are means ± SEM; Number of animals: 10-day-old = 4-6; 10-week-old = 4-6; adult = 4-5. Differences between regions within each age group are indicated by different lowercase letters above the bars, p at least ≤ 0.05.

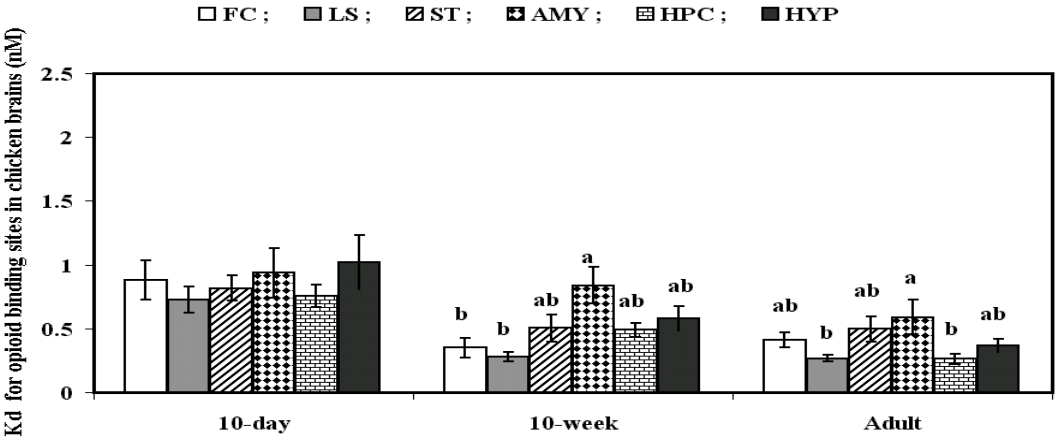

Figure 10. [^{3}H]DPN binding affinity detected in brain homogenates collected from 10-day-old, 10-week-old and adult control male chicken. Values are means ± SEM; Number of animals: 10-day-old = 4-6; 10-week-old = 4-6; adult = 4-5. Differences between regions within each age group are indicated by different lowercase letters above the bars, p at least ≤ 0.05.

5.3 Developmental changes characteristics of [^{3}H]diprenorphine binding in selected brain areas of male and female chicken

The development of the specific binding of [^{3}H]DPN in three age classes in six regions of the chicken brain are presented in Figures 11-14.

These changes can be summarized as following:

- Females

a) **Bmax**: Maximal binding capacity tended to decrease with the age in the FC, ST, and AMY. In the FC, Bmax values showed significant difference ($p \leq 0.05$) between the 10-week-old and adult chickens, whereas in the ST a significant difference ($p \leq 0.05$) was found in 10-day-old and adult chickens. In the AMY, the significant difference ($p \leq 0.05$) among ages was found in 10-day and 10-week-old. In contrast, no developmental change was observed in the LS, HPC and HYP (Figure 11).

b) **Kd:** Kd values tended to decrease from the day 10 of age to the week 10 of age. As shown in Figure 12, the significant differences were found in the FC ($p=0.018$), LS ($p=0.008$), ST ($p=0.001$), AMY ($p=0.004$) and HPC ($p=0.004$) regions.

- Males

a) **Bmax**: There was no significant changes during development in maximal binding capacity of [3H]DPN (Figure 13).

b) **Kd**: Affinity of [3H]DPN binding was decreased and significant differences among ages were seen in the FC ($p=0.007$), LS ($p \leq 0.001$), HPC ($p \leq 0.001$) and HYP ($p=0.040$) regions. In the FC and HYP, Kd was significantly different ($p \leq 0.05$) between 10-day-old and adult chicken; and Kd in LS was significantly different ($p \leq 0.05$) between 10-day-old and 10-week-old chickens (Figure 14).

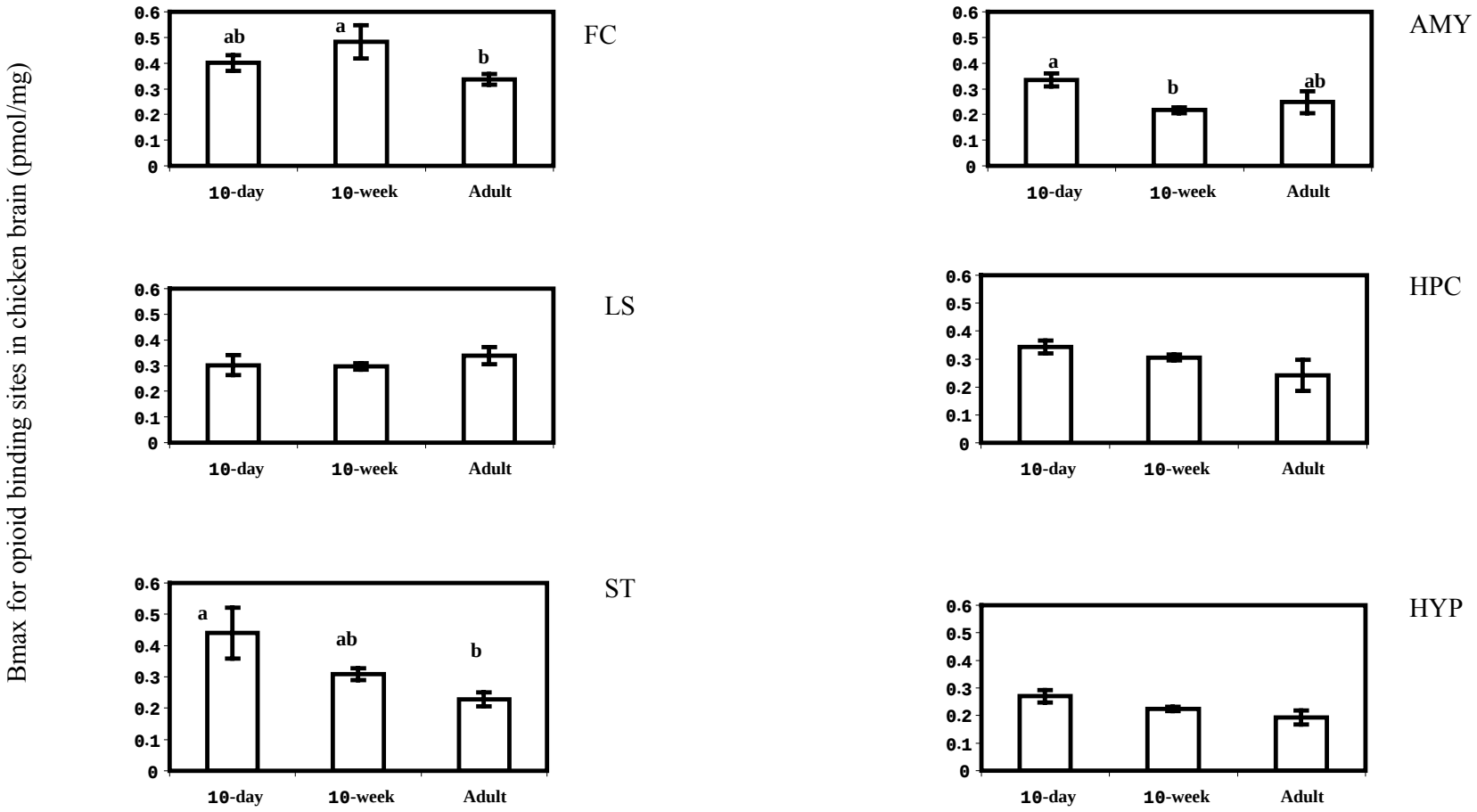

Figure 11. [^{3}H]DPN binding capacity in female chicken brains. Values are means ± SEM; Number of animals: 10-day-old = 5-6; 10-week-old = 4-6; adult = 4-6. Different letters indicate significant differences, p at least ≤ 0.05.

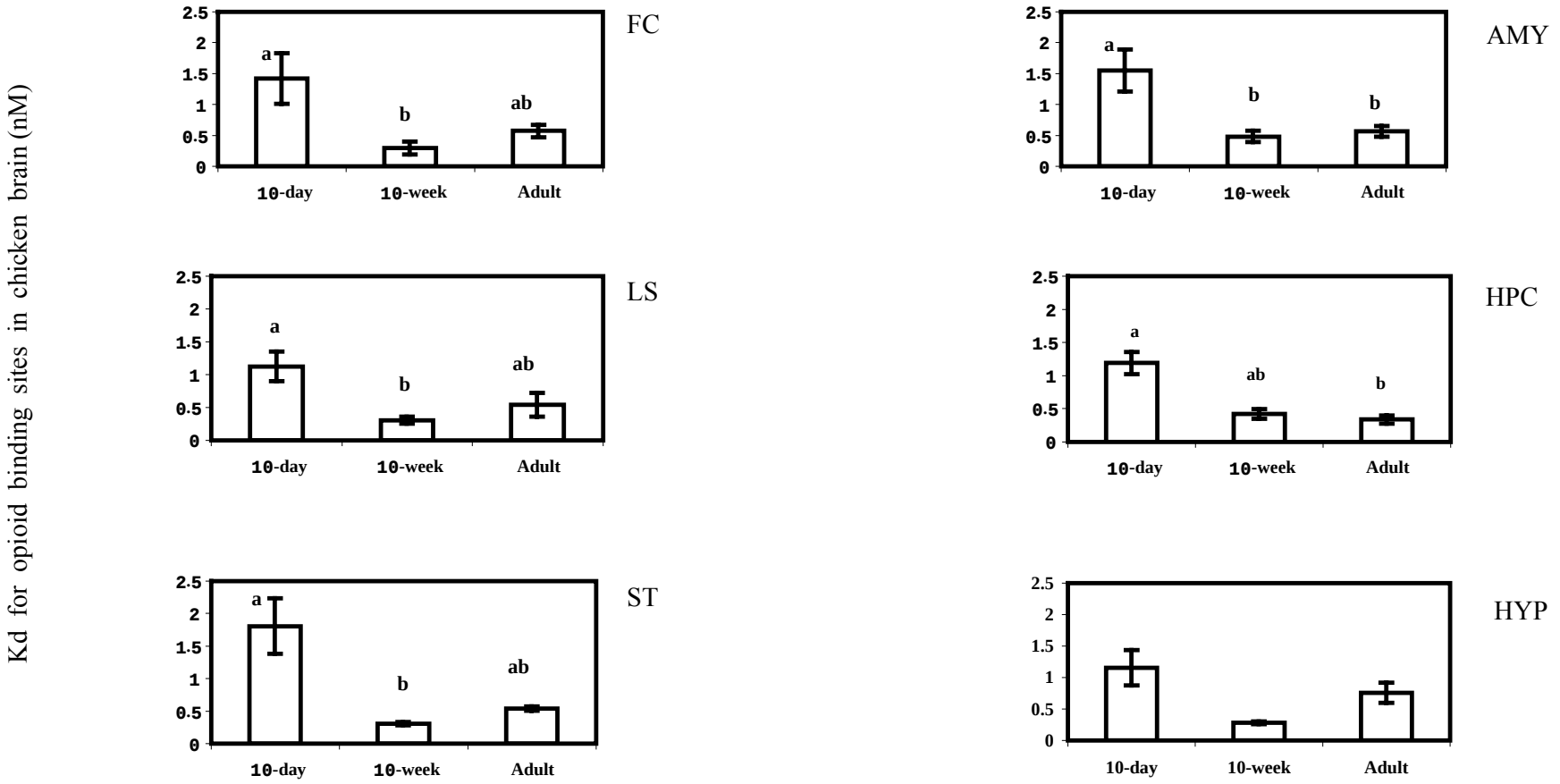

Figure 12. [^{3}H]DPN binding affinity in female chicken brains. Values are means ± SEM; Number of animals: 10-day-old = 5-6; 10-week-old = 4-6; adult = 4-6. Different letters indicate significant differences, p at least ≤ 0.05.

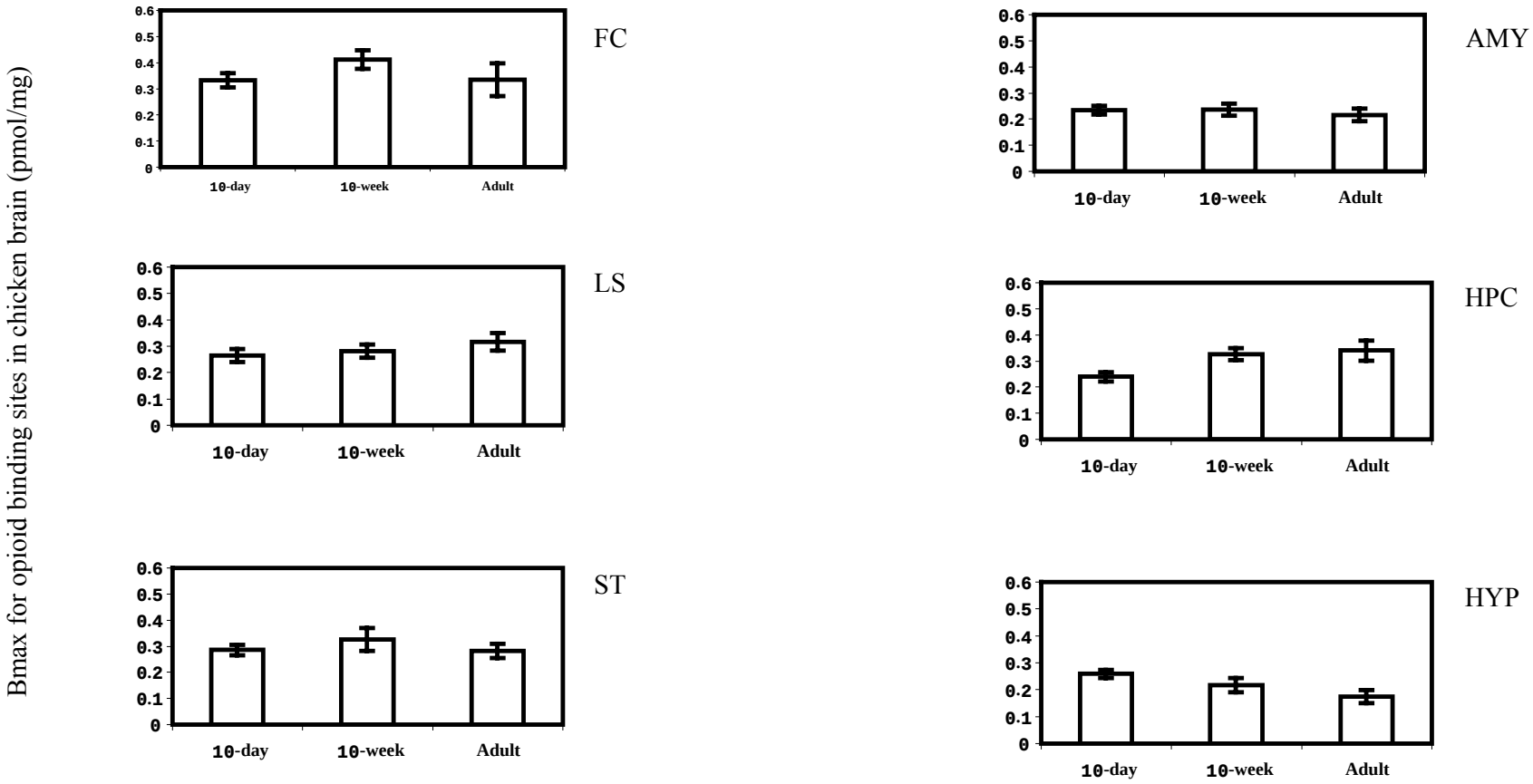

Figure 13. [^{3}H]DPN binding capacity in male chicken brains. Values are means ± SEM; Number of animals: 10-day-old = 4-6; 10-week-old = 4-6; adult = 4-5. Different letters indicate significant differences, p at least ≤ 0.05.

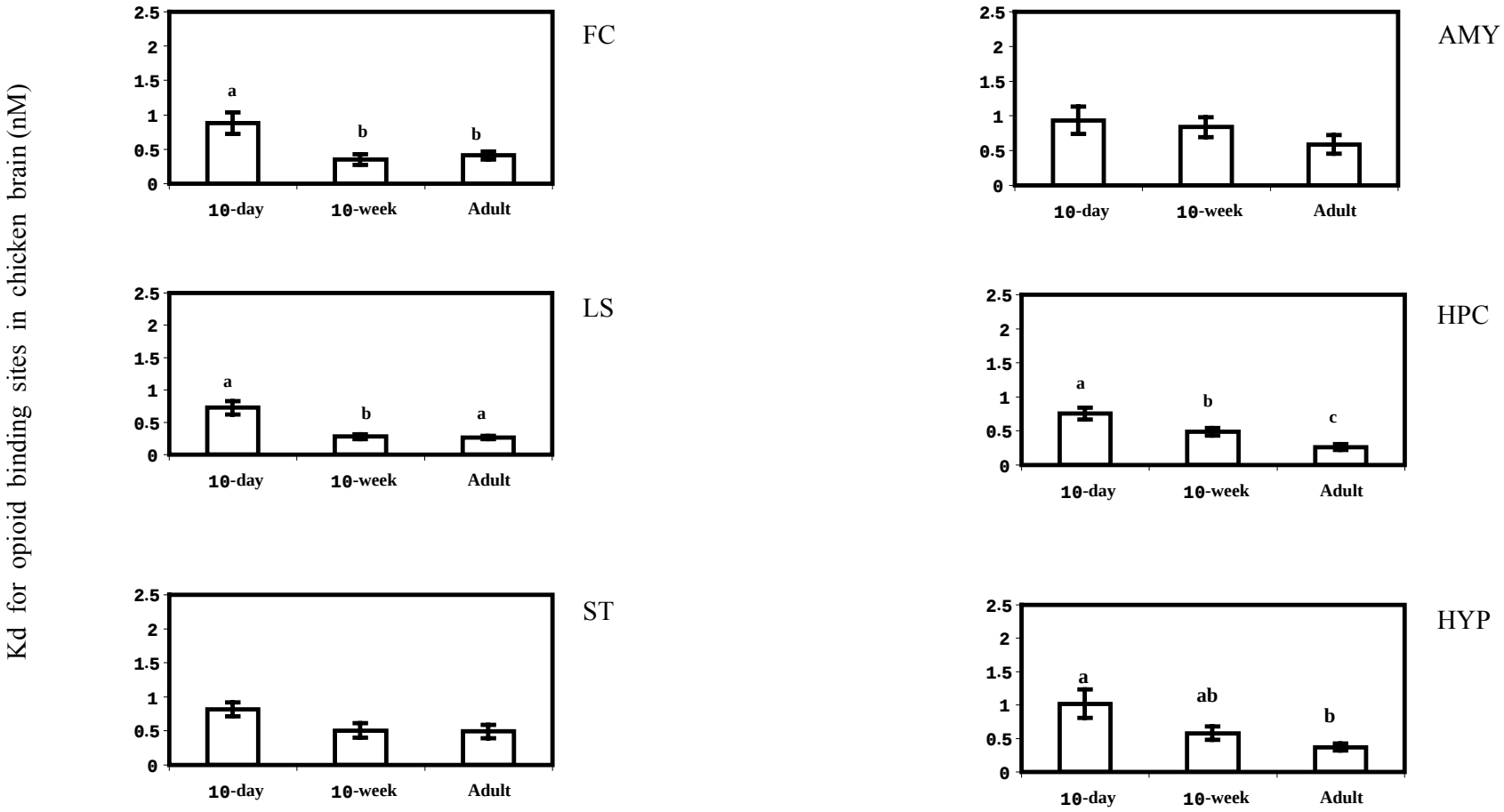

Figure 14. [^{3}H]DPN binding affinity in male chicken brains. Values are means ± SEM; Number of animals: 10-day-old = 4-6; 10-week-old = 4-6; adult = 4-5. Different letters indicate significant differences, p at least ≤ 0.05.

5.4 Characteristics of [^{3}H]diprenorphine binding in homogenates of dehydrated chicken

5.4.1 Effect of water deprivation on plasma osmolality

Initial plasma osmolalities (275.33 ± 2.60 and 280.33 ± 4.28 mosm/l for 10-day-old chicks male and female, 303 ± 2.08 and 300 ± 2.84 mosm/l for 10-week-old male and female chicks and 319.28 ± 1.47 and 318.83 ± 2.59 mosm/l for adult male and female chickens, respectively) were not significantly ($p \leq 0.05$) different (Figure 15). Following water deprivation, plasma osmolalities were highly increased and significantly ($p \leq 0.001$) different among groups. The levels increased to 316.33 ± 2.64 and 315.17 ± 2.90 mosm/l for 10-day-old chicks male and female, 334.17 ± 1.76 and 334.57 ± 1.86 mosm/l for 10-week-old chicks male and female and 345.17 ± 1.25 and 360.43 ± 5.44 mosm/l for adult male and female chickens, respectively. These values represent an increase in plasma osmolality of approximately 15 and 12.5% for 10-day-old male and female chicks, 10.3 and 11.5% for 10-week-old male and female chicks, and 8.1 and 13% for adult male and female chickens, respectively.

5.4.2 Effect of water deprivation on plasma vasotocin

Basal plasma AVT levels were 28.9 ± 7.1 (males) and 36.8 ± 12.5 (females) pg/ml in control 10-day-old chicks, 14.5 ± 2.7 (males) and 20.9 ± 4.2 (females) pg/ml in control 10-week-old chicks, 17.1 ± 2.1 (males) and 5.1 ± 1.0 pg/ml (females) in control adult chickens, respectively. In response to water deprivation these levels increased to 112.4 ± 15.3 (males) and 97.4 ± 11.0 (females) pg/ml in 10-day-old chicks, 49.4 ± 3.5 (males) and 60.5 ± 6.5 (females) pg/ml in 10-week-old chicks, 56.6 ± 6.8 (males), 79.6 ± 11.2 (females) pg/ml in adult chickens, respectively (Figures 16 and 17). Thus, the mean values of AVT increased in response to the dehydration by 288 % (males) and 165 % (females) in 10-day-old chicks, 239 % (males) and 188 % (females) in 10-week-old chicks, and 230 % (males) and 1,405 % (females) in adult chickens in comparison to the mean values in control birds. The response of plasma AVT levels to water deprivation was greatest in the adult female chickens. There was no sex-difference in AVT secretion in response to dehydration in 10-day- and 10-week-old male and female chickens. However, a significant ($p < 0.001$) sex-difference in plasma AVT levels was evident in adult chickens, with females reacting to dehydration nearly 6 times stronger than males.

To determine the osmotic sensitivity for AVT release, plasma osmolalities and plasma AVT concentration were plotted for each of the three age groups for both female and male chickens. The coefficient of this relationship, an indication of the osmotic sensitivity of AVT release, was not statistically significant different in males compared to females. However, in all age groups in control females and dehydrated male chickens, plasma AVT was significantly correlated with plasma osmolalities, $r = 0.562$, $p = 0.15$ vs. $r = 0.64$, $p = 0.005$, respectively.

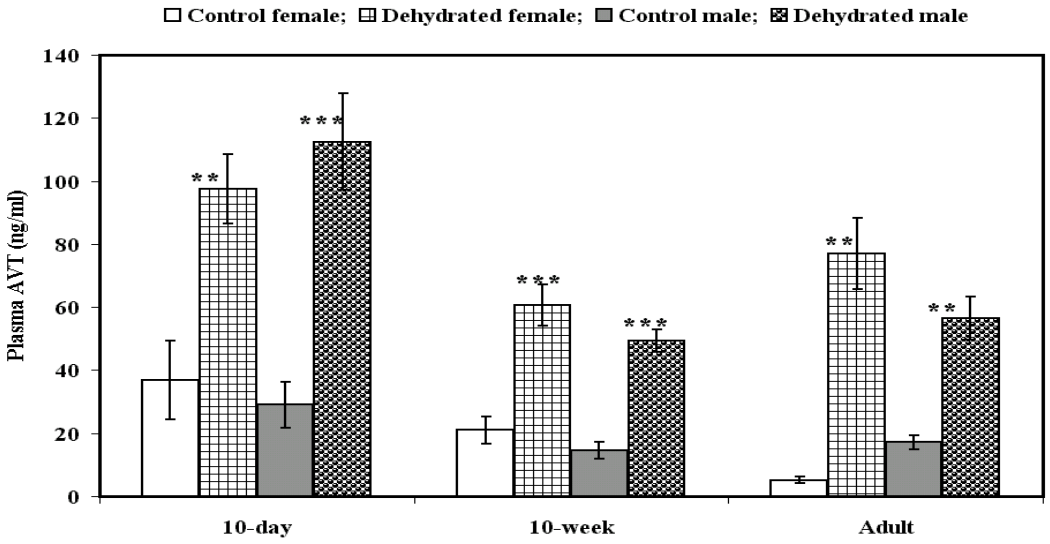

Figure 15. Mean plasma osmolality levels after water deprivation in female and male chickens. Values are means ± SEM; Number of animals: 10-day-old = 4-5; 10-week-old = 6-7; adult = 7-8. Statistical significance of dehydrated compared with control group was analyzed by Student's t-test; ** $p \leq 0.01$ and *** $p \leq 0.001$.

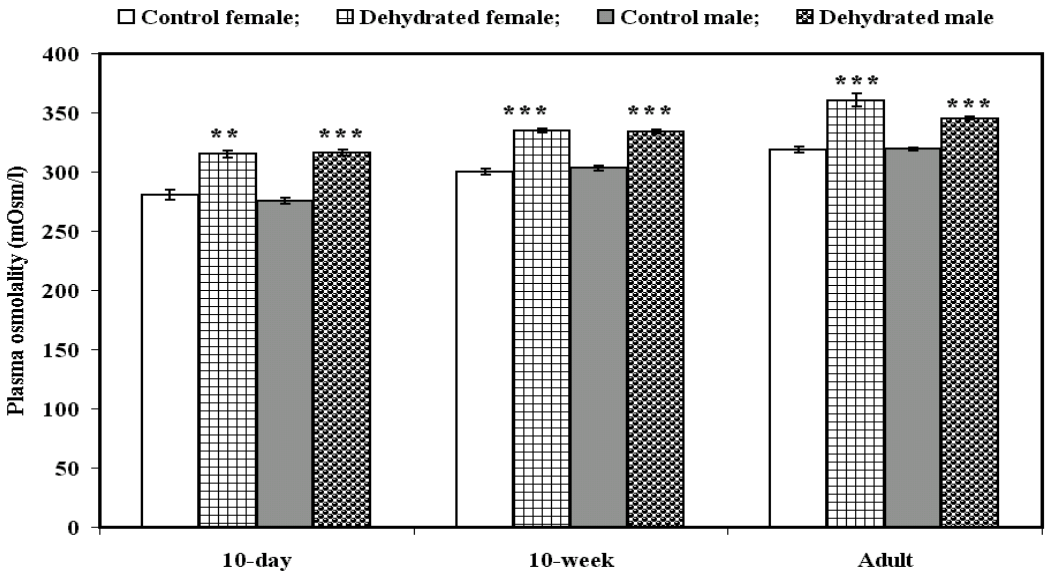

Figure 16. Mean plasma AVT levels after water deprivation in female and male chickens. Values are means ± SEM; Number of animals: 10-day-old = 4-5; 10-week-old = 6-7; adult = 7-8. ** $p \leq 0.01$ and *** $p \leq 0.001$; Student's t-test

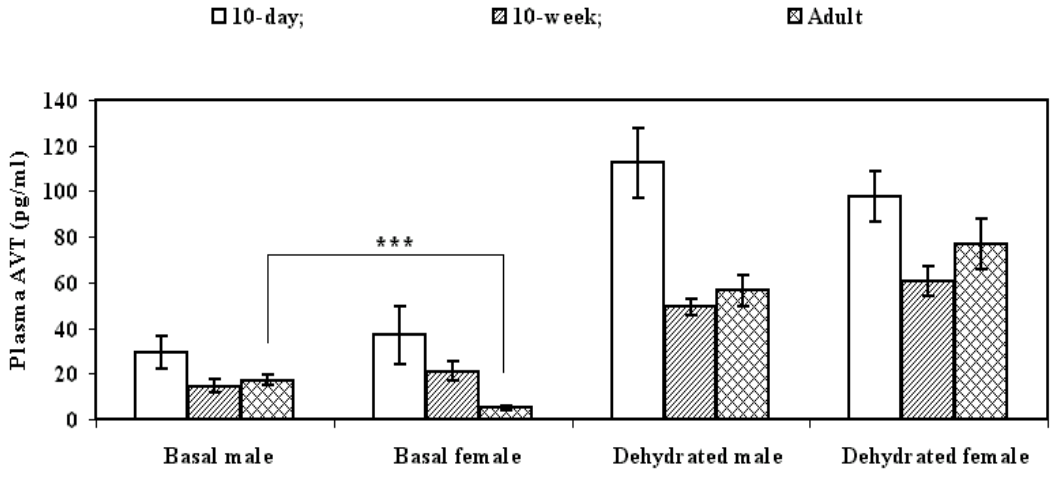

Figure 17. Mean plasma AVT levels after water deprivation in female and male chickens. Values are means ± SEM; Number of animals: 10-day-old = 4-5; 10-week-old = 6-7; adult = 7-8. *** $p \leq 0.001$; Student's t-test

5.4.3 Characteristics of [^{3}H]diprenorphine binding in brain of dehydrated chicken

In Tables 7-9, the binding profile of [^{3}H]DPN determined in brain homogenates from six regions of chicken brain are shown in the control and dehydrated groups. Most of the differences of Bmax and Kd values between control and dehydrated chickens were found to be in young female chickens. In response to the water deprivation, [^{3}H]DPN opioid biding was significantly decreased in the FC by 25% ($p \leq 0.01$), in the ST by 46% ($p \leq 0.05$) and in the AMY by 44% ($p \leq 0.001$) in 10-day-old females. In contrast, dehydration produced significant increments in opioid binding sites in the LS by 26% ($p \leq 0.01$), HPC by 18% ($p \leq 0.01$) and in the HYP by 21% ($p \leq 0.05$) in 10-week-old female chickens (Figure 18). In adult females was a 37% ($p \leq 0.05$) decrease in concentration of opioid binding in the LS (Figure 18).

In dehydrated males, a 22 % decline in Bmax was observed only in the HYP ($p \leq 0.05$) in 10-day-old group (Figure 20).

Compared to Kd values in control animals, [^{3}H]DPN Kd values were significantly decreased in the HPC, AMY ($p \leq 0.05$) and in the LS, AMY, HYP ($p \leq 0.05$) of 10-day-old and adult dehydrated female chickens, respectively (Figure 19). In contrast, an apparent significantly increased Kd value was only observed in 10-week-old female chickens in the HYP ($p \leq 0.05$) region. The only significant changes in Kd values in response to the dehydration occurred in the AMY and HYP in 10-week-old male chickens. Kd values declined ($p \leq 0.05$) in both of these brain regions after 2 days of water deprivation (Figure 21).

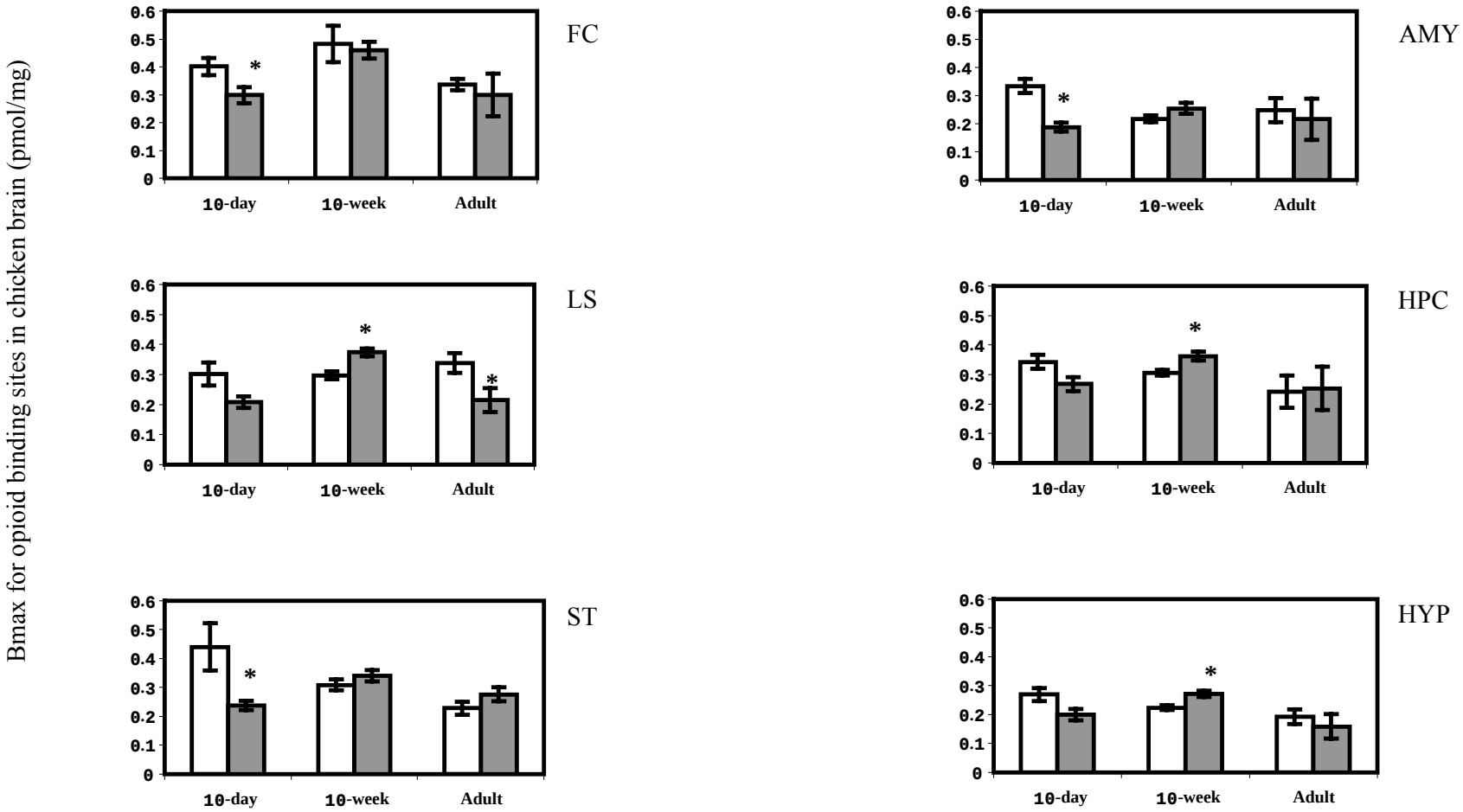

Figure 18. [^{3}H]DPN binding capacity in the control (open bars) and dehydrated (closed bars) female chicken brains. Values are means ± SEM; Number of animals: 10-day-old = 4-6; 10-week-old = 4-7; adult = 4-7. Statistical significance of the effects of dehydrated as compared with control was determined using a Student's t-test: * $p \leq 0.05$.

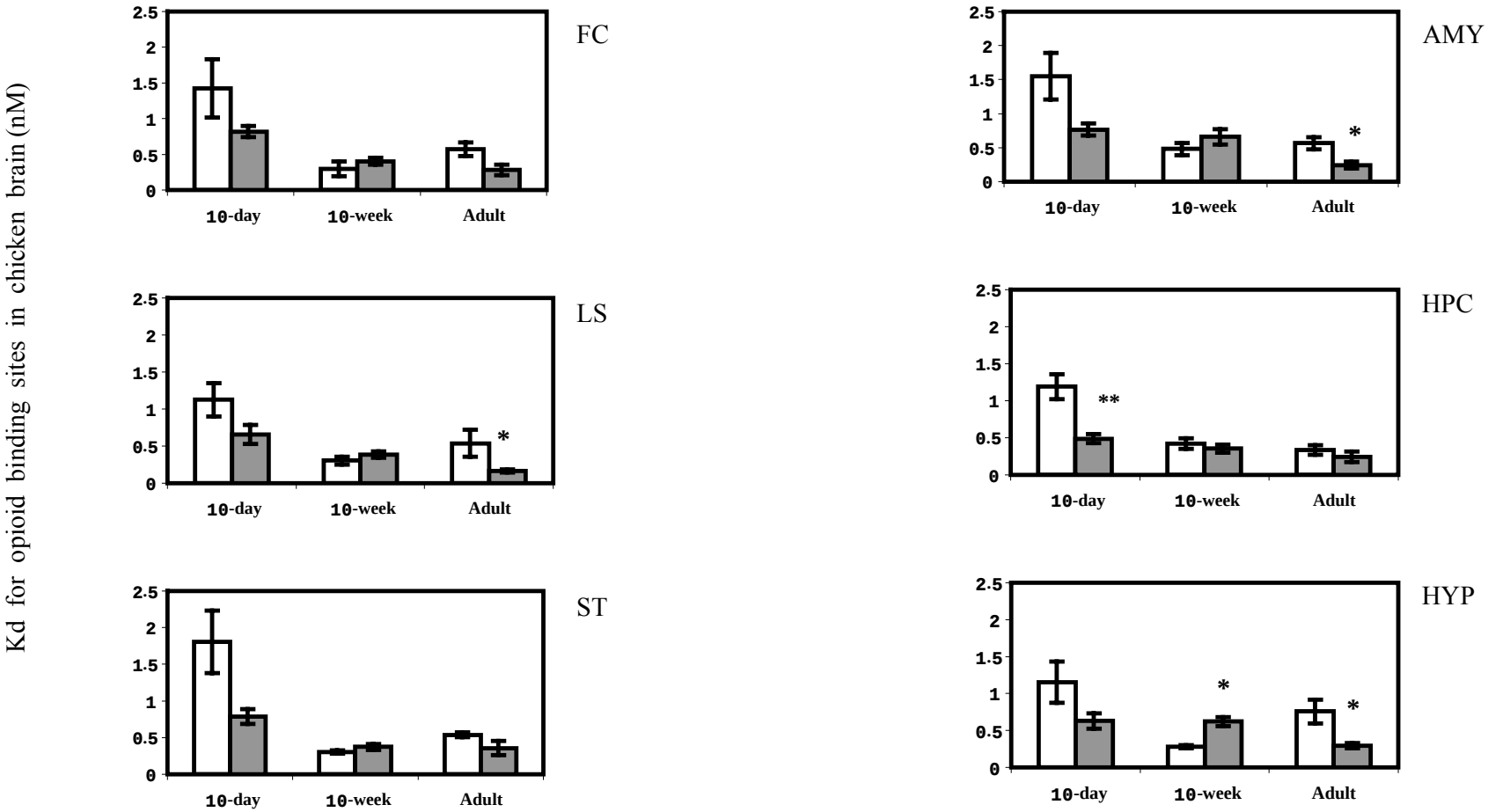

Figure 19. [^{3}H]DPN binding affinity in the control (open bars) and dehydrated (closed bars) female chicken brains. Values are means ± SEM; Number of animals: 10-day-old = 4-6; 10-week-old = 4-7; adult = 4-7. Statistical significance of the effects of dehydrated as compared with control was determined using a Student's t-test: * $p \leq 0.05$.

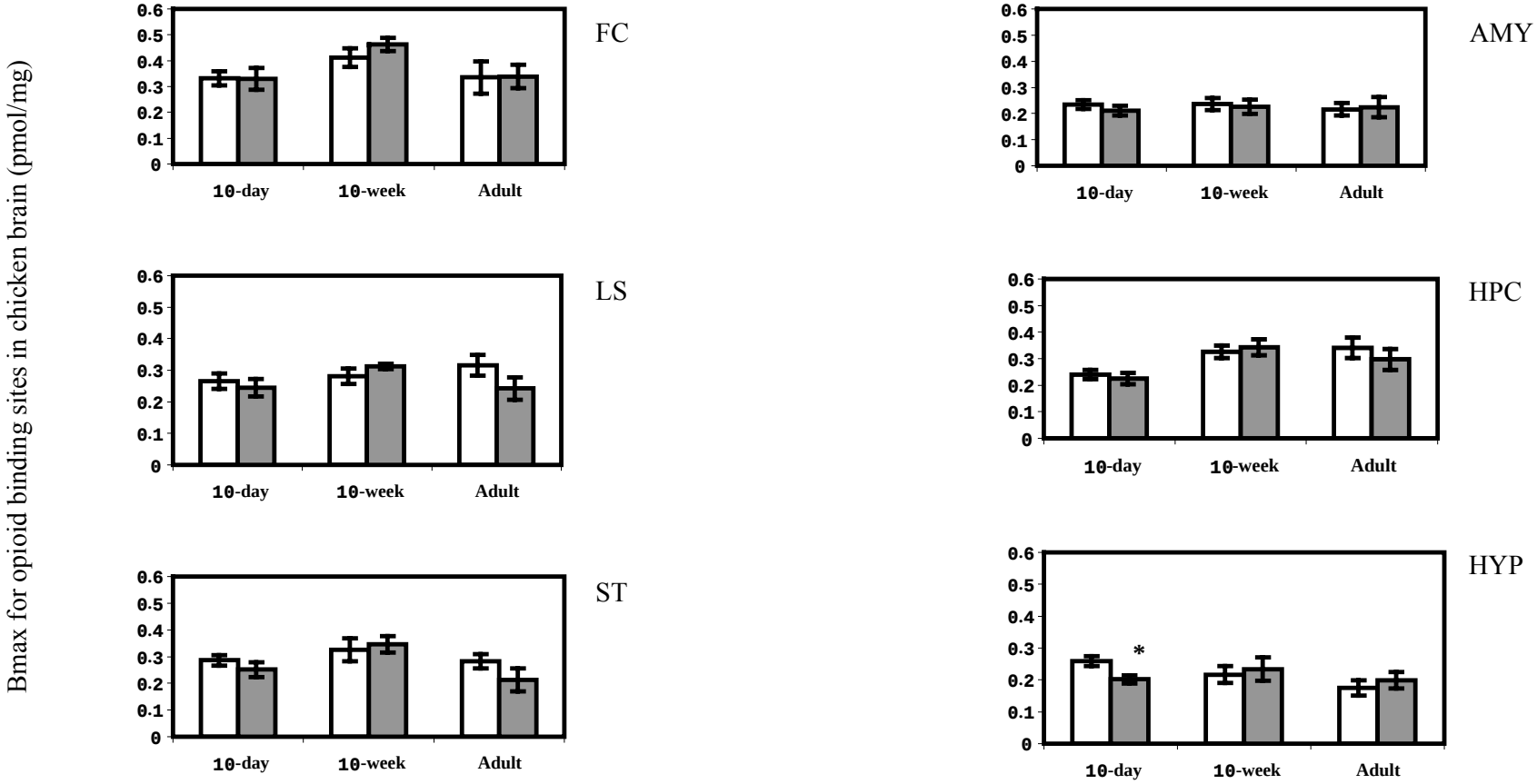

Figure 20. [^{3}H]DPN binding capacity in the control (open bars) and dehydrated (closed bars) male chicken brains. Values are means ± SEM; Number of animals: 10-day-old = 4-6; 10-week-old = 4-6; adult = 4-5. Statistical significance of the effects of dehydrated as compared with control was determined using a Student's t-test: * $p \leq 0.05$.

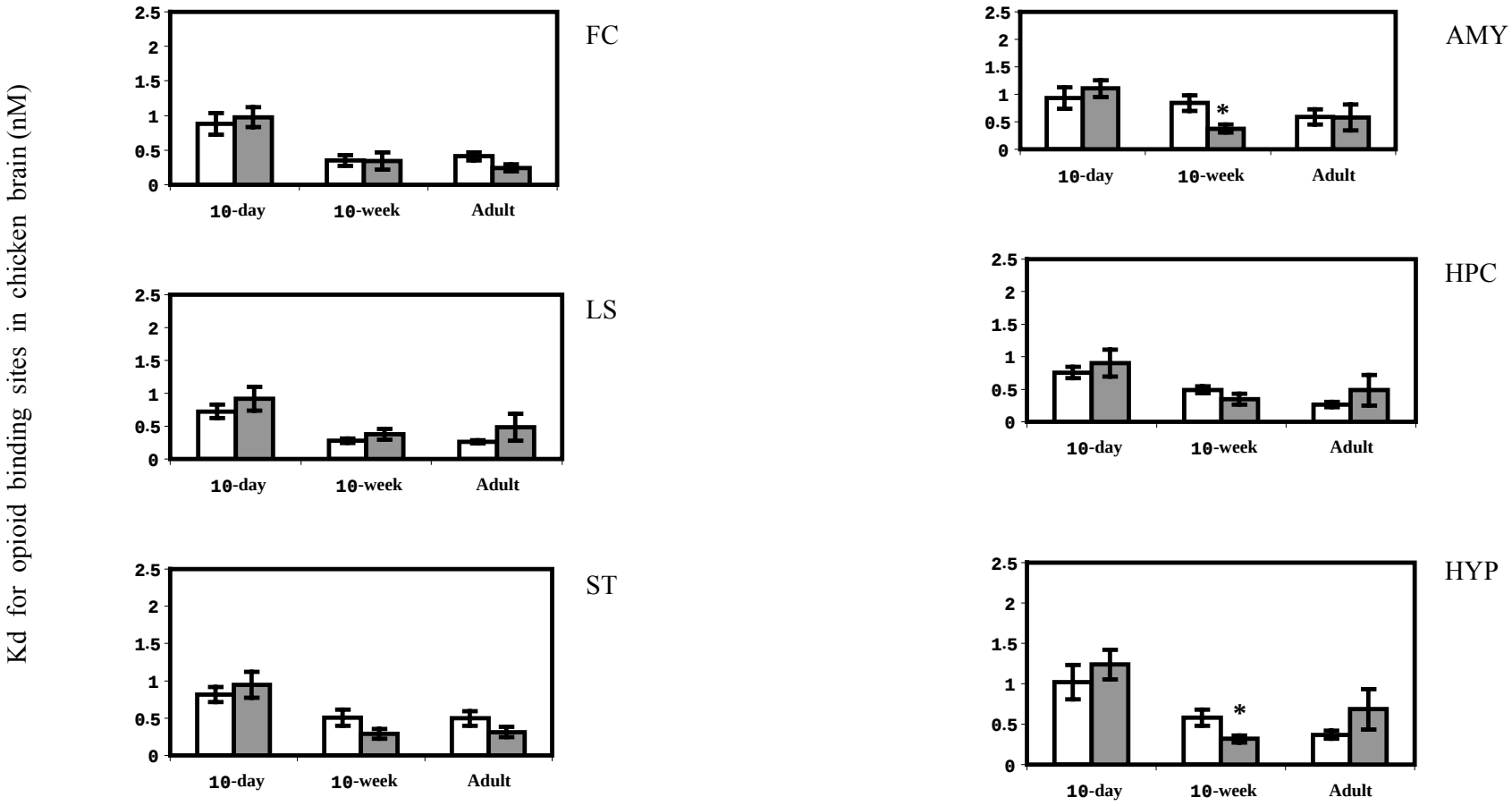

Figure 21. [^{3}H]DPN binding affinity in the control (open bars) and dehydrated (closed bars) male chicken brains. Values are means ± SEM; Number of animals: 10-day-old = 4-6; 10-week-old = 4-6; adult = 4-5. Statistical significance of the effects of dehydrated as compared with control was determined using a Student's t-test: * $p \leq 0.05$.

5.5 Characteristics of [^{3}H]diprenorphine binding in brain regions of castrated chicken

5.5.1 Plasma Testosterone concentrations during the development of the cockerel

Individual plasma testosterone level in 4-week-old chick was under 0.2 ng/ml (n= 10), and in adult (28-36 weeks) castrated cockerels ranged from 0.2 – 0.91 ng/ml (means 0.40 ± 0.1, n = 10). Castration caused a significant ($p = 0.004$) declination of plasma testosterone levels 7 months after surgery when compared to the sham-operated chickens (means 2.58 ± 0.8, n = 5) at the same age. The castrated male chickens treated with testosterone proprionate had increased concentration of plasma testosterone (mean 20.7 ± 2.6, n = 8).

5.5.2 Characteristics of [^{3}H]diprenorphine binding in brain regions of castrated and testosterone-substituted castrated chicken

Tables 10-12 show the distribution of opioid binding sites in various brain regions of castrated, sham-operated and castrated-testosterone substituted chickens. Both Bmax (Figure 22, $p < 0.001$ ANOVA) and Kd (Figure 23) values in all regions tended to be higher in castrated group compared to intact chickens. However, statistically significant higher binding capacities were measured in the FC ($p=0.022$), LS ($p=0.012$), HYP ($p=0.006$) and ST ($p=0.035$) regions (Figure 24). Whereas Kd values were significantly higher ($p \leq 0.001$) in the HPC (Figure 25).

After the testosterone substitution, specific [^{3}H]DPN binding was returned to the control levels in the FC, HYP, ST, AMY regions. But lower levels than in intact animal were observed in the LS ($p=0.006$) and HPC ($p=0.029$) (Figure 24). Dissociation constant values were returned to control values except in the HPC which remained significantly high ($p=0.037$) in comparison to castrated animals (Figure 25).

5.6 Characteristics of [^{3}H]diprenorphine binding in brain homogenates of castrated T-substituted chickens with or without naltrexone treatment

As can be seen in Figures 26 and 27, naltrexone treatment resulted in significant changes in Kd values in all investigated brain areas in castrated testosterone-substituted male chickens. Long-term naltrexone treatment, however, had little effects on Bmax, and the only significant change was seen in the HYP. The maximum binding capacity of [^{3}H]DPN decreased ($p = 0.015$) in this brain area (Figure 26).

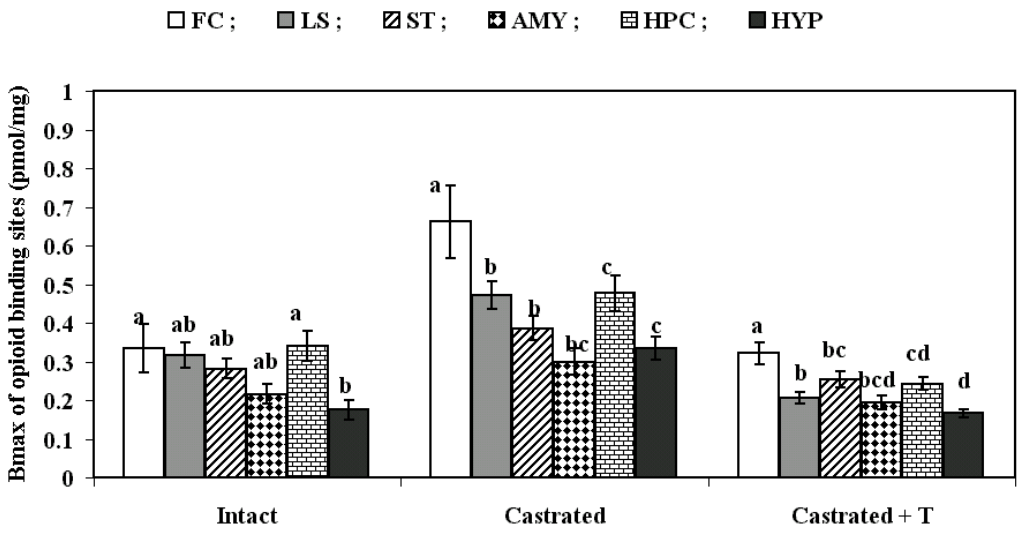

Figure 22. [^{3}H]DPN binding capacity detected in brain homogenates collected from intact, castrated and castrated T-substituted male chickens. Values are means ± SEM; Number of animals: control = 4-6; castrated = 6-7; T-substituted = 7-8. Statistical significance of the effects of regions of the brain in each group was determined using a one-way repeated measures analysis of variance. Differences between regions within each group are indicated by different lowercase letters above the bars, p at least ≤ 0.05.

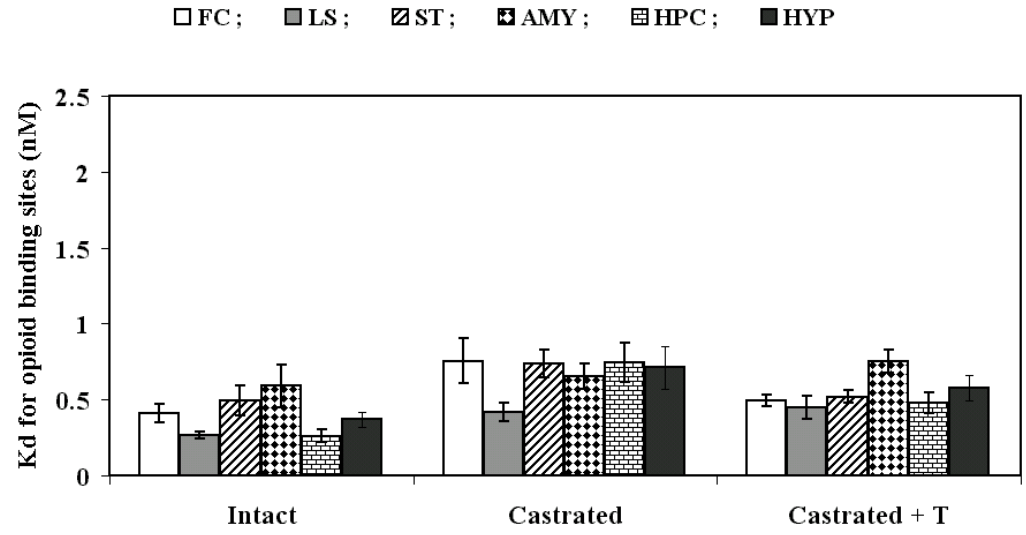

Figure 23. [^{3}H]DPN binding affinity detected in brain homogenates collected from intact, castrated and castrated T-substituted male chickens. Values are means ± SEM; Number of animals: control = 4-6; castrated = 6-7; T-substituted = 7-8. Statistical significance of the effects of regions of the brain in each group was determined using a one-way repeated measures analysis of variance.

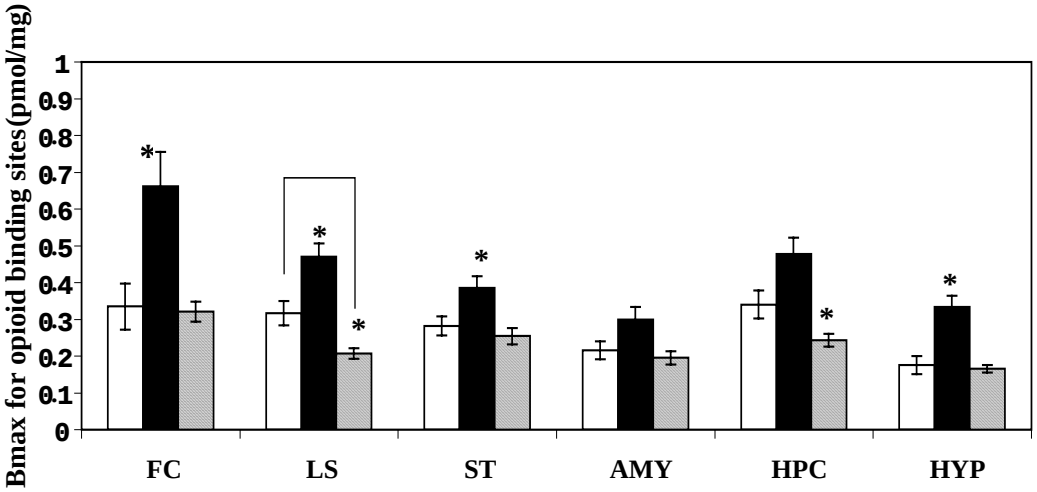

Figure 24. [^{3}H]DPN binding capacity detected in brain homogenates collected from intact (open bars), castrated (closed bars) and T-substituted (striated bars) male chicken. Values are means ± SEM; Number of animals: control = 4-6; castrated = 6-7; T-substituted = 7-8: * $p \leq 0.05$, Student's t-test between intact animals.

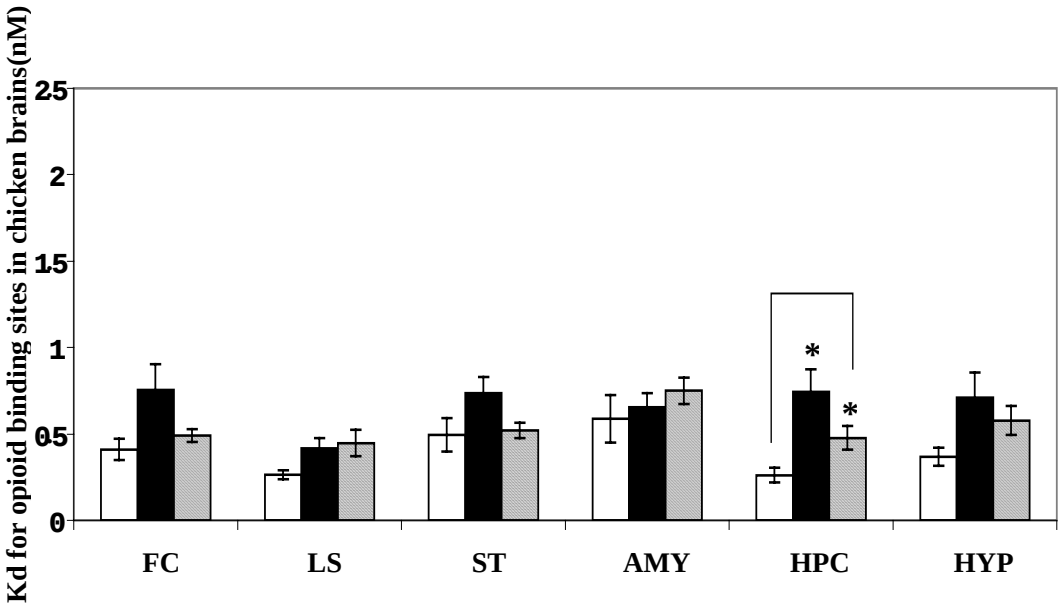

Figure 25. [^{3}H]DPN binding affinity detected in brain homogenates collected from control (open bars) castrated (closed bars) and T-substituted (striated bars) male chicken. Values are means ± SEM; Number of animals: control = 4-6; castrated = 6-7; T-substituted = 7-8: * $p \leq 0.05$, Student's t-test between intact animals.

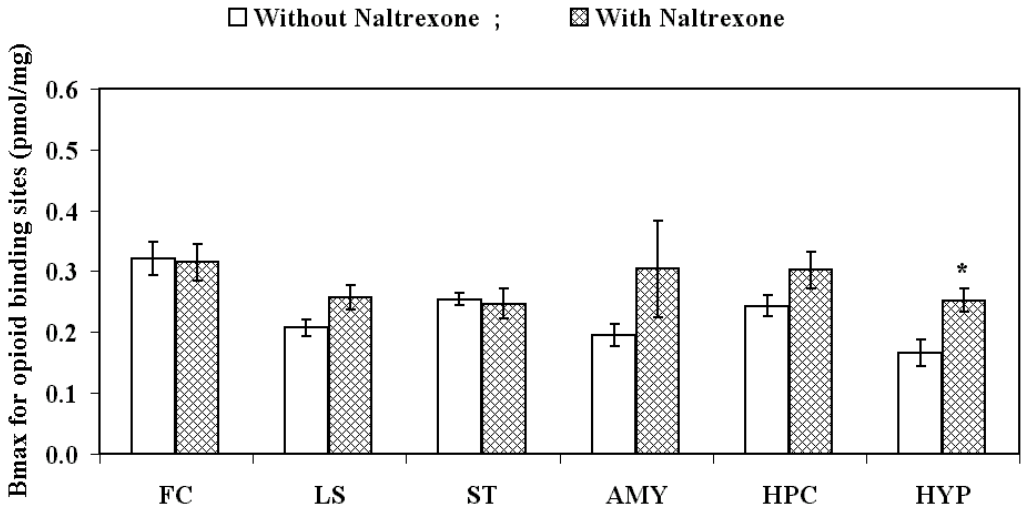

Figure 26. [^{3}H]DPN binding capacity detected in brain homogenates collected from T-substituted without naltrexone (open bars) and T-substituted with naltrexone (closed bars) castrated male chicken. Values are means ± SEM; Number of animals: castrated T-substituted with naltrexone = 10; castrated T-substituted without naltrexone = 7-8: * $p \leq 0.05$, Student's t-test.

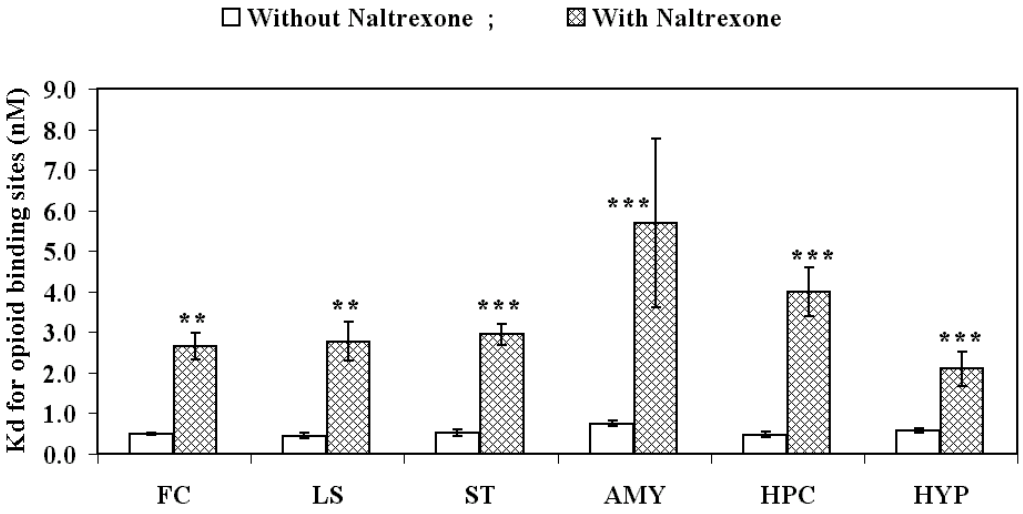

Figure 27. [^{3}H]DPN binding affinity detected in brain homogenates collected from T-substituted without naltrexone (open bars) and T-substituted with naltrexone (closed bars) castrated male chicken. Values are means ± SEM; number of animals: castrated T-substituted with naltrexone = 10; castrated T-substituted without naltrexone = 7-8: ** $p \leq 0.01$, *** $p \leq 0.001$, Student's t-test.

Table 7. Maximal binding capacity and dissociation constant (affinity) of [^{3}H]DPN binding in different brain regions in normal hydrated and dehydrated 10-day-old chickens

Brain region[a]	[^{3}H]DPN Binding					
	Control			Dehydration		
	Bmax (pmol/mg tissue)	Kd (nM)	n[b]	Bmax (pmol/mg tissue)	Kd (nM)	n
10-day-old female chicks						
FC	0.40 ± 0.03	1.42 ± 0.40	5	0.29 ± 0.03* [c]	0.81 ± 0.07	5
LS	0.30 ± 0.04	1.12 ± 0.22	5	0.20 ± 0.02	0.66 ± 0.12	4
ST	0.44 ± 0.08	1.81 ± 0.43	5	0.24 ± 0.01*	0.79 ± 0.10	5
AMY	0.33 ± 0.02	1.55 ± 0.34	5	0.19 ± 0.01***	0.76 ± 0.09*	6
HPC	0.34 ± 0.02	1.20 ± 0.17	6	0.27 ± 0.02	0.49 ± 0.06*	5
HYP	0.27 ± 0.02	1.15 ± 0.28	6	0.20 ± 0.02	0.69 ± 0.10	4
10-day-old male chicks						
FC	0.33 ± 0.02	0.88 ± 0.15	6	0.33 ± 0.04	0.97 ± 0.14	6
LS	0.26 ± 0.02	0.72 ± 0.10	6	0.24 ± 0.03	0.92 ± 0.18	6
ST	0.29 ± 0.02	0.82 ± 0.10	6	0.25 ± 0.02	0.95 ± 0.17	6
AMY	0.23 ± 0.02	0.93 ± 0.19	5	0.21 ± 0.02	1.10 ± 0.15	6
HPC	0.24 ± 0.02	0.76 ± 0.08	4	0.22 ± 0.02	0.90 ± 0.20	6
HYP	0.26 ± 0.01	1.02 ± 0.21	6	0.20 ± 0.01	1.23 ± 0.18	6

[a]Brain region: FC = Frontal cortex; LS = Lateral septum; HYP = Hypothalamus; AMY = Amygdala; HPC; Hippocampus; ST = Striatum
[b]Number of animals
[c]Student's t-test ; * = $p \leq 0.05$, *** = $p \leq 0.001$ compared with control group

Table 8. Maximal binding capacity and dissociation constant (affinity) of [^{3}H]DPN binding in different brain regions in normal hydrated and dehydrated 10-week-old chickens

Brain region[a]	[^{3}H]DPN Binding					
	Control			Dehydration		
	Bmax (pmol/mg tissue)	Kd (nM)	n[b]	Bmax (pmol/mg tissue)	Kd (nM)	n
10-week-old female chickens						
FC	0.48 ± 0.06	0.30 ± 0.10	6	0.46 ± 0.03	0.40 ± 0.05	7
LS	0.30 ± 0.01	0.30 ± 0.05	6	0.37 ± 0.01** [c]	0.38 ± 0.05	6
ST	0.30 ± 0.02	0.30 ± 0.02	6	0.34 ± 0.02	0.37 ± 0.04	7
AMY	0.22 ± 0.01	0.48 ± 0.09	6	0.25 ± 0.02	0.66 ± 0.11	6
HPC	0.30 ± 0.01	0.42 ± 0.07	6	0.36 ± 0.01**	0.35 ± 0.05	7
HYP	0.22 ± 0.01	0.28 ± 0.02	4	0.27 ± 0.01*	0.62 ± 0.06*	5
10-week-old male chickens						
FC	0.41 ± 0.03	0.35 ± 0.07	6	0.46 ± 0.02	0.34 ± 0.12	6
LS	0.28 ± 0.02	0.28 ± 0.03	4	0.31 ± 0.01	0.37 ± 0.08	5
ST	0.32 ± 0.04	0.50 ± 0.10	4	0.34 ± 0.03	0.29 ± 0.06	5
AMY	0.23 ± 0.02	0.84 ± 0.14	5	0.22 ± 0.02	0.37 ± 0.07*	5
HPC	0.32 ± 0.02	0.49 ± 0.05	5	0.34 ± 0.03	0.34 ± 0.08	5
HYP	0.21 ± 0.02	0.58 ± 0.09	5	0.23 ± 0.03	0.31 ± 0.04*	5

[a]Brain region: FC = Frontal cortex; LS = Lateral septum; HYP = Hypothalamus; AMY = Amygdala; HPC; Hippocampus; ST = Striatum
[b]Number of animals
[c]Student's t-test ; * = $p \leq 0.05$, ** = $p \leq 0.01$ compared with control group

Table 9. Maximal binding capacity and dissociation constant (affinity) of [^{3}H]DPN binding in different brain regions in normal hydrated and dehydrated 28-36 –week-old chickens

Brain region[a]	[^{3}H]DPN Binding					
	Control			Dehydration		
	Bmax (pmol/mg tissue)	Kd (nM)	n[b]	Bmax (pmol/mg tissue)	Kd (nM)	n
Adult female chickens						
FC	0.34 ± 0.02	0.57 ± 0.09	4	0.30 ± 0.07	0.28 ± 0.07	7
LS	0.34 ± 0.03	0.54 ± 0.18	5	0.21 ± 0.04* [c]	0.16 ± 0.02*	6
ST	0.23 ± 0.02	0.54 ± 0.03	5	0.27 ± 0.02	0.35 ± 0.09	4
AMY	0.25 ± 0.04	0.56 ± 0.09	5	0.21 ± 0.07	0.24 ± 0.05*	5
HPC	0.24 ± 0.05	0.34 ± 0.06	6	0.25 ± 0.07	0.24 ± 0.07	5
HYP	0.19 ± 0.02	0.76 ± 0.16	5	0.16 ± 0.04	0.29 ± 0.03*	6
Adult male chickens						
FC	0.33 ± 0.06	0.41 ± 0.06	5	0.34 ± 0.04	0.24 ± 0.05	5
LS	0.31 ± 0.03	0.26 ± 0.02	5	0.24 ± 0.03	0.48 ± 0.20	5
ST	0.28 ± 0.02	0.50 ± 0.09	5	0.21 ± 0.04	0.31 ± 0.07	5
AMY	0.21 ± 0.02	0.59 ± 0.13	5	0.22 ± 0.04	0.58 ± 0.23	5
HPC	0.34 ± 0.03	0.26 ± 0.04	5	0.30 ± 0.04	0.48 ± 0.24	5
HYP	0.17 ± 0.02	0.37 ± 0.05	4	0.20 ± 0.02	0.68 ± 0.25	5

[a]Brain region: FC = Frontal cortex; LS = Lateral septum; HYP = Hypothalamus; AMY = Amygdala; HPC; Hippocampus; ST = Striatum
[b]Number of animals
[c]Student's t-test ; * = $p \leq 0.05$ compared with control group

Table 10. Maximal binding capacity and dissociation constant (affinity) of [^{3}H]DPN binding in different brain regions in intact and castrated chickens

	[^{3}H]DPN Binding							
	Intact		Castrated		Intact		Castrated	
Brain region[a]	**Bmax (pmol/mg tissue)**	**n[b]**	**Bmax (pmol/mg tissue)**	**n**	**Kd (nM)**	**n**	**Kd (nM)**	**n**
FC	0.33 ± 0.14	5	0.66 ± 0.23*[c]	6	0.41 ± 0.13	5	0.75 ± 0.36	6
LS	0.31 ± 0.07	5	0.47 ± 0.08*	6	0.26 ± 0.05	5	0.42 ± 0.14	6
ST	0.28 ± 0.06	5	0.38 ± 0.08*	7	0.49 ± 0.22	5	0.73 ± 0.24	7
AMY	0.21 ± 0.05	5	0.30 ± 0.08	6	0.59 ± 0.30	5	0.65 ± 0.20	6
HPC	0.34 ± 0.08	5	0.48 ± 0.11	6	0.26 ± 0.09	5	0.74 ± 0.32*	6
HYP	0.17 ± 0.05	4	0.33 ± 0.07*	6	0.37 ± 0.10	4	0.71 ± 0.34	6

[a]Brain region: FC = Frontal cortex; LS = Lateral septum; HYP = Hypothalamus; AMY = Amygdala; HPC; Hippocampus; ST = Striatum
[b]Number of animals
[c]Student's t-test ; * = $p \leq 0.05$, compared with control group

Table 11. Maximal binding capacity and dissociation constant (affinity) of [^{3}H]DPN in different brain regions in sham-operated and castrated chickens

	[^{3}H]DPN Binding							
	Sham-operated		Castrated		Sham-operated		Castrated	
Brain region[a]	Bmax (pmol/mg tissue)	n[b]	Bmax (pmol/mg tissue)	n	Kd (nM)	n	Kd (nM)	n
FC	0.62 ± 0.08	5	0.66 ± 0.23	6	0.46 ± 0.25	5	0.75 ± 0.36	6
LS	0.40 ± 0.12	5	0.47 ± 0.08	6	0.41 ± 0.21	5	0.42 ± 0.14	6
ST	0.35 ± 0.10	5	0.38 ± 0.08	7	0.52 ± 0.26	5	0.73 ± 0.24	7
AMY	0.30 ± 0.07	5	0.30 ± 0.08	6	0.73 ± 0.30	5	0.65 ± 0.20	6
HPC	0.38 ± 0.08	5	0.47 ± 0.11	6	0.41 ± 0.13	5	0.74 ± 0.32	6
HYP	0.28 ± 0.07	5	0.33 ± 0.07	6	0.76 ± 0.34	5	0.71 ± 0.34	6

[a]Brain region: FC = Frontal cortex; LS = Lateral septum; HYP = Hypothalamus; AMY = Amygdala; HPC; Hippocampus; ST = Striatum
[b]Number of animals

Table 12. Maximal binding capacity and dissociation constant (affinity) of [^{3}H]DPN binding in different brain regions in intact and castrated testosterone-substituted chickens

	[^{3}H]DPN Binding							
	Intact		Castrated T-substituted		Intact		Castrated T-substituted	
Brain region[a]	**Bmax (pmol/mg tissue)**	**n[b]**	**Bmax (pmol/mg tissue)**	**n**	**Kd (nM)**	**n**	**Kd (nM)**	**n**
FC	0.33 ± 0.14	5	0.32 ± 0.07	7	0.41 ± 0.13	5	0.49 ± 0.10	7
LS	0.31 ± 0.07	5	0.21 ± 0.04** [c]	8	0.26 ± 0.05	5	0.44 ± 0.21	8
ST	0.28 ± 0.06	5	0.25 ± 0.05	7	0.49 ± 0.22	5	0.52 ± 0.11	7
AMY	0.21 ± 0.05	5	0.19 ± 0.05	7	0.59 ± 0.30	5	0.75 ± 0.20	7
HPC	0.34 ± 0.09	5	0.24 ± 0.04*	7	0.26 ± 0.09	5	0.48 ± 0.18*	7
HYP	0.17 ± 0.05	4	0.16 ± 0.02	7	0.37 ± 0.10	4	0.58 ± 0.22	7

[a]Brain region: FC = Frontal cortex; LS = Lateral septum; HYP = Hypothalamus; AMY = Amygdala; HPC; Hippocampus; ST = Striatum
[b]Number of animals
[c]Student's t-test ; * = $p \leq 0.05$, ** = $p \leq 0.01$ compared with intact group

Table 13. Maximal binding capacity and dissociation constant (affinity) of [^{3}H]DPN binding in different brain regions in castrated testosterone-substituted male chickens with or without naltrexone treatment

	[^{3}H]DPN Binding							
	Without naltrexone		With naltrexone		Without naltrexone		With naltrexone	
Brain region[a]	Bmax (pmol/mg tissue)	n[b]	Bmax (pmol/mg tissue)	n	Kd (nM)	n	Kd (nM)	n
FC	0.32 ± 0.07	7	0.31 ± 0.09	10	0.49 ± 0.10	7	2.65 ± 1.04*** [c]	10
LS	0.21 ± 0.04	8	0.25 ± 0.06	10	0.44 ± 0.21	8	2.76 ± 1.52***	10
ST	0.25 ± 0.06	7	0.24 ± 0.06	10	0.52 ± 0.11	7	2.94 ± 1.34***	10
AMY	0.19 ± 0.05	7	0.30 ± 0.25	10	0.75 ± 0.20	7	5.69 ± 6.58***	10
HPC	0.24 ± 0.04	7	0.30 ± 0.09	10	0.48 ± 0.18	7	3.98 ± 1.91***	10
HYP	0.16 ± 0.02	7	0.25 ± 0.08*	10	0.58 ± 0.22	7	2.09 ± 0.78***	10

[a]Brain region: FC = Frontal cortex; LS = Lateral septum; HYP = Hypothalamus; AMY = Amygdala; HPC; Hippocampus; ST = Striatum
[b]Number of animals
[c]Student's t-test ; * = $p \leq 0.05$, *** = $p \leq 0.001$ compared with control group

6 Discussion

6.1 Characteristics of [^{3}H]diprenorphine binding in homogenates of chicken brain

This study used the technique of radioactive ligand binding to measure binding sites for opioid peptides in the chicken brain. The maximum binding capacity and affinity (Kd) of [^{3}H]Diprenorphine were assessed in the chicken brain homogenates. The Bmax values reflect the density of receptor sites whereas Kd values reflect the receptor affinity for the radioligand. Affinity is a measure of the ability of ligand to bind reversibly to a receptor and indicating potency. The lower the Kd, the more effective is the ligand.

With the use of [^{3}H]DPN as a universal ligand, we did not determine the subtypes of the opioid receptor. However, μ-, δ-, and κ-subtypes are all possible contributors to the binding of [^{3}H]DPN (**SADÉE** 1982; **HÖLLT** et al. 2004). The values of Kd expressed as moles per liter obtained in the present study were in the order of 10^{-9}. Similar values were reported for the ewe hypothalamus, human brain and chicken neurohypophysis determined by the use of [^{3}H]DPN (**PFEIFFER** et al. 1982b; **YANG** et al. 1989; **MARTIN** et al. 1992). The Bmax values obtained in the present study were of a similar range reported in the ewe hypothalamus (**YANG** et al. 1989).

The present data demonstrated the binding of [^{3}H]DPN in 6 brain regions; frontal cortex, lateral septum, striatum, amygdala, hippocampus and hypothalamus. Scatchard plots indicated the interaction of [^{3}H]DPN with only one single binding site. These results indicate the same populations of opioid binding sites in all three age groups.

The development of opioid binding sites identified in chicken brains demonstrated marked regional variability with receptor concentrations dependent on region, sex and age. A striking feature was that the highest and lowest Bmax were in the frontal cortex and hypothalamus in 10-week-old male, adult male and adult female chickens, respectively. The regional distribution of [^{3}H]DPN binding in hypothalamus was similar to that obtained by the autoradiographic studies (**REINER** et al. 1989). In the chicken and pigeon, the binding capacity of the three opioid receptors – mu, delta and kappa - was generally low in the diencephalon (**REINER** et al. 1989; **CSILLAG** et al. 1990). In contrast, in rats and sheep, using naloxone, the other universal opioid antagonist, the highest opioid receptor concentrations were observed in the striatum and thalamus and the lowest in the

pons/medulla. The differences in the binding concentration are most probably due to species differences.

The limbic system (amygdala, striatum and hippocampus) in birds is involved in emotion, motivation, memory and learning (**KUENZEL** 2000). In birds, hippocampus and amygdala are relatively large and distinct cortical structure. The equivalent structure of the amygdala in birds includes nucleus Taenia, medial archistriatum and posterior archistriatum. The hippocampus of birds is also involved in the regulation of food storage behavior and homing (**KUENZEL** 2000). The high density of opioid binding sites observed in the limbic system suggests that opioids may be involved in the modulation of these functions in birds similar to mammals.

The opioid binding sites were decreasing in the frontal cortex, striatum and amygdala regions and constant in the lateral septum, hippocampus and hypothalamus regions from 10-day-old to adulthood in female chickens, while there was no significant differences in immature and adult male chickens. Bmax values of adult female compared to 10-day-old chickens changed in descending order by approximately 48.2, 29.4, 28.5, 25.7 and 15.9% in the striatum, hippocampus, hypothalamus, amygdala and frontal cortex, respectively. Age-dependent decrease of Bmax was concomitant with alterations in the Kd for [^{3}H]DPN in both sexes. Most of the changes in Kd values appeared in the hippocampus of both sexes. A sexual dimorphism in Bmax and Kd values were observed in the amygdala and hippocampus of 10-day-old chickens and in the hypothalamus of 10-week-old chickens. The homogenous distribution of Bmax in adult male chicken brains is in agreement with finding in the adult pig which shows no differences among brain areas (**KAHLE** and **PARVIZI** 1993). A previous study of the receptor development in midbrain and forebrain of posthatch (until 28 days) chick indicated that there was an age-related decrease in the maximal binding capacity without any alteration in receptor affinity for naloxone binding (**BARDO** et al. 1982). The study of the postnatal development of met-ENK binding to the rat brainstem indicated that the binding was stable between 1-6 days postpartum, then increased to reach a maximum by 14 days, and dropped to reach adult levels by 22 days (**TSANG** and **Ng** 1980).

A noticeable feature of the present study was the significant loss of opioid binding sites with age that occurred in the frontal cortex, striatum and amygdala. The decrease in binding sites was partly compensated by an increase in the affinity in the older animals.

These results are in agreement with studies in chicken (**BARDO** et al. 1982), rat (**KENT** et al. 1982), sheep (**VILLIGER** et al. 1982) and pig (**KAHLE** and **PARVIZI** 1993). Similar finding was reported regarding β-adrenergic and serotonin binding sites in human brain indicating that the developmental down regulation of receptors is not a sole property of opioids (**SCHOCKEN** and **ROTH** 1977; **SHIH** and **YOUNG** 1978).

Increasing evidence suggests that neuropeptides exert trophic actions during development (**HÖKFELT** 1991). The latter role can explain receptor down-regulation such as those observed in the chickens. Trophic actions of opioids have been shown, for examples, there is evidence for involvement of opioid peptides during CNS development and neuronal growth (**VÉRTES** et al. 1982; **ZAGON** and **MCLAUGHLIN** 1983). Evidence supporting a trophic role for opioid receptors also comes from the study of [^{3}H]Etorphine binding to tissues from chicken embryos (**GIBSON** and **VERNADAKIS** 1982). Once synaptogenesis is complete the need for such trophic receptors is eliminated and only those receptors necessary to perform a role in neuromodulation are maintained. Another possible explanation is that some of the neuronal elements upon which opioid receptors are located may degenerate with age. It is known that sprouting neuronal elements in developing brain may degenerate if they fail to make functional connections (**LEVI-MONTALCINI** 1964). In chickens, the rate of neuronal sprouting declines after hatching (**LEVI** and **MORISI** 1971).

6.2 Characteristics of [^{3}H]diprenorphine binding in the brain of dehydrated chicken

Water deprivation has been shown to be an effective stimulus for AVT synthesis and secretion in the domestic chicken (**ARAD** et al. 1985; **NOUWEN** et al. 1984). Withdrawal of water resulted in measurable increases in plasma osmolality and AVT levels (**KOIKE** et al. 1977; **STALLONE** and **BRAUN** 1986; **BENTLEY** 2002). The correlation between osmolality and plasma AVT is highest 24 – 48 hours after removal of the water (**ARNASON** et al. 1986). If dehydration is continued more than 72 and 96 hours, plasma AVT level decreased and returned to the initial values (**NOUWEN** et al. 1984; **KOIKE** 1989).

The present experiments provide support for a sexual dimorphism in the osmotic control of AVT release in the chickens. Besides the control of water balance, AVT is strongly involved in the mechanisms leading to laying egg in female birds. Thus from physiological point of view it makes sense that the female chickens response stronger to the water deprivation than males. Confirmingly, a line of evidence indicate a prominent the sexual

dimorphism in the AVT expressing neurons in chickens (**VIGLIETTI-PANZICA** et al. 1992, 1994; **JURKEVICH** et al. 1997) and Japanese quails (**CHATURVEDI** et al. 2000). However, a sex-difference in basal plasma AVT concentrations was not reported in an earlier work published by ROBINSON et al. (1990). The osmotic control of AVT release is determined by the correlation between plasma osmolality and plasma AVT concentration. This correlation, however, was relatively low (about 0.5-0.6) in all age groups of normohydrated female and dehydrated male chickens. The lack of correlation between these parameters after water deprivation in females is in agreement with data of **NOUWEN** et al. (1984) and in contrast to some other published data (**ARNASON** et al. 1986; **STALLONE** and **BRAUN** 1986; **ROBINSON** et al. 1990a and b; **CHATURVEDI** et al. 2000). The breed, sex and age as well as differences in the housing of the animals among the studies could be the reason for the discrepancies.

Changing of opioid binding characteristic in the brain and the rising plasma osmolality and AVT in response to dehydration indicate that the opioid receptors are linked to body fluid regulation. Opioid binding sites changed during water deprivation in the six brain regions. Hence it is likely that these regions contribute to the rise in the circulating AVT during dehydration. The present results are in corroboration to other reports concerning the opioid modulation of the osmolality-regulated release of AVP and AVT in mammals and birds (**VAN DE HEIJNING** et al. 1991; **SASAKI** et al. 2000). The site of opioid receptors involving in the regulation of AVT release is not clear in the chickens. In addition to the hypothalamus, the neurons that synthesize AVT are widely distributed in paraventricular nucleus, supraoptic nucleus, bed nucleus of stria terminalis, parvocellular neurons of the suprachiasmatic nucleus and also in extrahypothalamic sites in the CNS such as limbic system, autonomic centers of the reticular formation and circumventricular organ (**BONS** 1980; **KORF** 1984; **KOIKE** 1989; **JURKEVICH** et al. 1999). These regions have been also shown to possess opioid receptors.

Water deprivation caused a down-regulation of κ-opioid receptors in the rat pituitary gland (**BRADY** and **HERKENHAM** 1987) and up-regulation of δ-opioid receptors but no change in μ-opioid receptors in rat medulla (**HWANG** et al. 1986). As stated before, most probably μ- and κ-opioid receptors are involved in the control of AVT release in the chicken. There is considerable evidence indicating that the chicken brain contains high density of

κ-receptors (**MANSOUR** et al. 1988; **TRANQUILI** 2002). Thus it could be speculated that the up- and down regulation of opioid binding sites in response to dehydration may mainly be because of changes of the κ-subtype. Therefore, changes of opioid binding sites caused by dehydration may reflect a profile of the physiological condition of endogenous opioid peptides in the chicken; and it represents a defensive or compensatory response to minimize the impact of dehydration.

6.3 Characteristics of [^{3}H]diprenorphine binding in brain regions of the castrated, testosterone-substituted castrated and testosterone + naltrexone substituted castrated chicken

Long-term castration (7 months) altered the Bmax and Kd values of [^{3}H]DPN opioid binding sites in discrete regions of the male chicken brain. Concentration of opioid binding in six studied regions of castrated chickens were higher than in the intact animals, however, the difference was statistically significant in the frontal cortex, hypothalamus, lateral septum and striatum only. The Bmax of opioid binding increased 2 folds in both the frontal cortex and hypothalamus when compared to the intact animals. Whereas the Kd of [^{3}H]DPN remained constant after castration in all brain areas but hippocampus, where Kd increased 2.8 folds in response to castration. To the author's knowledge, this is the first description of the distribution of opioid binding sites throughout the brain of adult castrated male chickens.

Also in the rat (**HAHN** and **FISHMAN** 1975, 1985; **TAKAYAMA** et al. (1990) and pig (**KAHLE** and **PARVIZI** 1993) have been reported that castration produced increase in the Bmax of opioid binding sites in the male brain. Furthermore, **WILKINSON** et al. (1985) and **VERTES** et al. (1986) have reported that both long-term ovariectomy and acute administration of estradiol altered both Kd and Bmax values of opioid receptors in the hypothalamus, but not in the whole brain of female rats. However, other studies failed to observe any effect of orchidectomy/ovariectomy on opioid receptor content in whole male and female rat brain (**WILKINSON** et al. 1981; **DIEZ** and **ROBERTS** 1982; **CICERO** et al. 1983; **CLARK** et al. 1984). Testosterone substitution of castrated chicken restored the status of the opioid binding sites to that seen in intact males hypothalamus, stratum and cortex. This shows that the opioid receptors are modulated by androgens in these brain regions. Interestingly, testosterone-substitution had no effect on opioid binding in the hippocampus and amygdala in castrated chicken. Indeed, it has been shown that hypothalamic mediated

regulation of LHRH by opioid is gonadal steroid-dependent (**STANFIELD** and **CUNNINGHAM** 1987a and b). Direct competition of testosterone for the opioid receptors is not being considered because of the absence of any affinity of the steroid for the opioid receptors (**LABELLA** et al. 1978). Additional research is needed to determine the metabolites of testosterone whether 5 α-dihydrotestosterone or 17-β-estradiol play a role in substituted castrated chickens.

Another interesting observation is that long-term (7 days) naltrexone administration in castrated testosterone-substituted chickens was accompanied by changes in the Kd values. The extent of the changes in opioid binding site varied throughout the brain, largest increases of Kd were observed in the hippocampus (935%), whereas Bmax was significantly increased in the hypothalamus only.

The chronic administration of drugs which block the action of neurotransmitters may produce an increase in the number of postsynaptic receptor sites for the given neurotransmitter. The occurrence of up-regulation has been described for many receptors including dopamine (**BURT** et al. 1977), noradrenaline (**WOLFE** et al. 1978) and acetyl choline (**BEN-BARAK** and **DUDAI** 1980) receptors. Hence the opioid system in chicken adapts to chronic receptor blockade in a manner which is not quite similar to that observed in a classical neurotransmitter system. The regional differences in naltrexone induction of changes imply that the drug does not have uniform effects throughout the brain. In addition, the regional differences could reflect different basal level of activity of opioid-containing neurons. The varying degree of up-regulation of binding sites can then be explained by different levels of tonic opioid activity in different neuronal pathways.

BARDO et al. 1984 reported also a change in the Kd of opioid receptors after naltrexone treatment in rats. Other works have mostly considered the concentration of opioid receptors in response to naltrexone or naloxone treatments (**LAHTI** and **COLLINS**, 1978; **TEMPEL** et al. 1982; **ZUKIN** et al. 1982).

7 Summary

The term “opioid” generally refers to any substance, endogenous or synthetic peptide or non-peptide, which acts through any of the four major types of opioid receptors present in the cell membrane. Its effects can be stereospecifically blocked by pure narcotic antagonists like naloxone. The four major classes of endogenous opioid peptides, β-endorphin, enkephalin, dynorphin and nociceptin have been described as potential ligands for the μ, δ, κ and opioid receptor like (ORL1) receptor binding sites. Opioid peptides and their receptors are implicated in the modulation of a number of behavioral and physiological functions in birds (e.g., reproduction, endocrinology, water balance, social behavior and painful stimulus) in a manner similar to that described for mammals.

Opioids are (neuro) peptides, which are synthesized in the central nervous system (CNS) in the whole animal kingdom. They play a role both as neurotransmitters and neuromodulators. Opioid peptides and their receptors are implicated in the modulation of a number of behavioral and physiological functions in birds (e.g., reproduction, endocrinology, water balance, social behavior and painful stimulus) in a manner similar to that described for mammals. Nowadays, more attention is being paid to the effect of the opioid system on the water balance in birds. Opioid drugs were also introduced in veterinary medicine for pain relieving and anesthesia of birds.

The studies reported in this dissertation were undertaken to further elucidate opioid receptors, the physiological intervention on receptor binding properties and their regulation. To this end, we evaluated the receptor binding profiles - the dissociation constant (Kd) and maximum binding capacity (Bmax) - in various brain regions. Following questions were addressed using the chicken as model.

Are opioid binding profiles gender-, and/or age-dependent in the chicken brain?

Are opioid binding profiles affected by dehydration in the chicken brain?

Are opioid binding profiles testosterone-dependent?, and

Are opioid binding profiles affected by opioid antagonist?

To achieve this, the opioid binding sites were determined in six brain regions - frontal cortex, lateral septum, striatum, amygdala, hippocampus and hypothalamus - of chicken using [^{3}H]diprenorphine ([^{3}H]DPN) as the radioligand. Three age groups of chickens, 10-day-, 10-week- and 26-38-week-old, were used. Following situations were considered (a) the effects of hydration (b) the effect of testosterone treatments (performed in castrated male chickens) and (c) the effect of opioid antagonist, naltrexone.

Experiment I, opioid binding sites were investigated in 10-day-, 10-week- and 26-38-week-old female and male chickens. The linear Scatchard plot of [^{3}H]DPN binding in all experiments indicated a single binding site with high affinity. The mean of Bmax was as following: 0.27-0.44 (female), 0.23-0.33 (male); 0.21-0.48 (female), 0.21-0.41 (male); 0.19-0.34 (female), 0.17-0.34 (male) pmol/mg in 10-day-, 10-week- and 26-38-week-old chickens, respectively. The mean of Kd was as follow: 1.13-1.80 (female), 0.72-1.01 (male); 0.28-0.48 (female), 0.27-0.83 (male); 0.33-0.75 (female), 0.26-0.58 (male) nM in 10-day-, 10-week- and 26-38-week-old chickens, respectively. The highest concentration of opioid binding sites was found in the frontal cortex and the lowest concentration in the hypothalamus both in males and females. The Bmax of opioid binding sites decreased significantly from 10-day-old to adulthood in the frontal cortex, striatum and amygdala regions. While there was no such a decrease in males. The data also indicated that the sex related change in opioid binding reflects a change in the Kd in males more than in females.

Experiment II, groups of 10-day-, 10-week- and 26-38-week-old chickens were deprived from water for 1 or 2 days. Dehydration induced an increase of plasma AVT levels and had some impacts on the characteristics of opioid binding sites in the brain. Most of the changes of opioid binding site were in young female chickens. The results also indicated that dehydration induced both decrease (frontal cortex, striatum and amygdala: 10-day-old females; lateral septum: adult females; and hypothalamus: 10-day-old males) and increase (lateral septum, hippocampus and hypothalamus: 10-week-old females) of Bmax in chicken brains. Such changes were accompanied with alterations in receptor affinity.

Experiment III, male chickens were castrated and sham-operated for 7 months and were treated with testosterone proprionate or testosterone proprionate and naltrexone. The Bmax value in the brain of castrated chickens was higher than that found in intact animals. This increase was testosterone-dependent, since testosterone substitution reduced the binding

capacity to the control levels. The extent of opioid binding site changes after naltrexone administration was more pronounced in the Kd than in the Bmax values. The regional differences in naltrexone-induce changes imply that the drug does not have uniform effects throughout the brain. The Bmax was significantly increased in the hypothalamus only.

In conclusion, these results demonstrate, that like in some mammals, opioid binding sites are involved in modulation of water balance and reproduction in chickens. Such modulations are age-, sex- and brain region dependent.

8 Zusammenfassung

Der Begriff “Opioide” bezeichnet allgemein alle körpereigenen oder synthetischen, Peptide oder Nichtpeptidstruktur, die durch eine Bindung an einem der vier Haupttypen der Opioidrezeptoren wirken. Der Begriff “Opiat” bezeichnet Verbindungen mit biologische Eigenschaften, die dem des Morphin entsprechen (Nichtpeptidstruktur). Der alte Begriff “Opiate” ist jetzt durch den Begriff Opioide ersetzt worden. Der Begriff “endogene Opioide” bezieht sich auf körpereigene Substanzen, die im Gehirn oder in anderen Organen vorkommen. Diese Opioide haben pharmakologische Eigenschaften, die denen des Opiatabkömmlings Morphin ähneln, und gehen natürliche Bindungen mit den Opioidrezeptoren ein. Die körpereigenen Opioide sind überwiegend Peptide, obwohl auch solche mit Nichtpeptidstruktur beschrieben sind. Abweichend von den Opiaten werden die Opioide mit Peptidstruktur schnell nach ihrer Freisetzung abgebaut und akkumulieren nicht in Mengen, die groß genug sind um, eine Toleranz auszulösen. Die vier Hauptklassen körpereigener Opioide sind Endorphin, Enkephalin, Dynorphin und Nociceptin. Sie sind Liganden für die μ-, δ-, κ- beziehungweise ORL-1 (Opioid Receptor like) - Bindungsstellen.

Opioide sind (Neuro-) Peptide, die im Zentralnervensystem (ZNS) synthetisiert oder und im gesamten Tierreich vorkommen. Sie spielen sowohl als Neurotransmitter als auch als Neuromodulatoren eine Rolle. Opioide sind an der Modulation einer Reihe physiologischer Funktionen bei Vögeln beteiligt (z.B. Fortpflanzung, Endokrinologie, Wasserhaushalt, Sozialverhalten). Derzeit wird dem Einfluss des Opioid-Systems auf den Wasserhaushalt bei Vögeln vermehrt Aufmerksamkeit geschenkt. Ausserdem wurden Opioid-Pharmaka in der Veterinärmedizin eingesetzt zur Anästhesie und zur Schmerzlinderung bei Vögeln.

Die in dieser Dissertation dargestellten Untersuchungen wurden durchgeführt, um die Opioidrezeptoren, die physiologische Beeinflussung der Rezeptorbindungseigenschaften und deren Regulation näher aufzuklären. Zu diesem Zweck wurden die Rezeptorbindungsprofile- bestimmt als Dissoziationskonstante (Kd) und maximale Bindungskapazität (Bmax)-in verschiedenen Gehirnabschnitten des Huhnes als Modell benutzt.

Die Fragestellungen waren im einzelnen:

1. Sind die Opioidbindungsprofile im Hühnergehirn geschlechts- und/oder altersabhängig?

2. Werden die Opioidbindungsprofile durch Wasserentzug beeinflusst?

3. Sind die Opioidbindungsprofile testosteronabhängig?

4. Werden die Opioidbindungsprofile durch Opioidantagonisten beeinflusst?

Opioidbindungsstellen wurden in sechs Gehirnregionen – Frontaler Cortex, Laterales Septum, Striatum, Amygdala, Hippocampus und Hypothalamus untersucht. Als Ligand wurde [^{3}H]Diprenorphin ([^{3}H]DPN) eingesetzt. Es wurden drei Altersgruppen von Küken untersucht: 10 Tage-alt, 10 Wochen-alt und 26 bis 38 Wochen-alt. Folgende Aspekte wurden untersucht: a) der Einfluss von Wasserentzug, b) der Einfluss von Kastration und Testosteronsubstitution, und c) der Einfluss des Opioidantagonisten Naltrexon.

Experiment I: Opioidbindungsstellen wurden bei 10 Tage-, 10 Wochen- und 26 bis 38 Wochen-alten weiblichen und männlichen Küken untersucht. In allen Versuchen war der Scatchard plot der [^{3}H]DPN-Bindungen linear und wies auf eine Bindungsstelle mit hoher Affinität hin. Sowohl bei männlichen als auch bei weiblichen Tieren wurden die höchste Konzentrationen von Opioidbindungsstellen im Frontalen Cortex ermittelt. Die niedrigsten Werte wurden im Hypothalamus gemessen. Eine signifikante Abnahme der Bmax war in der Amygdala, im Striatum und im Frontalen Cortex mit steigendem Alter (Tag 10 bis erwachsenem Alter) bei Hennen zu beobachten. Keine altersbedingte Veränderung der Dichte der Opioidbindungsstellen war bei männlichen Tieren nachweisbar. Diese Geschlechtsdifferenzierung war zum Teil auch bei Kd-Werten vorhanden. Die Mittelwerte der maximalen Bindungskapazität betrugen bei am Tag 10: 0,27-0,44 (weiblich), 0,23-0,33 (männlich); bei 10 Wochen alten Tieren: 0,21-0,48 (weiblich), 0,21-0,41 (männlich); bei erwachenen Tieren: 0,19-0,34 (weiblich), 0,17-0,34 (männlich) pmol/mg. Die Mittelwerte der ermittelten Dissoziationskonstante betrugen bei am Tag 10: 1,13-1,80 (weiblich), 0,72-1,01 (männlich); bei 10 Wochen alten Tieren: 0,28-0,48 (weiblich), 0.27-0.83 (männlich); bei erwachenen Tieren: 0,33-0,75 (weiblich), 0,26-0,58 (männlich) nM. Die gemessenen Bmax-Werte der Opioidbindungsstellen im Frontalen Cortex, Striatum und Amygdala fielen vom 10-Tage-alten Küken bis zur erwachsenen Henne signifikant ab, während solcher Abfall

bei den männlichen Tieren nicht beobachtet wurde. Die Ergebnisse weisen außerdem darauf hin, dass die geschlechtsgebundene Veränderung der Opioidbindungen eine Veränderung des Kd – Wertes bei männlichen Tieren stärker als bei weiblichen widerspiegelt.

Experiment II: Küken wurden in verschiedenen Altersgruppen einem eintägigen (10-Tage-alte) oder zweitägigen (10-Wochen-alte und adulte Tiere) Wasserentzug ausgesetzt. Diese Dehydration bewirkte einen Anstieg des AVT-Plasmawerte. Darüber hinaus verursachte der Wasserentzug Veränderungen der Konzentration von Opioidbindungsstellen in den untersuchten Gehirngebieten. Die stärksten Veränderungen der Opioidbindungsstellen wurden bei 10 Tagealten und 10 Wochenalten weiblichen Hühnen gefunden. Die Ergebnisse liessen außerdem erkennen, dass der Wasserentzug einen Bmax Abfall (Frontaler Cortex, Striatum und Amygdala: 10-Tagealten weiblich; Laterales Septum: 26 bis 38 Wochenalten weiblich; und Hypothalamus: 10-Tagenalten männlich) oder Anstieg (Laterales Septum, Hippocampus und Hypothalamus: 10-Wochenalten weiblich) im Gehirn der Tiere bewirkte. Diese Veränderungen wurden von einem Anstieg oder Abfall der Bindungsaffinität begleitet.

Experiment III: Männliche Küken wurden in Alter von 3-4 Wochen kastriert und schein-operiert. 7 Monate nach der Kastration wurden die Tiere in drei Gruppen geteilt.

Gruppe 1 diente als unbehandelte Kontrollgruppe.

Gruppe 2 erhielt über einen 10 Tage-Zeitraum im Abstand von jeweils 3 Tagen intramuskuläre Injektionen von Testosteronproprionat (TP, 10mg/kg)

Gruppe 3 erhielt die gleiche Testosteronbehandlung wie Gruppe 2 und wurde zusätzlich zu TP auch mit Naltrexon behandelt (0,1mg/kg); von Tag 3 nach Beginn der Testosteroninjektionen für 7 Tage im 8 Stunden-Intervall.

Die Kastration bewirkte eine signifikante Zunahme der Opioidbindungsstellen im Gehirn. Diese Zunahme war testosteronabhängig, da sie durch eine Testosteronsubstitution reversibel war. Die Veränderungen bei den Rezeptorbindungsprofilen waren bei den Kd–Werten ausgeprägter als bei den Bmax–Werten. Die festgestellten Unterschiede der Veränderungen in den untersuchten

Gehirnregionen nach Applikation des Präparates Naltrexon zeigten, dass dieser Opioidantagonist nicht einheitlich im gesamten Gehirn wirkt. Nach der Applikation von Naltrexon zeigten die Bmax–Werte nur im Hypothalamus einen signifikanten Anstieg.

Die Schlussfolgerungen aus den vorstehend dargestellten Untersuchungen lassen sich wie folgt zusammenfassen:

a) Ebenso wie bei einigen Säugetierspezies sind auch bei Hühnern die Opioidbindungsstellen an der Regulation des Wasserhaushaltes und der Fortpflanzung modulierend beteiligt.

b) Die einhergehenden Veränderungen die Rezepterbindungsprofile ist abhängig vom Alter und vom Geschlecht der Tiere. Außerdem sind in den untersuchten Gehirnregionen unterschiedliche Veränderung auf die osmotische Stimulation zu finden.

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Brain Res. 245, 285-292

10 Appendix

10.1 Glossary of abbreviations

ACTH	adrenocorticotropin hormone
Aib	aminoisobutyric acid
Ala	alanine
AMY	amygdala
ANOVA	analysis of variance
Aq. Dest.	aqua destillata
Arg	arginine
Asn	asparagines
Asp	aspartic acid
ATP	adenosine triphosphate
AVT	arginine vasotocin
Bmax	maximum binding capacity
BSA	bovine serum albumin
°C	celsius degree
Ca^{2+}	calcium ion
cAMP	cyclic adenosine monophosphate
Ci	curie
CLIP	corticotropin-like intermediate lobe peptide
CMO	carboxymethyloxime
CNS	central nervous system
CRH	corticotropin-releasing hormone
δ	delta
DMSO	dimethyl sulfoxide
DOR	delta opioid receptor
dpm	disposable per minute
DPN	diprenorphine
DYN	dynorphine
EM-1	endomorphin-1
EM-2	endomorphin-2
END	endorphin

ENK	enkephalin
EOP	endogenous opioid peptide
ε	epsilon
Et al.	et alii
FC	frontal cortex
FSH	follicle stimulating hormone
GH	growth hormone
Gln	glutamine
Glu	glutamic acid
Gly	glycine
GnRH	gonaddotropin releasing hormone
GTP	guanine triphosphate
H	hydrogen
^{3}H	tritrium
HPC	hippocampus
HRP	horse radish peroxidase
HYP	hypothalamus
ι	iota
Ile	isoleucine
κ	kappa
Kd	dissociation constant
KOR	kappa opioid receptor
λ	lambda
Leu	leucine
LH	luteinizing hormone
LHLH	luteinizing hormone releasing hormone
LPH	lipotropin
LS	lateral septum
LSL	lohman-selected leghorn
Lys	lysine
M	molar
Met	methionine

MOR	mu opioid receptor
mRNA	messenger ribonucleic acid
μ	mu
μl	microlitre
MSH	melanocyte stimulating hormone
MAC	minimal anesthetic concentration
mg	milligram
ml	millilitre
MT	mesotocin
n	number of animals
NAL	naloxone hydrochloride
NLTRX	naltrexone
NOR	nociceptin opioid receptor
NSB	nonspecific binding
OFQ/N	orphanin FQ/Nociceptin
ORL	opioid receptor like
OT	oxytocin
p	probability of being wrong
PBS	phosphate buffer saline
Phe	phenyalanine
PDYN	preprodynorphin
PENK	preproenkephalin
PNS	peripheral nervous system
POMC	proopiomelanocortin
Pro	proline
PVN	paraventricular nucleus
RIA	radioimmunoassay
σ	sigma
SEM	standard error mean
Ser	serine
ST	striatum
T	testosterone

TB	total binding
TBM	tetramethylbenzidine
Thr	threonine
Tic	tetrahydroisoquinoline
TRH	thyrotropin releasing hormone
Tris	tris-[hydroxymethyl]aminomethane
Tyr	tyrosine
UV	ultraviolet
Val	valine
ζ	zeta

10.2 Tables

Table 1. δ (OP_1) Opioid receptor ligands and chemical structures

δ (OP_1) Opioid receptor ligands	
Agonists Peptides	Chemical structure
DADLE	Tyr-D-Ala-Gly-Phe-D-Leu
DSLET	Tyr-D-Ser-Gly-Phe-Leu-Thr
DTLET	Tyr-D-Thr-Gly-Phe-Leu-Thr
DSTBULET	Tyr-D-Ser(OtBu)-Gly-Phe-Leu-Thr
BUBU	Tyr-D-Ser(OtBu)-Gly-Phe-Leu-Thr(OtBu)
BUBUC	Tyr-D-Cys(StBu)-Gly-Phe-Leu-Thr(OtBu)
DPLPE	Tyr-D-Pen-Gly-Phe-L-Pen
DPDPE	Tyr-D-Pen-Gly-Phe-D-Pen
Deltorphine	Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2
Deltorphin I	Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2
Deltorphin II	Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2
Agonists Non-peptides	
BW 373U86	(±)4-[(α-R)-α-[2S,5R)4-allyl-2,5-dimethyl-1-piperazinyl]-3-hydroxybenzyl]-N,N-diethyl-benzamide
SCN 80	(±)4-[(α-R)-α-[2S,5R)4-allyl-2,5-dimethyl-1-piperazinyl]-3-methoxybenzyl]-N,N-diethyl-benzamide
SIOM	7-spiroindanyloxymorphone
Antagonists Peptides	
ICI 154,129	CH_2=CH- CH_2 N-Tyr-Gly-ψ-CH_2-S)Phe-Leu CH_2=CH- CH_2
ICI 174,864	CH_2=CH- CH_2 N-Tyr-Gly-ψ-CH_2-S)Phe-Leu CH_2=CH- CH_2
TIPP	H-Tyr-Tic-Phe-Phe-OH
TIPP[ψ]	H-Tyr-Ticψ[CH_2-NH]Phe-Phe-OH
DALCE	Tyr-D-Ala-Gly-Phe-Leu-Cys

Table 1. (Cont.). δ (OP_1) Opioid receptor ligands and chemical structures

δ (OP_1) Opioid receptor ligands	
Antagonists Non-peptides	Chemical structure
Naltridone (NTI)	17-cyclo-propylmethyl-6,7-dehydro-4,5-epoxy-3,14-dihydroxy-6,7,2',3'-indolmorphinan
BNTX	7-benzylidenenaltrexone
Naltrindole 5'-isothiocyanate (5'NTI)	
Naltriben (NTB)	naltrindole benzofuran
N-benzylnaltridole (BNTI)	

After **DHAWAN** et al. (1996)

Table 2. κ (OP_2) Opioid receptor ligands and chemical structures

OP_2 (κ) Opioid receptor ligands	
Agonists Peptides	Chemical structure
Dyn(1-11)	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-D-Pro-Lys
E-2078	[N-methyl-Tyr,N-methyl-Arg,D-Leu]dynorphin-A(1-8) ethylamide
Dynorphin la	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Tyr-Leu-Phe-Asn-Gly-Pro
Agonists Non-peptides	
Ketocyclazocine	3-(cyclopropylmethyl)-8-keto-1,2,3,4,5,6-hexahydro-6,11-dimethyl-2,6-methano-3-benzazocin-8-ol
Ethylketocyclazocine	3-(cyclopropylmethyl)-8-keto-1,2,3,4,5,6-hexahydro-6-methyl-11-ethyl-2,6-methano-3-benzazocin-8-ol
Bremazocine	(±)4-ethyl-1,2,3,4,5,6-hexahydro-3-[(1-hydroxyclopropyl)-Methyl)]-11,11-dimethyl-2,6-methano-3-benzazocin-8-ol
Tifluadom	(±)-N-[(5-(O-fluorophenyl)-2,3-dihydro-1-methyl]-1H-1,4-Benzodiazepin-2yl)methyl]-3-thiophenecarboxamide
U-50,488	trans-3,4-dichloro-N-methyl-N[2-(1-pyrrolidinyl)-cyclo-hexyl]-Benzeneacetamide

Table 2. (Cont.). κ (OP_2) Opioid receptor ligands and chemical structures

OP_2 (κ) Opioid receptor ligands	
Agonists Non-peptides	Chemical structure
U-69,593	(5α,7α,8β)-(-)-N-methyl-N[7-(1-pyrrolidinyl)-oxaspirol(4,5)dec-8-yl]-phenyl-benzeneacetamide
U-62,066 (Spiradoline)	(5α,7α,8β)-(±)-3,4-dichloro-N-[7-(1--pyrrolidinyl)-oxaspirol(‘,5)dec –8-yl]methan sulfonate
PD 117302	(±)-trans-N-methyl-N[2-1-pyrrolidinyl)-cyclohexyl]benzo[b]thiophene-4-acetamide
CI-977 (PD 129290,Enadoline)	(5R)-(5α,7α,8β)-N-methyl-N[7-(1-pyrrolidinyl)-1-oxaspirol(4,5)dec –8-yl]thiophene-4-acetamide
ICI 197067	(2S)-N-[2-(N-methyl-3,4-dichlorophenylacetamido)-3-methylbutyl]-pyrrolidine
ICI 199441	2-(3,4-dichlorophenyl- N-methyl-N-[(1S)-1-phenyl-2-(1- pyrrolidinyl)-ethyl]acetamide
ICI 204448	2-[3-(1-3,4-dichlorophenyl-N-methylacetamido)-2-Pyrrolidinoethyl)-phenoxy]acetic acid
EMD 61753	N-methyl-N-[(1S)-1-phenyl-2((3S)-3-hydroxypyrrolidine-1-yl)-ethyl]-2,2-diphenylacetamide
EMD 60400	N-methyl-N-[(1S)-1-phenyl-2((3S)-3-hydroxypyrrolidine-1-yl)-ethyl]-2-amino-phenylacetamide
GR 89696	methyl-4-[(3,4-dichlorophenyl)acetyl]-3-(1-pyrrolidinylmethyl)-1-piperazinecarboxylate
Antagonists Peptides	
None	
Antagonists Non-peptides	
TENA	(6β,6’β-[ethylenebis (oxyethyleneimino)]bis[17-(cyclopropylmethyl)-4,5α-epoxymorphinan-3,14-diol]
NorBN (Nor-BNI)	17,17’-bis(cyclo-propylmethyl)-6,6’,7,7’-tetrahydro-4,5,4’5’-diepoxy-6,6’-(imino)[7,7’-bimorphinan]-3,3’,14,14’-tetrol
UPHIT	(1S,2S)-trans-2-isothiocyanato-4,5-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetzmide
DIPPA	2-(3,4-dichlorophenyl)-N-methyl-N-[(1S)-1-(3-isothiocyanatophenyl)-2-(1-pyrrodinyl)ethylacetamide

After **DHAWAN** et al. (1996)

Table 3. μ (OP_3) Opioid receptor ligands and chemical structures

μ (OP_3) Opioid receptor ligands	
Agonists Peptides	Chemical structure
CDRI 82-205	Tyr-D-Ala-Gly-MePhe-Gly-NHC_3H_7
DAMGO	Tyr-D-Ala-Gly-MePhe-Gly-ol
FK 33-824	Tyr-D-Ala-Gly-MePhe-Gly-Met(O)-ol
PL 017	Tyr-Pro-τ-MePhe-D-Pro-NH_2
Dermorphin	Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2
Morphiceptin	Tyr-Pro-Phe-Pro-NH_2
TRIMU-5	Tyr-D-Ala-Gly-NH-$(CH_2)_2$-$CH(CH_3)_2$
Agonists Non-peptides	
Sufetanyl	N-[4-(methoxymethyl)-1-[2-(2-thienyl)ethyl]-4-piperidinyl]-N-phenyl-propanamide
Ohmefentanyl	N-[1-(β-hydroxy-β-phenethyl)-3-methyl-4-piperidyl]-N-phenylpropionamide
Etonitazene	2[(4-ethoxyphenyl)methyl]-N,N-diethyl-5-nitro-1H-benzimidazole-1-ethan-amine
Meptazinol	m-(3-ethyl-1-methyl-hexahydro-1-H-azepin-3-yl)phenol
Antagonists Peptides	
CTOP	D-Phe-Cys-Tyr-D-Trp-Om-Thr-Pen-Thr-NH_2
CTP	D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2
SMS-201,995	D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol
CTAP	D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2
TCTOP (D-Tic-CTOP)	D-Tic-Cys-Tyr-D-Trp-Om-Thr-Pen-Thr-NH_2
Antagonists Non-peptides	
Naloxonazine	bis[5-α-4,5-epoxy-3,14-dihydroxy-17(2-propenyl)-Morphinan-6-ylidene]hydrazine
β-Funaltrexamine (β-FNA)	(E)-4[[5α,6β)-17-(cyclo-propylmethyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-yl]amino]-4-oxo-2-butenoic acid methyl ester
N-CPM-MET-CAMO	N-cyclopropylmethylnor-5β-methyl-14 (ρ-nitrocinnamoylamino)-7,8-dihydromorphinone
MET-CAMO	5β-methyl-14β(p-nitrocinnamoylamino)-7,8-dihydromorphinone

After **DHAWAN** et al. (1996)

Table 4. N/OFQ (OP_4) Opioid receptor ligands and chemical structures

Agonist Peptides	Chemical structure
N/OFQ-(1-13)NH_2	Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-NH_2
Agonist Non-peptides	
R0646198	(1S,3As)-8-(2,3,3a,4.5.6-hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triazaspirol[4,5]decan-4-one
Antagonist Non-peptides	
J113397	1-[(3R,4R)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2H-benzimidazol-2-one

After **DHAWAN** et al. (1996); **ALEXANDER** et al. (2004)

Table 5. Amino acid sequences of some opioid peptides

Peptide	Amino acid sequence
[Leu^5]-enkephalin	**Tyr-Gly-Gly-Phe-Leu**
[Met^5]-enkephalin	**Tyr-Gly-Gly-Phe-Met**
MERF (heptapeptide)	**Tyr-Gly-Gly-Phe-Met**-Arg^6-Phe^7
MERGL(octapeptide)	**Tyr-Gly-Gly-Phe-Met**-Arg^6-Gly^7-Leu^8
Peptide E	**Tyr-Gly-Gly-Phe-Met**-Lys-Lys-Met-Asp-Glu-Leu-Tyr-Pro-Leu-Glu-Val-Glu-Glu-Ala-Asn-Gly-Gly-Glu-Val-Leu
BAM-22P	**Tyr-Gly-Gly-Phe-Met**-Lys-Lys-Met-Asp-Glu-Leu-Tyr-Pro-Leu-Glu-Val-Glu-Glu-Glu-Ala-Asn-Gly-Gly
Metrophamide	**Tyr-Gly-Gly-Phe-Met**-Arg-Val-NH_2
Peptide F	**Tyr-Gly-Gly-Phe-Met**-Lys-Lys-Met-Asp-Glu-Leu-Tyr-Pro-Leu-Glu-Val-Glu-Glu-Glu-Ala-Asn-Gly-Gly-Glu-Val Leu-Gly-Lys-Arg-Tyr-Gly-Gly-Phe-Met
Dynorphin A	**Tyr-Gly-Gly-Phe-Leu**-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln
α-Neoendorphin	**Tyr-Gly-Gly-Phe-Leu**-Arg-Lys-Tyr-Pro-Lys
β-Neoendorphin	**Tyr-Gly-Gly-Phe-Leu**-Arg-Lys-Tyr-Pro
Dynorphin B (Rimorphin)	**Tyr-Gly-Gly-Phe-Leu**-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr
Leumorphin (DynorphinB-29)	**Tyr-Gly-Gly-Phe-Leu**-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr Arg-Ser-Gln-Gln-Pro-Asn-Ala-Tyr-Tyr-Glu-Glu-Leu-Phe Asp-Val
Dynorphin AB-32	**Tyr-Gly-Gly-Phe-Leu**-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys Trp-Asp-Asn-Gln-Lys-Arg-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr
β-Endorphin	**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-His-Lys-Lys-Gly-Gln
Endomorphin-1	Tyr-Pro-Trp-Phe-NH_2
Endomorphin-2	Tyr-Pro-Phe-Phe-NH_2
OrphaninFQ/N	**Phe-Gly-Gly-Phe**-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Glp
OrphaninFQ2	Phe-Ser-Glu-Phe-Met-Arg-Gln-Tyr-Leu-Val-Leu-Ser-Met-Gln-Ser-Ser-Gln
Nocistatin	Thr-Glu-Pro-Gly-Leu-Glu-Glu-Val-Gly-Glu-Ile-Glu-Gln-Lys-Gln-Leu-Gln

Modified from **HUNTER** et al. (1994); **CALO** et al.(2002)

Table 6. Plasma osmolality, arginine vasotocin and testosterone in chickens

Chicken No.	Sex	Age	Status	Osmolality	AVT	T
				(mmol/l)	(pg/ml)	(ng/ml)
16	Female	26-38-week	Dehydration	378	64.52	-
17	Female	26-38-week	Dehydration	375	113.34	-
19	Female	26-38-week	Dehydration	374	89.98	-
26	Female	26-38-week	Dehydration	346	116.70	-
27	Female	26-38-week	Dehydration	349	56.86	-
28	Female	26-38-week	Dehydration	351	43.12	-
29	Female	26-38-week	Dehydration	350	54.13	-
30	Male	26-38-week	Dehydration	346	71.65	-
31	Male	26-38-week	Dehydration	343	48.52	-
32	Male	26-38-week	Dehydration	349	59.36	-
33	Male	26-38-week	Dehydration	344	38.86	-
34	Male	26-38-week	Dehydration	348	79.98	-
35	Male	26-38-week	Dehydration	341	41.26	-
36	Female	26-38-week	Control	321	3.65	-
37	Female	26-38-week	Control	320	3.59	-
38	Female	26-38-week	Control	322	3.14	-
39	Female	26-38-week	Control	322	10.08	-
40	Female	26-38-week	Control	322	4.62	-
41	Female	26-38-week	Control	306	5.59	-
46	Male	26-38-week	Control	321	11.21	-
47	Male	26-38-week	Control	326	21.18	-
48	Male	26-38-week	Control	321	15.24	-
49	Male	26-38-week	Control	314	13.92	-
50	Male	26-38-week	Control	316	12.31	-
51	Male	26-38-week	Control	318	27.45	-
52	Male	26-38-week	Control	319	18.92	-
53	Male	26-38-week	Castration	-	14.09	0.2
54	Male	26-38-week	Castration	-	8.40	0.2
55	Male	26-38-week	Castration	-	8.78	0.2
56	Male	26-38-week	Castration	-	11.35	0.2
57	Male	26-38-week	Castration	-	17.59	0.2
58	Male	26-38-week	Castration	299	28.61	0.72
59	Male	26-38-week	Castration	302	25.72	0.91
60	Male	26-38-week	Castration	305	17.73	0.85
61	Male	26-38-week	Castration	308	7.08	0.2
62	Male	26-38-week	Castration	306	10.35	0.83
63	Male	26-38-week	Sham-operated	-	11.81	5.34
64	Male	26-38-week	Sham-operated	-	18.56	3.56
65	Male	26-38-week	Sham-operated	-	16.04	1.82
66	Male	26-38-week	Sham-operated	-	14.63	1.49
69	Male	26-38-week	Sham-operated	-	23.31	0.71

Table 6. (Cont.) Plasma osmolality, arginine vasotocin and testosterone in chickens

Chicken No.	Sex	Age	Status	Osmolality	AVT	T
				(mmol/l)	(pg/ml)	(ng/ml)
71	Male	26-38 weeks	Castrated + T & NLTRX	310	14.79	34.3
72	Male	26-38 weeks	Castrated + T & NLTRX	299	7.59	24.8
73	Male	26-38 weeks	Castrated + T & NLTRX	309	40.74	32
74	Male	26-38 weeks	Castrated + T & NLTRX	312	21.15	32.8
75	Male	26-38 weeks	Castrated + T & NLTRX	310	19.31	42.5
76	Male	26-38 weeks	Castrated + T & NLTRX	316	21.52	40.6
77	Male	26-38 weeks	Castrated + T & NLTRX	307	15.69	30.7
78	Male	26-38 weeks	Castrated + T & NLTRX	293	12.69	32.6
79	Male	26-38 weeks	Castrated + T & NLTRX	306	24.21	19.7
80	Male	26-38 weeks	Castrated + T & NLTRX	295	32.72	24.1
81	Male	26-38 weeks	Castrated + T & NLTRX	316	22.33	26.1
82	Female	10-day	Control	275	16.68	-
83	Female	10-day	Control	278	14.97	-
84	Female	10-day	Control	280	31.47	-
85	Male	10-day	Control	271	9.86	-
86	Male	10-day	Control	270	47.75	-
87	Male	10-day	Control	274	37.23	-
88	Female	10-day	Control	272	95.34	-
89	Female	10-day	Control	301	43.39	-
90	Male	10-day	Control	272	7.99	-
91	Female	10-day	Control	276	19.00	-
92	Male	10-day	Control	278	46.10	-
93	Male	10-day	Control	287	25.04	-
94	Male	10-day	Dehydration	312	150.21	-
95	Male	10-day	Dehydration	312	93.49	-
96	Female	10-day	Dehydration	314	137.2	-
97	Female	10-day	Dehydration	318	73.52	-
98	Male	10-day	Dehydration	326	137.23	-
99	Male	10-day	Dehydration	321	124.69	-
100	Female	10-day	Dehydration	311	93.03	-
101	Female	10-day	Dehydration	312	116.02	-
102	Male	10-day	Dehydration	309	123.02	-
103	Male	10-day	Dehydration	318	46.25	-
104	Female	10-day	Dehydration	308	64.13	-
105	Female	10-day	Dehydration	328	101.08	-

Table 6. (Cont.) Plasma osmolality, arginine vasotocin and testosterone in chickens

Chicken No.	Sex	Age	Status	Osmolality	AVT	T
				(mmol/l)	(pg/ml)	(ng/ml)
106	Male	26-38-week	Castrated + T	307	10.37	12.7
107	Male	26-38-week	Castrated + T	312	26.69	35.4
108	Male	26-38-week	Castrated + T	302	-	-
109	Male	26-38-week	Castrated + T	298	25.81	22.1
110	Male	26-38-week	Castrated + T	305	18.43	17.5
111	Male	26-38-week	Castrated + T	307	21.61	18.6
112	Male	26-38-week	Castrated + T	306	18.10	18
113	Male	26-38-week	Castrated + T	306	16.84	21.1
114	Male	10-week	Control	310	25.92	-
115	Male	10-week	Control	304	16.17	-
116	Male	10-week	Control	297	6.2	-
117	Male	10-week	Control	305	10.05	-
118	Male	10-week	Control	305	12.49	-
119	Male	10-week	Control	297	16.65	-
120	Female	10-week	Control	297	34.75	-
121	Female	10-week	Control	310	23.82	-
122	Female	10-week	Control	296	29.87	-
123	Female	10-week	Control	291	8.36	-
124	Female	10-week	Control	300	17.75	-
125	Female	10-week	Control	306	11.42	-
126	Male	10-week	Dehydration	333	37.03	-
127	Female	10-week	Dehydration	340	86.74	-
128	Male	10-week	Dehydration	342	43.01	-
129	Female	10-week	Dehydration	336	64.26	-
130	Male	10-week	Dehydration	333	55.62	-
132	Female	10-week	Dehydration	334	68.42	-
133	Male	10-week	Dehydration	333	53.10	-
134	Male	10-week	Dehydration	335	47.26	-
135	Female	10-week	Dehydration	339	60.53	-
136	Female	10-week	Dehydration	325	38.04	-
137	Male	10-week	Dehydration	329	60.49	-
138	Female	10-week	Dehydration	333	66.97	-
139	Female	10-week	Dehydration	335	38.59	-

Table 7. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the control 10-day-old female chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
FC	83	2	0.937	0.5067	1.83	0	0.4581	1.236	0
	84	2	0.96465	0.3391	1.041	0.9817	0.3365	1.035	0.9974
	88	1.97	0.96324	0.4038	0.7297	1.02	0.4045	0.7465	1.016
	89	1.97	0.94131	0.3411	0.6773	0.9766	0.3287	0.5923	1.096
	91	2.01	0.94783	0.4126	2.837	0	0.3722	2.384	0
	Mean	**1.99**	**0.9508**	**0.4007**	**1.423**	**0.5957**	**0.38**	**1.1988**	**0.6219**
	SD	0.0187	0.0126	0.0684	0.9148	0.544	0.0531	0.708	0.5689
	SEM	0.0083	0.0056	0.0306	0.4091	0.2433	0.0238	0.3166	0.2544
LS	83	2	0.93399	0.3374	1.583	0	0.3119	1.074	0
	84	1.99	0.9792	0.197	0.7769	1.108	0.2022	0.8683	1.015
	88	1.97	0.96229	0.3179	0.8906	0.8911	0.2944	0.6771	1.013
	89	2	0.98619	0.2438	0.6347	0.9068	0.2369	0.5584	0.9668
	91	1.76	0.93183	0.4153	1.746	0	0.3984	1.565	0
	Mean	**1.944**	**0.9587**	**0.3023**	**1.1262**	**0.5812**	**0.2888**	**0.9486**	**0.599**
	SD	0.1036	0.0251	0.0848	0.503	0.5374	0.0754	0.3963	0.5471
	SEM	0.0463	0.0112	0.0379	0.2249	0.2403	0.0337	0.1772	0.2447
HYP	82	1.73	0.97916	0.3413	0.7127	0.9728	0.3394	0.6958	0.9863
	83	1.97	0.97917	0.253	1.724	0	0.243	1.625	0
	84	2	0.97052	0.2486	0.8452	1.077	0.2572	0.9667	0.983
	88	2.01	0.97187	0.2013	0.6849	0.9181	0.1952	0.6014	0.9824
	89	1.97	0.96265	0.2438	0.6683	1.079	0.256	0.8095	0.9521
	91	1.76	0.93137	0.3311	2.292	0	0.2873	1.62	0
	Mean	**1.907**	**0.9658**	**0.2698**	**1.1545**	**0.6745**	**0.263**	**1.0531**	**0.6506**
	SD	0.1266	0.018	0.0547	0.6879	0.5261	0.048	0.4576	0.5041
	SEM	0.0517	0.0073	0.0223	0.2808	0.2148	0.0196	0.1868	0.2058

Table 8. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the control 10-day-old female chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
AMY	82	1.73	0.94904	0.3308	0.9985	1.075	0.3781	1.458	0.8182
	83	1.78	0.7887	0.2954	2.515	0	0.271	2.091	0
	84	1.74	0.8067	0.3425	1.073	0.9695	0.3925	1.909	0.7807
	88	1.76	0.94126	0.2769	0.9294	1.025	0.2892	1.071	0.9496
	91	2	0.93314	0.424	2.244	0	0.3698	1.541	0
	Mean	**1.802**	**0.8838**	**0.3339**	**1.552**	**0.6139**	**0.3401**	**1.614**	**0.5097**
	SD	0.1123	0.079	0.0569	0.7632	0.5617	0.0558	0.3997	0.4695
	SEM	0.0502	0.0353	0.0254	0.3413	0.2512	0.0249	0.1788	0.21
HPC	82	1.74	0.96336	0.3703	0.7075	1.245	0.3937	0.7332	1.006
	83	1.76	0.97654	0.3256	1.492	0	0.3245	1.747	0
	84	1.99	0.99545	0.3261	1.424	0.999	0.3278	1.44	0.9919
	88	1.73	0.98536	0.3569	0.955	0.9903	0.3546	0.9309	1.003
	89	1.74	0.98633	0.2512	0.8472	0.9387	0.2392	0.6041	1.057
	91	1.77	0.90967	0.4254	1.725	0	0.4366	1.524	0
	Mean	**1.7883**	**0.9695**	**0.3426**	**1.1918**	**0.6955**	**0.3461**	**1.1632**	**0.6763**
	SD	0.0999	0.0312	0.0579	0.4093	0.5491	0.0675	0.4688	0.5244
	SEM	0.0408	0.0127	0.0236	0.1671	0.2242	0.0275	0.1914	0.2141
ST	82	1.75	0.99105	0.4636	0.7164	1.074	0.4762	0.797	0.9952
	83	1.99	0.94353	0.3248	2.915	0	0.2742	1.861	0
	84	2	0.99373	0.2607	0.9363	1.044	0.2661	1.009	0.9964
	89	2.01	0.93204	0.7358	2.463	0.8381	0.8262	3.189	0.7833
	91	2.01	0.94356	0.4134	2.002	0	0.3683	1.409	0
	Mean	**1.952**	**0.9608**	**0.4397**	**1.8065**	**0.5912**	**0.4422**	**1.653**	**0.555**
	SD	0.1132	0.0292	0.1832	0.9544	0.5473	0.2309	0.9499	0.514
	SEM	0.0506	0.0131	0.0819	0.4268	0.2448	0.1033	0.4248	0.2299

Table 9. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the dehydrated 10-day-old female chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
FC	96	1.73	0.92574	0.3792	0.9642	0.9799	0.3871	1.046	0.9366
	100	2.01	0.98777	0.2491	0.6755	0.9219	0.2426	0.597	0.9925
	101	1.96	0.96578	0.2347	0.5994	0.8564	0.2281	0.5165	0.9336
	104	2	0.93044	0.2745	0.9934	0.8912	0.2566	0.7881	1.018
	105	1.97	0.91096	0.3535	0.8502	0.7522	0.3183	0.5087	0.9456
	Mean	**1.934**	**0.9441**	**0.2982**	**0.8165**	**0.8803**	**0.2865**	**0.6913**	**0.9653**
	SD	0.1159	0.0316	0.0645	0.1741	0.0848	0.0659	0.228	0.0379
	SEM	0.0518	0.0141	0.0288	0.0779	0.0379	0.0295	0.102	0.0169
LS	97	1.98	0.96901	0.2477	0.8387	0.8761	0.2436	0.7709	0.9221
	100	2	0.95352	0.21	0.779	0.9184	0.202	0.6726	1.032
	101	1.75	0.96475	0.2193	0.2815	0.6909	0.2099	0.2338	1.03
	105	1.97	0.96283	0.157	0.7276	0.8527	0.15	0.5926	0.9713
	Mean	**1.925**	**0.9625**	**0.2085**	**0.6567**	**0.8345**	**0.2014**	**0.5675**	**0.9889**
	SD	0.1173	0.0065	0.0379	0.2542	0.0995	0.0387	0.2341	0.0527
	SEM	0.0587	0.0032	0.0189	0.1271	0.0498	0.0194	0.117	0.0263
HYP	100	1.97	0.97119	0.1786	0.8767	0.9083	0.1675	0.6077	1.125
	101	1.97	0.98653	0.2143	0.3902	0.9284	0.2131	0.4078	0.9531
	104	1.76	0.95098	0.2485	0.7017	0.8277	0.2389	0.5856	0.916
	105	1.76	0.95576	0.1598	0.5455	1.229	0.162	0.5912	1.109
	Mean	**1.865**	**0.9661**	**0.2003**	**0.6285**	**0.9734**	**0.1954**	**0.5481**	**1.0258**
	SD	0.1212	0.0161	0.0393	0.2087	0.1759	0.037	0.094	0.1066
	SEM	0.0606	0.008	0.0196	0.1043	0.088	0.0185	0.047	0.0533

Table 10. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the dehydrated 10-day-old female chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
AMY	96	2.01	0.94186	0.2196	0.674	0.7609	0.2066	0.4497	0.9142
	97	1.75	0.96492	0.1767	0.9971	1.079	0.1787	1.047	1.034
	100	1.75	0.98373	0.1753	0.7364	1.057	0.1755	0.7495	1.051
	101	1.95	0.95787	0.2479	0.7596	0.7264	0.2178	0.5057	1.113
	104	2	0.97526	0.1544	1.017	0.9684	0.1753	0.5423	0.956
	105	1.73	0.97654	0.1512	0.4114	1.067	0.1508	0.4081	1.089
	Mean	**1.865**	**0.9667**	**0.1875**	**0.7659**	**0.9431**	**0.1841**	**0.6171**	**1.0262**
	SD	0.135	0.0152	0.0384	0.2243	0.1597	0.0242	0.2417	0.077
	SEM	0.0551	0.0062	0.0157	0.0916	0.0652	0.0098	0.0987	0.0314
HPC	96	1.75	0.95168	0.3331	0.4665	0.5019	0.2966	0.2994	0.7967
	100	2.01	0.98506	0.2629	0.6792	0.9504	0.2617	0.661	0.9629
	101	2.01	0.99089	0.2415	0.3782	1.041	0.2433	0.3955	1.002
	104	1.75	0.96665	0.306	0.5605	0.828	0.2217	0.5935	1.185
	105	1.98	0.98321	0.1939	0.3553	0.8976	0.1914	0.3267	0.9705
	Mean	**1.9**	**0.9755**	**0.2675**	**0.4879**	**0.8438**	**0.2429**	**0.4552**	**0.9834**
	SD	0.1375	0.0161	0.0545	0.1341	0.2063	0.0398	0.1627	0.1382
	SEM	0.0615	0.0071	0.0244	0.06	0.0923	0.0178	0.0727	0.0618
ST	96	2	0.95501	0.2743	0.7713	1.086	0.2839	0.619	0.9255
	100	1.75	0.98528	0.2587	1.162	0.8823	0.2377	0.7738	1.042
	101	1.99	0.97148	0.2389	0.5326	1.086	0.2355	0.5655	1.198
	104	1.99	0.95905	0.2407	0.7566	0.7752	0.262	0.4923	0.9898
	105	1.78	0.94879	0.1786	0.717	0.717	0.1582	0.4752	1.023
	Mean	**1.902**	**0.9639**	**0.2382**	**0.7879**	**0.9093**	**0.2355**	**0.5852**	**1.0357**
	SD	0.1256	0.0145	0.0363	0.2299	0.1719	0.0475	0.1202	0.101
	SEM	0.0562	0.0065	0.0163	0.1028	0.0769	0.0212	0.0538	0.0452

Table 11. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the control 10-day-old male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
FC	85	1.98	0.95116	0.3906	0.7209	1.358	0.4163	0.7899	0.9829
	86	1.97	0.96382	0.3648	0.9629	0.8394	0.3431	0.7531	0.9551
	87	1.99	0.95894	0.2979	0.7039	0.8394	0.2797	0.5382	0.9816
	90	2	0.98125	0.3918	0.5152	0.8765	0.3828	0.4563	0.9555
	92	2	0.95868	0.3318	1.595	0.9386	0.3065	1.27	1.049
	93	1.98	0.97014	0.2161	0.7709	0.898	0.214	0.729	0.9427
	Mean	**1.9867**	**0.964**	**0.3322**	**0.8781**	**0.9583**	**0.3237**	**0.7561**	**0.9778**
	SD	0.0121	0.0105	0.0674	0.3793	0.1994	0.0731	0.284	0.0384
	SEM	0.0049	0.0042	0.0275	0.1549	0.0814	0.0298	0.116	0.0157
LS	85	1.72	0.96252	0.2814	0.5502	1.226	0.2877	0.6153	1.047
	86	1.72	0.93093	0.3416	0.5664	0.7933	0.333	0.4936	0.8754
	87	1.99	0.92789	0.2941	0.6348	0.7792	0.2733	0.4593	0.9399
	90	1.98	0.92111	0.2802	0.5646	0.782	0.2629	0.4062	1.018
	92	1.99	0.97214	0.1702	0.8666	0.9523	0.1652	0.7755	1.035
	93	2.01	0.92759	0.2242	1.166	0.8184	0.2046	0.8349	0.9685
	Mean	**1.9017**	**0.9404**	**0.2653**	**0.7248**	**0.8919**	**0.2545**	**0.5975**	**0.9806**
	SD	0.1411	0.0213	0.0598	0.2466	0.1762	0.0603	0.176	0.0658
	SEM	0.0576	0.0087	0.0244	0.1007	0.0719	0.0246	0.0718	0.0269
HYP	85	1.74	0.91824	0.3183	1.985	0.9544	0.2961	1.226	1.05
	86	1.75	0.92045	0.2719	0.9755	0.8278	0.2605	0.8196	0.9107
	87	1.75	0.97629	0.2496	0.5123	0.8728	0.2457	0.4672	0.9228
	90	2.01	0.96896	0.2739	0.6426	1.575	0.2822	0.7447	1.125
	92	1.99	0.99366	0.2263	0.9058	0.9831	0.2251	0.8872	0.9959
	93	1.97	0.98541	0.2115	1.089	1.017	0.2157	1.167	0.9738
	Mean	**1.8683**	**0.9605**	**0.2586**	**1.0184**	**1.0384**	**0.2542**	**0.8853**	**0.9964**
	SD	0.1339	0.033	0.0383	0.5198	0.2721	0.0316	0.2808	0.0808
	SEM	0.0547	0.0135	0.0156	0.2122	0.1111	0.0129	0.1146	0.033

Table 12. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the control 10-day-old male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
AMY	86	1.5	0.97821	0.296	0.8023	0.9735	0.288	0.7181	1.082
	87	1.71	0.96165	0.2082	0.5857	0.9031	0.2051	0.5423	0.956
	90	2	0.93979	0.2239	0.6892	0.9813	0.223	0.6807	0.9959
	92	1.5	0.98333	0.1968	0.9075	1.015	0.1942	0.8704	1.056
	93	1.99	0.9415	0.2476	1.691	0.9016	0.2301	1.333	0.9943
	Mean	**1.74**	**0.9609**	**0.2345**	**0.9351**	**0.9549**	**0.2281**	**0.8289**	**1.0168**
	SD	0.2481	0.0202	0.0393	0.4394	0.0504	0.0364	0.305	0.0511
	SE	0.11	0.009	0.0176	0.1965	0.0226	0.0163	0.1364	0.0228
HPC	86	2.01	0.95554	0.283	0.7707	0.8978	0.2797	0.7268	0.9286
	87	1.75	0.9764	0.2184	0.5479	1.393	0.2217	0.5935	1.185
	90	1.75	0.92776	0.2517	0.7349	0.7511	0.2478	0.4588	0.7876
	92	1.98	0.98341	0.2058	0.9724	0.9542	0.2002	0.8782	1.018
	Mean	**1.8725**	**0.9608**	**0.2397**	**0.7565**	**0.999**	**0.2374**	**0.6643**	**0.9798**
	SD	0.142	0.025	0.0347	0.174	0.2762	0.0343	0.1797	0.1665
	SEM	0.071	0.0125	0.0174	0.087	0.1381	0.0171	0.0899	0.0832
ST	85	1.96	0.94803	0.3815	0.8996	0.9408	0.3823	0.9016	0.9366
	86	2.01	0.95124	0.2856	0.8347	0.8186	0.2711	0.6667	0.9259
	87	1.99	0.98704	0.2687	0.5534	0.9101	0.262	0.4923	0.9898
	90	1.98	0.97373	0.2764	0.5451	0.8809	0.2709	0.489	0.9565
	92	2	0.96875	0.2427	1.209	0.8808	0.2246	0.7702	1.024
	93	1.74	0.94084	0.2615	0.8609	1.031	0.2588	0.8378	1.072
	Mean	**1.9467**	**0.9616**	**0.2861**	**0.8171**	**0.9104**	**0.2783**	**0.6929**	**0.9841**
	SD	0.1027	0.0177	0.049	0.2474	0.0716	0.0538	0.1749	0.0561
	SEM	0.0419	0.0072	0.02	0.101	0.0292	0.0219	0.0714	0.0229

Table 13. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the dehydrated 10-day-old male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
FC	94	1.98	0.94304	0.3212	0.7094	0.9008	0.3136	0.6472	0.9576
	95	1.97	0.92152	0.5226	1.267	0.9443	0.5187	1.237	0.9559
	98	2	0.95918	0.3509	1.401	0.8591	0.3261	1.071	0.9829
	99	2.01	0.98354	0.2897	1.118	1.033	0.291	1.143	1.022
	102	1.99	0.99135	0.2221	0.8733	0.999	0.2229	0.8858	0.9915
	103	2	0.97319	0.2687	0.4819	0.9817	0.2689	0.4839	0.9852
	Mean	**1.9917**	**0.962**	**0.3292**	**0.9751**	**0.953**	**0.3235**	**0.9113**	**0.9825**
	SD	0.0147	0.0263	0.1045	0.3496	0.0647	0.1023	0.2961	0.0244
	SEM	0.006	0.0107	0.0427	0.1427	0.0264	0.0418	0.1209	0.01
LS	94	1.98	0.96573	0.2374	0.6352	0.8667	0.2304	0.5596	0.9392
	95	1.99	0.96639	0.3422	1.005	1.096	0.3564	0.9434	0.9879
	98	1.99	0.93211	0.2398	1.682	0.8459	0.2138	1.077	1.024
	99	1.99	0.95695	0.2205	1.087	0.978	0.2214	1.101	0.9708
	102	1.98	0.97229	0.1364	0.5423	1.007	0.1333	0.4274	1.152
	103	1.75	0.98214	0.2875	0.5662	1.044	0.2892	0.4931	1.022
	Mean	**1.9467**	**0.9626**	**0.244**	**0.9196**	**0.9729**	**0.2408**	**0.7669**	**1.016**
	SD	0.0965	0.0171	0.0689	0.4392	0.0988	0.0755	0.3073	0.0739
	SEM	0.0394	0.0069	0.0281	0.1793	0.0403	0.0308	0.1255	0.0302
HYP	94	1.76	0.92528	0.2004	0.8414	0.8568	0.1902	0.692	0.9658
	95	1.76	0.93095	0.2234	0.9896	0.9147	0.2167	0.7532	0.9758
	98	1.97	0.95629	0.1543	1.449	1.005	0.1551	1.465	0.9947
	99	2	0.9125	0.2427	2.044	0.6915	0.1579	0.7963	1.251
	102	1.96	0.97234	0.1769	1.023	0.953	0.1761	0.9119	1.017
	103	1.75	0.98301	0.2116	1.077	0.9173	0.2034	0.6135	1.002
	Mean	**1.8667**	**0.9467**	**0.2016**	**1.2373**	**0.8897**	**0.1832**	**0.872**	**1.0344**
	SD	0.1213	0.0281	0.032	0.4439	0.1087	0.0247	0.3073	0.1077
	SE	0.0495	0.0115	0.0131	0.1812	0.0444	0.0101	0.1255	0.044

Table 14. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the dehydrated 10-day-old male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
AMY	94	1.5	0.97979	0.2237	0.8054	0.9941	0.2249	0.8257	0.9802
	95	1.96	0.98552	0.2354	1.076	0.986	0.2347	1.067	0.9926
	98	2.01	0.95282	0.1412	1.833	0.8905	0.1313	1.333	0.9996
	99	1.5	0.95049	0.2595	1.147	0.8737	0.2431	0.9076	0.9821
	102	1.99	0.96219	0.1746	0.8943	0.9906	0.1746	0.8975	0.9915
	103	1.99	0.95186	0.2327	0.8647	0.9521	0.226	0.7697	1.016
	Mean	**1.825**	**0.9638**	**0.2112**	**1.1034**	**0.9478**	**0.2058**	**0.9668**	**0.9937**
	SD	0.2522	0.0153	0.0442	0.3807	0.0533	0.0436	0.2055	0.0131
	SEM	0.103	0.0062	0.0181	0.1554	0.0218	0.0178	0.0839	0.0053
HPC	94	1.76	0.98608	0.2345	0.9113	0.957	0.2271	0.8152	1.036
	95	1.97	0.9927	0.2855	0.8485	0.9778	0.284	0.8304	0.9915
	98	1.99	0.97394	0.2572	1.804	0.9459	0.2447	1.551	1.025
	99	1.99	0.9502	0.1446	0.9646	0.828	0.1337	0.6354	0.9819
	102	1.98	0.94069	0.1759	0.5423	1.066	0.1797	0.5449	0.9683
	103	1.98	0.96687	0.2492	0.339	0.87	0.2472	0.3185	0.8997
	Mean	**1.945**	**0.9684**	**0.2245**	**0.9016**	**0.9408**	**0.2194**	**0.7826**	**0.9837**
	SD	0.0909	0.0202	0.0534	0.5034	0.0837	0.054	0.4213	0.0486
	SEM	0.0371	0.0082	0.0218	0.2055	0.0342	0.022	0.172	0.0198
ST	94	1.77	0.97207	0.2947	0.7197	0.9139	0.2912	0.6864	0.9407
	95	1.98	0.98241	0.338	0.9127	0.9222	0.3253	0.7888	1.008
	98	1.99	0.95208	0.2679	1.597	0.9778	0.2621	1.344	1.018
	99	2.01	0.96978	0.2269	0.7819	0.9812	0.2211	0.7102	1.071
	102	1.95	0.92062	0.1397	1.253	0.8436	0.1251	0.7163	1.015
	103	1.98	0.9817	0.2427	0.4108	1.133	0.2446	0.4348	1.082
	Mean	**1.9467**	**0.9631**	**0.2517**	**0.9459**	**0.962**	**0.2449**	**0.7801**	**1.0224**
	SD	0.0887	0.0235	0.0675	0.4204	0.0978	0.069	0.3017	0.0507
	SEM	0.0362	0.0096	0.0276	0.1716	0.0399	0.0282	0.1232	0.0207

Table 15. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the control 10-week-old female chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
FC	120	2	0.8462	0.7984	0.7808	0.2645	0.3637	0.2271	0.9707
	121	2.02	0.98424	0.3621	0.2421	0.9172	0.3593	0.229	0.9555
	122	1.78	0.93813	0.4813	0.2082	0.9244	0.4916	0.247	1.065
	123	1.99	0.96999	0.419	0.3051	0.8639	0.4173	0.2943	0.8771
	124	1.97	0.93279	0.4035	0.0382	0.8517	0.3919	0.03619	0.4452
	125	2	0.9305	0.4306	0.2029	1.013	0.4308	0.2057	1.006
	Mean	**1.96**	**0.9336**	**0.4825**	**0.2962**	**0.8058**	**0.4091**	**0.2065**	**0.8866**
	SD	0.0897	0.0481	0.1595	0.2533	0.2713	0.0494	0.0886	0.2249
	SEM	0.0366	0.0196	0.0651	0.1034	0.1107	0.0202	0.0362	0.0918
LS	120	2.01	0.90344	0.2559	0.4841	0.4323	0.198	0.3583	0.4337
	121	2.01	0.97412	0.2779	0.1623	0.8282	0.2755	0.1497	0.8825
	122	1.78	0.95812	0.2972	0.2407	0.8111	0.2877	0.1908	1.002
	123	2.01	0.9092	0.3026	0.4455	0.7343	0.2917	0.3966	0.8423
	124	1.98	0.96217	0.2971	0.2841	0.8135	0.2939	0.262	0.8628
	125	2	0.92304	0.3502	0.2179	1.241	0.348	0.2097	0.7471
	Mean	**1.965**	**0.9383**	**0.2968**	**0.3058**	**0.8101**	**0.2825**	**0.2612**	**0.7951**
	SD	0.0914	0.0301	0.0314	0.1299	0.2586	0.0484	0.0978	0.195
	SEM	0.0373	0.0123	0.0128	0.053	0.1056	0.0198	0.0399	0.0796
HYP	121	2.01	0.95608	0.2134	0.2251	0.8967	0.2123	0.2159	0.9269
	122	1.76	0.93227	0.2108	0.2845	0.6731	0.1995	0.2265	0.6801
	123	2.01	0.92332	0.2253	0.2892	0.9192	0.2233	0.2989	1.002
	125	1.77	0.92275	0.246	0.3294	0.7964	0.2368	0.2587	0.9646
	Mean	**1.8875**	**0.9336**	**0.2239**	**0.2821**	**0.8214**	**0.218**	**0.25**	**0.8934**
	SD	0.1415	0.0156	0.016	0.043	0.1123	0.0159	0.0373	0.1455
	SEM	0.0708	0.0078	0.008	0.0215	0.0562	0.0079	0.0187	0.0727

Table 16. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the control 10-week-old female chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
AMY	120	2	0.92796	0.2308	0.6709	0.9716	0.2319	0.6853	0.9587
	121	2.02	0.95451	0.1828	0.3475	0.7912	0.1779	0.2944	0.8701
	122	1.72	0.96605	0.1782	0.2997	0.7158	0.1672	0.2443	0.5497
	123	1.75	0.88667	0.2375	0.7965	0.5365	0.1648	0.3122	0.5985
	124	2.01	0.9474	0.2328	0.253	0.9131	0.2297	0.2258	0.605
	125	1.75	0.93657	0.2407	0.523	0.9113	0.2304	0.4255	1.112
	Mean	**1.875**	**0.9365**	**0.2171**	**0.4818**	**0.8066**	**0.2003**	**0.3646**	**0.7823**
	SD	0.1484	0.0278	0.0286	0.2191	0.1618	0.0335	0.172	0.231
	SEM	0.0606	0.0114	0.0117	0.0894	0.0661	0.0137	0.0702	0.0943
HPC	120	2	0.98407	0.3014	0.451	0.9866	0.304	0.4708	0.9551
	121	1.98	0.96476	0.3149	0.2785	0.8959	0.3115	0.2582	0.9541
	122	1.99	0.99391	0.2626	0.7508	0.9892	0.261	0.7323	1.002
	123	2	0.94376	0.301	0.3343	1.032	0.3001	0.3297	1.078
	124	2.01	0.95161	0.3355	0.3201	0.5248	0.309	0.2225	0.5762
	125	1.78	0.97931	0.3185	0.3924	1.03	0.3188	0.3968	1.025
	Mean	**1.96**	**0.9696**	**0.3057**	**0.4212**	**0.9098**	**0.3007**	**0.4017**	**0.9317**
	SD	0.0888	0.0195	0.0246	0.1723	0.1949	0.0205	0.1855	0.1803
	SEM	0.0362	0.008	0.0101	0.0704	0.0796	0.008	0.0757	0.0736
ST	120	2	0.9576	0.2778	0.2657	0.73	0.2708	0.2185	0.8253
	121	2.01	0.94127	0.2948	0.2524	0.7534	0.2869	0.2093	0.875
	122	1.97	0.94386	0.2531	0.32	1.114	0.2582	0.3657	1.002
	123	1.98	0.95437	0.327	0.3218	1.056	0.3313	0.4124	0.9616
	124	1.99	0.95566	0.3885	0.2749	0.7311	0.3786	0.227	0.8374
	125	1.76	0.92553	0.3125	0.3979	0.8866	0.3127	0.3939	0.8849
	Mean	**1.9517**	**0.9464**	**0.309**	**0.3054**	**0.8785**	**0.3064**	**0.3045**	**0.8977**
	SD	0.095	0.0122	0.0468	0.0536	0.1711	0.0444	0.0958	0.07
	SEM	0.0388	0.005	0.0191	0.0219	0.0699	0.0181	0.0391	0.0286

Table 17. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the dehydrated 10-week-old female chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
FC	127	2	0.97179	0.5314	0.4651	0.8129	0.5163	0.3953	0.9034
	129	1.99	0.98599	0.545	0.3979	0.8909	0.5354	0.3596	0.9667
	132	1.76	0.91603	0.4399	0.6403	0.9565	0.4409	0.6402	0.9475
	135	2	0.92902	0.315	0.4125	0.7606	0.2993	0.3131	0.9067
	136	2	0.95288	0.4351	0.3266	0.84	0.4326	0.3134	0.8657
	138	1.75	0.97095	0.4456	0.3004	0.8231	0.4415	0.276	0.861
	139	1.98	0.94731	0.5063	0.282	0.8278	0.5028	0.3209	0.8587
	Mean	**1.9257**	**0.9534**	**0.4598**	**0.4035**	**0.8445**	**0.4527**	**0.3741**	**0.9014**
	SD	0.1169	0.025	0.0782	0.1233	0.0626	0.0792	0.1234	0.0431
	SEM	0.0442	0.0094	0.0296	0.0466	0.0237	0.0299	0.0466	0.0163
LS	127	1.98	0.96839	0.4353	0.4408	0.8073	0.4267	0.3901	0.8709
	129	1.76	0.90237	0.3782	0.4642	0.4408	0.3325	0.2616	0.6428
	132	1.74	0.96481	0.3636	0.3363	0.2678	0.2979	0.1802	0.044
	136	1.77	0.94136	0.3422	0.4076	0.692	0.331	0.3259	0.7879
	138	1.73	0.94578	0.3702	0.1822	0.5043	0.3688	0.1342	0.5113
	139	2	0.9458	0.3531	0.4851	1.039	0.363	0.7415	0.9149
	Mean	**1.83**	**0.9448**	**0.3738**	**0.386**	**0.6252**	**0.3533**	**0.3389**	**0.6286**
	SD	0.1249	0.0235	0.0327	0.1126	0.2776	0.0441	0.2181	0.323
	SEM	0.051	0.0096	0.0133	0.046	0.1133	0.018	0.0891	0.1319
HYP	129	1.76	0.92401	0.2997	0.8002	0.7383	0.2813	0.4692	0.8795
	132	1.77	0.95239	0.2389	0.467	0.2337	0.2256	0.3641	0.275
	135	1.75	0.97639	0.2628	0.7232	0.9186	0.254	0.6328	0.9683
	136	1.99	0.96349	0.2657	0.4984	0.9027	0.2635	0.477	0.9341
	139	1.89	0.93425	0.2898	0.61	1.02	0.2993	0.7784	0.9117
	Mean	**1.832**	**0.9501**	**0.2714**	**0.6198**	**0.7627**	**0.2647**	**0.5443**	**0.7937**
	SD	0.105	0.0213	0.024	0.1427	0.3125	0.0279	0.1622	0.2918
	SEM	0.0469	0.0095	0.0107	0.0638	0.1397	0.0125	0.0726	0.1305

Table 18. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the dehydrated 10-week-old female chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
AMY	127	1.99	0.95962	0.1857	0.8706	0.8936	0.182	0.7987	0.9371
	129	1.96	0.97583	0.212	0.5031	1.12	0.2112	0.496	1.154
	132	1.77	0.97203	0.2668	0.4779	0.5826	0.2406	0.3313	0.5143
	135	1.75	0.96362	0.2607	0.6229	0.8655	0.2463	0.4884	0.9918
	138	2	0.97729	0.2901	0.3753	0.579	0.2658	0.2597	0.5129
	139	2.01	0.925	0.3092	1.099	1.078	0.3371	1.591	0.9161
	Mean	**1.9133**	**0.9622**	**0.2541**	**0.6581**	**0.8531**	**0.2472**	**0.6609**	**0.8377**
	SD	0.1201	0.0195	0.0469	0.2747	0.2332	0.053	0.4921	0.2645
	SEM	0.049	0.0079	0.0191	0.1121	0.0952	0.0216	0.2009	0.108
HPC	127	2	0.99799	0.3662	0.4282	0.94	0.3649	0.419	0.9548
	129	1.75	0.92115	0.4097	0.4566	0.8413	0.3956	0.3979	1.022
	132	2	0.95084	0.4029	0.2533	0.7245	0.3794	0.1958	0.5047
	135	2	0.97935	0.3032	0.5844	1.14	0.3125	0.6658	1.021
	136	1.77	0.92859	0.3342	0.3033	0.64	0.3239	0.2373	0.74
	138	1.78	0.9395	0.3737	0.1681	0.7056	0.3724	0.1683	0.7223
	139	1.98	0.95695	0.3474	0.2848	0.7576	0.3411	0.2958	0.8238
	Mean	**1.8971**	**0.9535**	**0.3625**	**0.3541**	**0.8213**	**0.3557**	**0.34**	**0.8269**
	SD	0.1226	0.0275	0.0377	0.1421	0.1711	0.0306	0.1725	0.189
	SEM	0.0463	0.0104	0.0143	0.0537	0.0647	0.0116	0.0652	0.0714
ST	127	2.01	0.94773	0.2925	0.4593	1.021	0.2958	0.5412	0.9582
	129	1.75	0.92027	0.3473	0.4365	0.7287	0.3356	0.3556	0.8475
	132	1.77	0.92464	0.3367	0.3164	0.5414	0.3304	0.2773	0.5735
	135	1.97	0.96409	0.2678	0.4496	0.9037	0.258	0.3758	1.038
	136	2	0.9684	0.4266	0.4633	0.7828	0.411	0.374	0.8945
	138	1.76	0.9849	0.3445	0.2605	1.049	0.3444	0.2612	1.05
	139	2.02	0.93911	0.3658	0.2274	0.8823	0.3636	0.2158	0.9706
	Mean	**1.8971**	**0.9499**	**0.3402**	**0.3733**	**0.8441**	**0.3341**	**0.343**	**0.9046**
	SD	0.1293	0.0238	0.0512	0.1021	0.1765	0.0486	0.107	0.1629
	SEM	0.0489	0.009	0.0193	0.0386	0.0667	0.0184	0.0404	0.0616

Table 19. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the control 10-week-old male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
FC	114	1.99	0.99008	0.3731	0.3479	1.036	0.378	0.3763	0.978
	115	1.99	0.92027	0.2847	0.3274	0.8728	0.2801	0.3006	0.9325
	116	1.99	0.92546	0.504	0.703	0.7326	0.4341	0.4449	1.152
	117	1.75	0.97346	0.4469	0.2457	1.142	0.4518	0.2686	1.045
	118	2.01	0.96975	0.4974	0.3111	0.9656	0.5004	0.3253	0.935
	119	1.99	0.95994	0.3659	0.164	0.8649	0.365	0.159	0.8865
	Mean	**1.953**	**0.9565**	**0.412**	**0.3499**	**0.9356**	**0.4016**	**0.3125**	**0.9882**
	SD	0.0999	0.0279	0.0858	0.1855	0.144	0.0775	0.0974	0.0964
	SEM	0.0408	0.0114	0.035	0.0757	0.0588	0.0316	0.0397	0.0393
LS	114	2	0.98475	0.2728	0.3701	0.5839	0.2504	0.2731	0.8301
	117	2	0.96593	0.3213	0.2174	0.4772	0.3013	0.161	0.548
	118	1.99	0.97972	0.316	0.2885	0.9123	0.3154	0.2833	0.9241
	119	2	0.94773	0.2147	0.2338	0.6965	0.2113	0.2018	0.7755
	Mean	**1.9975**	**0.9695**	**0.2812**	**0.2774**	**0.6675**	**0.2696**	**0.2298**	**0.7694**
	SD	0.005	0.0166	0.0494	0.0688	0.1862	0.0479	0.0585	0.1599
	SEM	0.0025	0.0083	0.0247	0.0344	0.0931	0.0239	0.0292	0.0799
HYP	114	2	0.9739	0.1969	0.4099	0.9098	0.1932	0.3684	0.9801
	115	1.96	0.92284	0.1241	0.4024	1.072	0.1205	0.3491	1.5
	116	2	0.93149	0.2799	0.5307	0.733	0.2334	0.3072	0.8956
	118	1.96	0.93092	0.2265	0.6275	0.8537	0.2177	0.5155	0.9889
	119	1.99	0.93292	0.2562	0.9311	1.184	0.2807	1.309	0.9172
	Mean	**1.982**	**0.9384**	**0.2167**	**0.5803**	**0.9505**	**0.2091**	**0.5698**	**1.0564**
	SD	0.0205	0.0202	0.0605	0.217	0.1786	0.0589	0.4206	0.2512
	SEM	0.0092	0.009	0.027	0.0971	0.0799	0.0264	0.1881	0.1123

Table 20. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the control 10-week-old male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
AMY	114	2	0.95944	0.2078	1.015	1.036	0.2204	1.226	0.9428
	115	1.98	0.96819	0.1729	0.7575	1.095	0.1832	0.9319	0.9506
	116	1.75	0.95704	0.2963	1.288	1.146	0.3637	2.562	0.8572
	118	2.01	0.96022	0.2795	0.6268	0.9227	0.2808	0.6448	0.9253
	119	1.99	0.96618	0.2203	0.5069	1.085	0.22	0.5079	1.097
	Mean	**1.946**	**0.9622**	**0.2354**	**0.8388**	**1.0569**	**0.2536**	**1.1745**	**0.9546**
	SD	0.1101	0.0047	0.0514	0.3141	0.0846	0.0708	0.8233	0.0877
	SEM	0.0493	0.0021	0.023	0.1405	0.0378	0.0317	0.3682	0.0392
HPC	114	2	0.94473	0.2981	0.488	0.8073	0.2803	0.3552	0.9718
	115	1.75	0.94535	0.2813	0.5538	0.7951	0.2668	0.4334	0.9024
	116	2	0.92509	0.3308	0.5865	1.23	0.3536	0.8891	0.9275
	117	1.97	0.98087	0.4124	0.2842	1.126	0.4171	0.3076	1.033
	118	1.96	0.94522	0.3084	0.534	0.7712	0.2963	0.4293	0.8775
	Mean	**1.936**	**0.9483**	**0.3262**	**0.4893**	**0.9459**	**0.3228**	**0.4829**	**0.9424**
	SD	0.1055	0.0202	0.0514	0.1201	0.2154	0.0622	0.2331	0.0615
	SEM	0.0472	0.009	0.023	0.0537	0.0963	0.0278	0.1042	0.0275
ST	114	1.99	0.94157	0.2038	0.5178	0.983	0.2031	0.5138	1.002
	116	1.98	0.95327	0.4101	0.7854	0.8309	0.3825	0.583	0.9654
	117	2	0.9746	0.3329	0.2766	0.9235	0.3278	0.249	1.027
	118	2.01	0.96193	0.3566	0.4409	0.9307	0.3495	0.3951	1.035
	Mean	**1.995**	**0.9578**	**0.3258**	**0.5052**	**0.917**	**0.3157**	**0.4352**	**1.0074**
	SD	0.0129	0.0139	0.0875	0.2122	0.0632	0.0784	0.1464	0.0313
	SEM	0.0064	0.007	0.0438	0.1061	0.0316	0.0392	0.0732	0.0157

Table 21. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the dehydrated 10-week-old male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
FC	126	1.99	0.90452	0.3923	0.5564	0.7126	0.3645	0.3694	0.9944
	128	1.74	0.98293	0.5192	0.1231	0.8315	0.5157	0.1561	0.8835
	130	1.97	0.93156	0.3936	0.07218	0.4508	0.3942	0.08039	0.4598
	133	2	0.91838	0.5221	0.8514	0.9542	0.6441	1.308	0.9575
	134	1.74	0.95991	0.4423	0.2035	0.7797	0.439	0.1877	0.8212
	137	1.97	0.95802	0.5091	0.2539	0.8098	0.4945	0.2106	0.9646
	Mean	**1.9017**	**0.9426**	**0.4631**	**0.3434**	**0.7564**	**0.4753**	**0.3854**	**0.8468**
	SD	0.1258	0.0294	0.0617	0.301	0.1694	0.1007	0.4619	0.1998
	SEM	0.0513	0.012	0.0252	0.1229	0.0692	0.0411	0.1886	0.0816
LS	126	2	0.9605	0.3007	0.4437	0.9083	0.31	0.5355	1.18
	130	1.97	0.91268	0.3345	0.2679	0.5476	0.3315	0.3162	0.5765
	133	2	0.94722	0.2865	0.6623	0.9556	0.4554	2.121	0.8139
	134	1.75	0.96597	0.3141	0.2528	0.8642	0.3123	0.2393	0.9037
	137	1.77	0.98662	0.3234	0.2493	0.9379	0.3221	0.2418	0.9689
	Mean	**1.898**	**0.9546**	**0.3118**	**0.3752**	**0.8427**	**0.3463**	**0.6908**	**0.8886**
	SD	0.1268	0.0274	0.0188	0.1799	0.1686	0.0616	0.8086	0.2206
	SEM	0.0567	0.0122	0.0084	0.0805	0.0754	0.0276	0.3616	0.0986
HYP	126	1.97	0.95448	0.1683	0.2321	0.672	0.1675	0.2231	0.6894
	128	1.99	0.9265	0.1911	0.2086	0.8307	0.1889	0.2673	0.8908
	130	1.75	0.90928	0.3589	0.4456	0.7344	0.3775	0.8065	0.935
	134	1.99	0.96419	0.1775	0.3278	0.7925	0.1713	0.3015	0.9678
	137	2	0.93774	0.2729	0.3704	0.9841	0.2674	0.3341	1.139
	Mean	**1.94**	**0.9384**	**0.2337**	**0.3169**	**0.8027**	**0.2345**	**0.3865**	**0.9244**
	SD	0.1068	0.0219	0.0813	0.0981	0.1178	0.0896	0.2384	0.1616
	SEM	0.0477	0.0098	0.0364	0.0439	0.0527	0.0401	0.1066	0.0723

Table 22. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the dehydrated 10-week-old male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
AMY	126	2.01	0.91757	0.1839	0.5802	0.7935	0.1739	0.4312	0.9657
	128	1.75	0.92798	0.2604	0.1563	1.141	0.2562	0.1771	1.268
	130	2.01	0.99985	0.3049	0.4945	1.134	0.331	1.01	0.8838
	134	1.74	0.97507	0.1573	0.2775	1.046	0.1584	0.3397	0.9558
	137	2.01	0.95651	0.2231	0.3643	0.8453	0.2191	0.3243	0.9527
	Mean	**1.904**	**0.9554**	**0.2259**	**0.3746**	**0.992**	**0.2277**	**0.4565**	**1.0052**
	SD	0.1452	0.0337	0.059	0.1687	0.1629	0.0694	0.3226	0.1505
	SEM	0.0649	0.0151	0.0264	0.0754	0.0729	0.031	0.1443	0.0673
HPC	126	1.98	0.95206	0.3587	0.6304	0.8039	0.3385	0.4736	0.9435
	128	1.75	0.91709	0.3249	0.1472	0.7305	0.3205	0.1224	0.6948
	130	2	0.9839	0.4388	0.3667	0.8683	0.4301	0.3274	0.9062
	134	1.98	0.91996	0.2512	0.2336	0.7951	0.2483	0.2071	0.9248
	137	2.01	0.95982	0.3441	0.3518	0.7415	0.3331	0.2886	0.8438
	Mean	**1.944**	**0.9466**	**0.3435**	**0.3459**	**0.7879**	**0.3341**	**0.2838**	**0.8626**
	SD	0.1092	0.0282	0.0674	0.1827	0.0553	0.0648	0.1322	0.101
	SEM	0.0488	0.0126	0.0301	0.0817	0.0247	0.029	0.0591	0.0452
ST	126	1.99	0.93957	0.2706	0.5152	0.9572	0.2674	0.4837	1.035
	128	1.74	0.92569	0.3992	0.2926	0.9275	0.3968	0.3271	0.9652
	130	2	0.93296	0.3958	0.1326	0.6228	0.3934	0.1252	0.6566
	134	1.75	0.93608	0.2684	0.2643	0.9931	0.2678	0.2604	1.021
	137	2.01	0.95151	0.3965	0.2423	0.7924	0.3881	0.208	0.9301
	Mean	**1.898**	**0.9372**	**0.3461**	**0.2894**	**0.8586**	**0.3427**	**0.2809**	**0.9216**
	SD	0.1399	0.0095	0.0699	0.14	0.1521	0.0686	0.1353	0.1541
	SEM	0.0626	0.0042	0.0313	0.0626	0.068	0.0307	0.0605	0.0689

Table 23. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the control adult female chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
FC	37	2.05	0.96856	0.3063	0.4814	0.8686	0.2897	0.4445	0.9765
	38	2.22	0.99478	0.3287	0.5273	1.019	0.3313	0.5459	1.001
	39	2.02	0.98375	0.3970	0.4157	0.9130	0.3771	0.4070	1.124
	41	2.00	0.93068	0.3141	0.8593	0.8792	0.2838	0.5516	1.006
	Mean	**2.0702**	**0.969**	**0.337**	**0.571**	**0.920**	**0.320**	**0.487**	**1.027**
	SD	0.100	0.0280	0.0414	0.198	0.0687	0.0433	0.0727	0.0660
	SEM	0.0502	0.0140	0.0207	0.0988	0.0343	0.0216	0.0363	0.0330
LS	37	2.05	0.96319	0.2486	0.3287	0.8418	0.2363	0.2610	0.9502
	38	2.28	0.91530	0.3207	0.9036	0.5698	0.2506	0.4623	0.6900
	39	1.71	0.93312	0.3419	0.1696	0.7854	0.3949	0.2893	0.9258
	40	1.70	0.91118	0.4543	0.2521	2.049	0.5140	0.5097	0.8612
	41	1.50	0.92446	0.3226	1.0380	0.6275	0.2133	0.3697	0.9610
	Mean	**1.848**	**0.929**	**0.338**	**0.538**	**0.975**	**0.322**	**0.378**	**0.878**
	SD	0.312	0.0207	0.0743	0.402	0.611	0.129	0.107	0.112
	SEM	0.140	0.00925	0.0332	0.180	0.273	0.0576	0.0480	0.0500
HYP	36	1.74	0.96675	0.1201	0.8187	0.9660	0.1139	0.6596	1.070
	37	1.99	0.98762	0.2096	0.6769	0.9868	0.2156	0.8098	0.9457
	38	2.26	0.93471	0.1774	0.2534	0.9837	0.1866	0.3591	0.8421
	40	1.72	0.94744	0.2743	0.7902	1.241	0.3555	1.8050	0.8697
	41	1.75	0.92311	0.1823	1.2510	1.111	0.2251	2.1180	0.8940
	Mean	**1.892**	**0.952**	**0.193**	**0.758**	**1.058**	**0.219**	**1.150**	**0.924**
	SD	0.233	0.0257	0.0560	0.357	0.118	0.0877	0.766	0.0899
	SEM	0.104	0.0115	0.0251	0.160	0.0526	0.0392	0.343	0.0402

Table 24. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the control adult female chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
AMY	36	1.74	0.92686	0.1241	0.3632	1.203	0.1354	0.5722	0.9035
	37	2.05	0.94439	0.2292	0.6609	0.9355	0.2296	0.7336	0.9335
	38	2.24	0.93837	0.2720	0.5925	0.6352	0.2044	0.2614	0.9536
	40	1.50	0.92837	0.3895	0.3822	1.075	0.3998	0.4309	0.9814
	41	1.98	0.92941	0.2252	0.8298	1.277	0.2116	0.6881	1.381
	Mean	**1.903**	**0.933**	**0.248**	**0.566**	**1.025**	**0.236**	**0.537**	**1.031**
	SD	0.287	0.00757	0.0959	0.196	0.254	0.0982	0.194	0.198
	SEM	0.128	0.00339	0.0429	0.0878	0.113	0.0439	0.0866	0.0885
HPC	36	2.00	0.98112	0.1278	0.2149	0.6948	0.1182	0.1642	0.6220
	37	2.05	0.96441	0.1755	0.5092	0.9137	0.1767	0.6410	0.9030
	38	2.28	0.99222	0.1109	0.3603	0.9123	0.1167	0.3060	0.8995
	39	2.20	0.90906	0.2416	0.1594	0.7592	0.2522	0.2153	0.6859
	40	1.50	0.94021	0.4646	0.2511	0.9348	0.4974	0.3650	1.005
	41	2.00	0.94237	0.3292	0.5310	1.208	0.3540	0.6941	0.9486
	Mean	**2.005**	**0.955**	**0.242**	**0.338**	**0.904**	**0.253**	**0.398**	**0.844**
	SD	0.272	0.0305	0.136	0.156	0.178	0.150	0.221	0.153
	SEM	0.111	0.0124	0.0553	0.0637	0.0726	0.0612	0.0902	0.0626
ST	36	2.00	0.97788	0.1799	0.4830	0.9045	0.1698	0.3844	1.014
	37	2.03	0.95115	0.2764	0.5269	1.117	0.3060	0.7354	0.9125
	38	2.00	0.95221	0.1841	0.6751	0.9516	0.1726	0.7440	0.9468
	40	1.50	0.94843	0.2858	0.4910	1.175	0.3023	0.6082	0.9717
	41	1.72	0.96011	0.2124	0.5123	0.9903	0.2160	0.5445	0.9522
	Mean	**1.850**	**0.958**	**0.228**	**0.538**	**1.028**	**0.233**	**0.603**	**0.959**
	SD	0.233	0.0120	0.0504	0.0788	0.114	0.0672	0.149	0.0372
	SEM	0.104	0.00535	0.0225	0.0352	0.0510	0.0301	0.0666	0.0166

Table 25. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the dehydrated adult female chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
FC	16	1.99	0.90062	0.09327	0.1285	1.062	0.09878	0.1676	0.8918
	17	1.99	0.99945	0.09432	0.2646	1.193	0.1037	0.2403	0.9737
	19	1.75	0.96356	0.1546	0.3520	1.112	0.2985	1.8300	0.7233
	26	1.73	0.97761	0.4057	0.4374	1.048	0.4145	0.4774	0.9819
	27	2.00	0.97872	0.2813	0.6043	1.064	0.2985	0.8202	0.9363
	28	2.03	0.94102	0.4408	0.1804	1.342	0.4587	0.2302	0.9612
	29	2.00	0.94038	0.6242	0.0139	0.4261	0.7234	0.3831	0.5502
	Mean	**1.972**	**0.957**	**0.299**	**0.283**	**1.035**	**0.342**	**0.593**	**0.860**
	SD	0.129	0.0328	0.201	0.200	0.288	0.218	0.588	0.163
	SE	0.0486	0.0214	0.0760	0.0754	0.109	0.0822	0.222	0.0616
LS	16	1.99	0.92878	0.1311	0.2038	0.6427	0.1841	0.8223	0.6789
	17	2.00	0.90918	0.07536	0.1064	0.9825	0.0779	0.1772	0.8463
	26	1.75	0.96473	0.2619	0.1399	1.332	0.2751	0.2239	0.9291
	27	2.00	0.95575	0.2046	0.2240	1.252	0.1880	0.2168	2.315
	28	2.04	0.95179	0.2763	0.1961	0.6316	0.2524	0.1840	0.5812
	29	1.72	0.96796	0.3422	0.0998	0.8111	0.3471	0.1149	1.202
	Mean	**1.917**	**0.946**	**0.215**	**0.162**	**0.942**	**0.221**	**0.290**	**1.092**
	SD	0.142	0.0229	0.0988	0.0533	0.301	0.0925	0.264	0.637
	SEM	0.0580	0.00933	0.0403	0.0218	0.123	0.0377	0.108	0.260
HYP	16	2.00	0.93768	0.08359	0.1401	0.9564	0.08994	0.2576	0.8118
	17	1.96	0.96070	0.04607	0.2880	0.6478	0.03788	0.1697	0.8278
	19	1.50	0.80637	0.2002	0.3794	0.4073	1.9690	28.09	0.3748
	26	2.01	0.97417	0.1872	0.2591	0.8762	0.2155	0.6237	0.8476
	27	1.99	0.92485	0.1059	0.3656	0.9606	0.1878	1.0700	0.7934
	29	1.50	0.95836	0.3331	0.3357	1.088	0.3468	0.3916	0.9426
	Mean	**1.827**	**0.927**	**0.159**	**0.295**	**0.823**	**0.474**	**5.110**	**0.766**
	SD	0.254	0.0617	0.104	0.0884	0.250	0.740	11.267	0.199
	SE	0.104	0.0252	0.0425	0.0361	0.102	0.302	4.600	0.0811

Table 26. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the dehydrated adult female chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
AMY	17	2.18	0.99121	0.08802	0.2411	0.5507	0.1049	0.4659	0.6185
	19	1.50	0.96083	0.1119	0.2526	1.254	0.1246	0.4903	0.8882
	26	1.74	0.90733	0.2394	0.1897	0.6640	0.2673	0.5324	0.8030
	27	1.99	0.92778	0.1419	0.4158	0.7638	0.1343	0.3462	0.8707
	29	1.50	0.96244	0.4990	0.1220	0.7136	0.4617	0.1653	1.009
	Mean	**1.782**	**0.950**	**0.216**	**0.244**	**0.789**	**0.219**	**0.400**	**0.838**
	SD	0.301	0.0327	0.168	0.109	0.272	0.150	0.148	0.143
	SEM	0.135	0.0146	0.0735	0.0487	0.121	0.0672	0.0663	0.0641
HPC	17	1.96	0.95961	0.08505	0.1910	0.7053	0.09129	0.1516	0.6456
	19	1.50	0.91739	0.1489	0.3090	1.654	0.1766	0.5572	0.8699
	26	1.76	0.96507	0.4255	0.1417	0.9670	0.4800	0.3983	0.8104
	27	2.00	0.96680	0.1757	0.4854	0.9181	0.1738	0.5044	0.9616
	29	1.73	0.97036	0.4307	0.0931	1.046	0.4611	0.1194	0.9578
	Mean	**1.790**	**0.956**	**0.253**	**0.244**	**1.058**	**0.277**	**0.346**	**0.849**
	SD	0.201	0.0218	0.163	0.157	0.356	0.180	0.201	0.130
	SEM	0.0899	0.00977	0.0729	0.0702	0.159	0.0807	0.0899	0.0582
ST	26	1.75	0.94452	0.2993	0.4385	1.145	0.3647	1.093	0.7624
	27	1.73	0.98946	0.2053	0.5930	0.9266	0.2004	0.6230	0.9757
	28	1.96	0.92974	0.2890	0.2081	1.186	0.2971	0.3153	0.9890
	29	1.72	0.97175	0.3120	0.1789	0.8213	0.3162	0.1987	0.7990
	Mean	**1.790**	**0.959**	**0.276**	**0.355**	**1.020**	**0.295**	**0.558**	**0.882**
	SD	0.114	0.0268	0.0483	0.197	0.175	0.0689	0.399	0.118
	SEM	0.0570	0.0934	0.0242	0.0984	0.0873	0.0345	0.200	0.0588

Table 27. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the control adult male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
FC	46	1.99	0.90129	0.2411	0.2405	1.064	0.2526	0.2988	0.9257
	47	2.00	0.96773	0.2570	0.4493	0.9014	0.2705	0.6036	0.8224
	48	1.98	0.96452	0.2383	0.5926	1.028	0.2453	0.6500	0.9701
	50	2.00	0.98375	0.3717	0.4516	1.182	0.4019	0.3944	0.9631
	51	1.98	0.98216	0.5659	0.3171	1.051	0.5841	0.3373	0.9618
	Mean	**1.990**	**0.960**	**0.335**	**0.410**	**1.045**	**0.351**	**0.457**	**0.929**
	SD	0.01000	0.0338	0.140	0.136	0.100	0.145	0.160	0.0618
	SEM	0.00447	0.0151	0.0628	0.0608	0.0448	0.0649	0.0714	0.0277
LS	46	1.99	0.93639	0.2191	0.2967	1.057	0.2302	0.3620	0.9355
	47	1.99	0.94228	0.2819	0.1742	1.120	0.2956	0.2764	0.9018
	49	1.75	0.97841	0.3663	0.2503	1.134	0.3898	0.2443	0.8877
	50	1.99	0.89665	0.3011	0.3174	0.6703	0.2710	0.1947	0.8715
	51	1.95	0.95452	0.4096	0.2848	0.7860	0.3667	0.1722	0.9952
	Mean	**1.934**	**0.942**	**0.316**	**0.265**	**0.953**	**0.311**	**0.250**	**0.918**
	SD	0.104	0.0299	0.0743	0.0561	0.212	0.0665	0.0748	0.0490
	SEM	0.0466	0.0134	0.0332	0.0251	0.0947	0.0297	0.0335	0.0219
HYP	46	1.99	0.98783	0.1608	0.5146	1.005	0.1608	0.5194	1.005
	47	2.01	0.90571	0.1219	0.2855	1.056	0.1234	0.3111	1.004
	50	2.00	0.93204	0.1771	0.3706	1.022	0.1822	0.4958	0.8913
	51	1.97	0.95943	0.2385	0.3058	1.144	0.2448	0.3456	1.030
	Mean	**1.992**	**0.946**	**0.175**	**0.369**	**1.057**	**0.178**	**0.418**	**0.983**
	SD	0.0171	0.0353	0.0485	0.104	0.0619	0.0508	0.105	0.0620
	SEM	0.00854	0.0177	0.0243	0.0518	0.0310	0.0254	0.0524	0.0310

Table 28. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the control adult male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
AMY	46	2.00	0.92003	0.2554	0.3644	1.011	0.2580	0.3850	0.9876
	47	1.98	0.97264	0.2161	0.5223	1.057	0.2207	0.5706	1.006
	48	1.50	0.93759	0.1272	1.062	1.085	0.1643	2.083	0.8410
	49	2.00	0.90688	0.2173	0.7033	0.870	0.1963	0.3821	1.0150
	51	1.73	0.94232	0.2662	0.2951	0.9747	0.2590	0.2733	1.067
	Mean	**1.842**	**0.936**	**0.216**	**0.589**	**1.000**	**0.220**	**0.739**	**0.983**
	SD	0.223	0.0249	0.0547	0.307	0.0839	0.0407	0.759	0.0848
	SEM	0.0996	0.0112	0.0245	0.137	0.0375	0.0182	0.339	0.0379
HPC	46	1.97	0.96070	0.2220	0.2757	1.018	0.2288	0.3128	0.9445
	47	2.00	0.97894	0.2747	0.1694	0.9600	0.2715	0.1836	1.022
	49	2.00	0.94751	0.4029	0.1644	0.6898	0.3852	0.1250	0.8000
	50	1.75	0.95243	0.4126	0.3256	0.8595	0.4021	0.2874	0.9508
	51	2.00	0.96255	0.3862	0.3738	1.083	0.4083	0.4537	0.9549
	Mean	**1.944**	**0.960**	**0.340**	**0.262**	**0.922**	**0.339**	**0.273**	**0.934**
	SD	0.109	0.0120	0.0859	0.0933	0.154	0.0831	0.127	0.0814
	SEM	0.0488	0.00538	0.0384	0.0417	0.0687	0.0372	0.0567	0.0364
ST	47	1.73	0.96532	0.2393	0.3694	0.8778	0.2304	0.3114	0.9523
	48	1.50	0.94365	0.3121	0.8657	0.9823	0.3109	0.8524	0.9883
	49	1.75	0.96338	0.3035	0.3049	1.133	0.3167	0.3697	0.9525
	50	1.98	0.95591	0.2037	0.4957	1.025	0.2122	0.5692	0.9545
	51	1.97	0.93493	0.3503	0.4371	0.9020	0.3215	0.3366	1.049
	Mean	**1.786**	**0.953**	**0.282**	**0.495**	**0.984**	**0.278**	**0.488**	**0.979**
	SD	0.199	0.0131	0.0591	0.219	0.102	0.0526	0.228	0.0418
	SEM	0.0888	0.00584	0.0264	0.0982	0.0458	0.0235	0.102	0.0187

Table 29. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the dehydrated adult male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
FC	31	2.01	0.94310	0.2756	0.4147	0.9272	0.2400	0.2453	1.104
	32	1.99	0.92268	0.3468	0.2092	0.7817	0.3858	0.3463	0.9707
	33	1.77	0.93658	0.3421	0.2552	0.7873	0.4162	0.7625	0.7980
	34	2.00	0.97106	0.5014	0.0821	1.107	0.6131	0.4376	0.7173
	35	2.00	0.92963	0.2274	0.2632	0.9274	0.2673	0.5797	0.8663
	Mean	**1.954**	**0.941**	**0.339**	**0.245**	**0.906**	**0.384**	**0.474**	**0.891**
	SD	0.103	0.0187	0.104	0.119	0.133	0.148	0.203	0.151
	SEM	0.0461	0.00834	0.0463	0.0534	0.0595	0.0663	0.0906	0.0675
LS	30	1.74	0.96600	0.2953	1.3090	0.9535	0.2840	1.1650	1.003
	31	2.00	0.93868	0.1858	0.2352	1.142	0.1663	0.1560	0.8753
	32	2.00	0.97507	0.2667	0.2358	1.142	0.2782	0.2858	0.9725
	33	1.76	0.96294	0.3246	0.3298	1.140	0.3627	0.6104	0.8697
	35	2.25	0.98856	0.1376	0.3019	0.9919	0.1391	0.3158	0.9629
	Mean	**1.950**	**0.966**	**0.242**	**0.482**	**1.074**	**0.417**	**0.507**	**0.937**
	SD	0.209	0.0183	0.0780	0.464	0.0934	0.329	0.404	0.0605
	SEM	0.0936	0.00821	0.0349	0.207	0.0418	0.147	0.181	0.0270
HYP	30	1.50	0.97707	0.2393	1.5530	1.029	0.2598	2.016	0.9396
	32	1.76	0.96011	0.1961	0.4349	0.9708	0.1914	0.3995	1.022
	33	1.76	0.95250	0.2167	0.9251	1.021	0.2186	0.8628	1.008
	34	1.99	0.98145	0.2420	0.1936	1.644	0.2716	0.4472	0.8557
	35	1.98	0.98259	0.0984	0.3235	0.9917	0.1012	0.3894	0.9314
	Mean	**1.798**	**0.971**	**0.199**	**0.686**	**1.131**	**0.209**	**0.823**	**0.951**
	SD	0.201	0.0136	0.0590	0.558	0.288	0.0680	0.695	0.0669
	SEM	0.0899	0.00609	0.0264	0.250	0.129	0.0304	0.311	0.0299

Table 30. Parameter of saturation assay of [^{3}H]DPN binding on membranes of different regions of the dehydrated adult male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM)	nH
AMY	30	2.00	0.97981	0.2745	0.7149	0.7144	0.2347	0.5074	0.9903
	31	2.03	0.95302	0.1457	0.1648	0.6669	0.1356	0.1245	0.9889
	32	1.76	0.95565	0.1905	0.2851	0.8905	0.1813	0.2316	1.017
	33	1.99	0.94943	0.3499	1.4460	0.9893	0.3269	1.0350	1.054
	35	2.22	0.96408	0.1572	0.2872	1.059	0.1623	0.3325	0.9552
	Mean	**2.000**	**0.960**	**0.224**	**0.580**	**0.864**	**0.208**	**0.446**	**1.001**
	SD	0.164	0.0121	0.0868	0.528	0.170	0.0757	0.358	0.0368
	SEM	0.0731	0.00524	0.0388	0.236	0.0760	0.0338	0.160	0.0165
HPC	30	1.50	0.96760	0.4402	1.4330	0.7504	0.3354	0.7690	1.017
	31	2.02	0.97399	0.2722	0.2674	0.8422	0.2645	0.2269	0.9205
	32	1.76	0.95856	0.2193	0.1803	0.9464	0.2116	0.1533	1.185
	33	1.76	0.99793	0.3089	0.3393	0.9935	0.3126	0.3899	0.9660
	35	2.22	0.93114	0.2432	0.2111	1.004	0.2536	0.2953	0.8659
	Mean	**1.852**	**0.966**	**0.297**	**0.486**	**0.907**	**0.276**	**0.367**	**0.991**
	SD	0.276	0.0243	0.0869	0.533	0.109	0.0491	0.241	0.122
	SEM	0.123	0.0109	0.0389	0.238	0.0486	0.0220	0.108	0.0546
ST	31	2.02	0.95943	0.1830	0.2107	0.8022	0.1755	0.1628	0.9696
	32	1.75	0.97993	0.2647	0.2975	1.230	0.2998	0.5700	0.9029
	33	1.96	0.97949	0.1491	0.2719	0.8995	0.1478	0.2575	0.9260
	34	1.73	0.92623	0.3504	0.1983	1.043	0.3721	0.2934	0.8700
	35	2.22	0.95669	0.1183	0.5793	0.9906	0.1230	0.6869	0.9379
	Mean	**1.936**	**0.960**	**0.213**	**0.312**	**0.993**	**0.224**	**0.394**	**0.921**
	SD	0.203	0.0202	0.0942	0.155	0.161	0.107	0.233	0.0374
	SEM	0.0909	0.00982	0.0421	0.0694	0.0720	0.0480	0.0997	0.0167

Table 31. Results of binding assay of [^{3}H]DPN binding on membranes of different regions of the castrated adult male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM0	nH
FC	53	1.96	0.93316	0.6139	1.257	0.8407	0.5442	0.801	1.013
	54	1.98	0.96235	0.7345	0.5787	0.8748	0.7166	0.5152	0.9482
	55	2	0.94563	0.4692	0.3989	0.7159	0.447	0.2502	0.8948
	56	2	0.9288	0.5488	1.012	0.7927	0.4815	0.5871	1.011
	57	2	0.95378	0.516	0.3487	0.8522	0.5094	0.3202	0.9154
	60	1.97	0.95674	1.092	0.9286	0.7335	0.9339	0.5957	0.9882
	Mean	**1.985**	**0.9467**	**0.6624**	**0.754**	**0.8016**	**0.6054**	**0.5116**	**0.9618**
	SD	0.0176	0.0134	0.2297	0.3664	0.0656	0.1864	0.2007	0.0501
	SEM	0.00718	0.00547	0.0938	0.1496	0.0268	0.0761	0.082	0.0205
LS	53	2	0.94391	0.4076	0.3626	0.7721	0.4042	0.2518	0.8157
	54	1.75	0.94745	0.5291	0.3521	1.114	0.5337	0.3169	0.983
	56	1.98	0.96819	0.3924	0.7016	0.8871	0.3766	0.5939	0.9757
	57	2.01	0.99781	0.4043	0.4304	0.5812	0.3712	0.315	1.013
	58	2	0.95531	0.4813	0.3351	0.7789	0.4996	0.3625	0.6694
	61	1.75	0.98752	0.609	0.3242	1.206	0.6054	0.3136	1.356
	Mean	**1.915**	**0.9667**	**0.4706**	**0.4177**	**0.8899**	**0.4651**	**0.3589**	**0.9688**
	SD	0.1282	0.022	0.0862	0.144	0.2331	0.0959	0.1204	0.2303
	SEM	0.0523	0.00898	0.0352	0.0588	0.0952	0.0391	0.0491	0.094
HYP	53	2	0.94417	0.2761	0.6136	0.7947	0.264	0.4285	0.9213
	54	1.75	0.9789	0.4297	1.367	0.8541	0.4253	1.594	0.8717
	57	2	0.93941	0.233	0.4956	1.269	0.2368	0.5528	1.115
	58	1.96	0.93214	0.3062	0.4666	1.186	0.3087	0.4966	1.107
	59	2.02	0.91883	0.3676	0.5022	1.184	0.4118	0.8229	0.8549
	61	2	0.93584	0.3943	0.8271	0.775	0.3355	0.5877	0.904
	Mean	**1.955**	**0.9415**	**0.3345**	**0.712**	**1.0105**	**0.3304**	**0.7471**	**0.9623**
	SD	0.1023	0.0202	0.0752	0.3473	0.2255	0.0765	0.436	0.1175
	SEM	0.0418	0.00825	0.0307	0.1418	0.0921	0.0313	0.178	0.048

Table 32. Results of binding assay of [^{3}H]DPN binding on membranes of different regions of the castrated adult male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM0	nH
AMY	53	2	0.93837	0.4039	0.4968	1.839	0.3974	0.4672	0.8283
	54	1.95	0.95642	0.3438	0.7636	0.8222	0.3334	0.5886	0.8903
	55	2	0.9573	0.241	0.532	0.6284	0.2313	0.395	0.7915
	56	2	0.98556	0.2256	0.9453	0.9854	0.2202	0.869	1.047
	57	2.01	0.97064	0.2043	0.4282	1.014	0.2039	0.4265	1.024
	61	2	0.93017	0.373	0.7619	0.9085	0.3536	0.6083	1.122
	Mean	**1.9933**	**0.9564**	**0.2986**	**0.6546**	**1.0329**	**0.29**	**0.5591**	**0.9505**
	SD	0.02161	0.0203	0.0851	0.1995	0.4185	0.0815	0.1745	0.1326
	SEM	0.0088	0.0083	0.0347	0.0815	0.1709	0.0333	0.0712	0.0541
HPC	53	2	0.98512	0.3591	0.6863	0.4961	0.3268	0.3314	0.6186
	54	1.97	0.94951	0.5587	0.4476	0.7536	0.5322	0.3392	0.9041
	55	2	0.94729	0.3869	0.6342	0.6284	0.3169	0.1058	0.7915
	56	1.75	0.92716	0.4077	0.7368	0.8418	0.3755	0.4039	1.051
	57	2	0.96913	0.504	0.5926	0.5972	0.4446	0.3726	0.8335
	60	1.76	0.90592	0.6456	1.362	0.8482	0.5724	0.9156	1.04
	Mean	**1.9133**	**0.9474**	**0.477**	**0.7433**	**0.6942**	**0.4281**	**0.4114**	**0.8731**
	SD	0.1232	0.0284	0.1119	0.3188	0.1428	0.1071	0.2686	0.1634
	SEM	0.0503	0.0116	0.0457	0.1301	0.0583	0.0437	0.1097	0.0667
ST	53	2.01	0.94487	0.3856	0.5272	0.5285	0.3605	0.3935	0.62
	54	2	0.93939	0.3727	0.9654	0.9727	0.3584	0.834	1.135
	55	2	0.93515	0.3499	1.142	0.6284	0.2544	0.2493	0.7915
	56	1.76	0.96502	0.2444	0.4682	1.033	0.2415	0.4472	1.106
	58	2	0.98541	0.4462	0.5682	0.8918	0.4358	0.5036	0.9681
	60	1.97	0.98173	0.5052	0.7797	0.8623	0.4781	0.5536	1.047
	61	1.75	0.96857	0.397	0.7001	0.9154	0.3889	0.6377	0.9748
	Mean	**1.9271**	**0.96**	**0.3859**	**0.7358**	**0.8332**	**0.3597**	**0.517**	**0.9489**
	SD	0.1183	0.0204	0.0811	0.2463	0.1849	0.0873	0.1864	0.1839
	SEM	0.0447	0.00769	0.0307	0.0931	0.0699	0.033	0.0704	0.0695

Table 33. Results of binding assay of [^{3}H]DPN binding on membranes of different regions of the sham-operated adult male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM0	nH
FC	63	2	0.96986	0.6232	0.5991	0.7574	0.5812	0.3403	0.9697
	64	2	0.96909	0.5463	0.1365	0.8435	0.5589	0.1654	0.7863
	65	1.99	0.9406	0.6515	0.2647	0.4671	0.6197	0.242	0.5501
	66	2.01	0.99789	0.7351	0.5756	0.7877	0.6761	0.4528	0.9738
	69	2	0.99375	0.5371	0.7334	0.6981	0.5432	0.7671	0.6807
	Mean	**2**	**0.9742**	**0.6186**	**0.46189**	**0.7108**	**0.5958**	**0.3935**	**0.7921**
	SD	0.007	0.023	0.0815	0.2501	0.146	0.0533	0.235	0.1841
	SEM	0.0032	0.0103	0.0364	0.1119	0.0653	0.0238	0.1051	0.0823
LS	63	1.97	0.97242	0.3898	0.6727	0.8639	0.3716	0.4895	1.045
	64	2.02	0.94931	0.3384	0.1595	0.6782	0.3761	0.2048	0.6221
	65	1.99	0.9401	0.5348	0.2442	0.3327	0.4895	0.1799	0.4859
	66	2.02	0.97736	0.5206	0.5196	0.8838	0.5047	0.4459	0.6972
	69	1.97	0.94251	0.2417	0.4768	0.4791	0.2324	0.3827	0.543
	Mean	**1.994**	**0.95634**	**0.4051**	**0.4146**	**0.6475**	**0.3949**	**0.3406**	**0.6786**
	SD	0.0251	0.0174	0.124	0.2095	0.2403	0.1099	0.1408	0.2198
	SEM	0.0112	0.0077	0.0555	0.0937	0.1075	0.0491	0.063	0.0983
HYP	63	1.96	0.95447	0.3635	1.346	0.9423	0.3457	1.142	1.034
	64	2.01	0.97222	0.293	0.6619	1.147	0.3116	0.8467	0.9582
	65	1.99	0.98269	0.2972	0.5079	0.4052	0.2478	0.2806	0.5067
	66	2.01	0.99136	0.3119	0.7516	1.058	0.3162	0.7976	1.017
	69	1.98	0.97333	0.1554	0.5439	0.2826	0.1357	0.318	0.4238
	Mean	**1.99**	**0.9748**	**0.2842**	**0.7623**	**0.767**	**0.2714**	**0.677**	**0.7879**
	SD	0.0212	0.0138	0.0773	0.3403	0.3954	0.0838	0.3693	0.2974
	SEM	0.0094	0.0061	0.0346	0.1522	0.1768	0.0375	0.1652	0.133

Table 34. Results of binding assay of [^{3}H]DPN binding on membranes of different regions of the sham-operated adult male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM0	nH
AMY	63	2.02	0.98653	0.3648	1.15	1.019	0.3639	1.15	1.025
	64	2	0.94892	0.2661	0.486	0.7927	0.2774	0.54	1.055
	65	1.74	0.95803	0.3205	0.9281	0.8587	0.308	0.6414	0.9572
	66	1.77	0.97108	0.3475	0.5887	0.7421	0.3187	0.447	1.01
	69	2	0.96269	0.182	0.4905	0.772	0.1788	0.4299	0.8396
	Mean	**1.906**	**0.9655**	**0.2962**	**0.7287**	**0.8369**	**0.2894**	**0.6417**	**0.9774**
	SD	0.1385	0.0142	0.074	0.2969	0.1104	0.0692	0.2964	0.0848
	SEM	0.0619	0.0063	0.0331	0.1328	0.0494	0.0309	0.1326	0.0379
HPC	63	1.96	0.90873	0.4349	0.5461	0.7659	0.3964	0.3785	1.569
	64	2.02	0.92621	0.4253	0.3921	0.4958	0.3975	0.2965	0.5992
	65	1.96	0.98199	0.3446	0.241	0.7539	0.3405	0.2175	0.8132
	66	1.97	0.99754	0.4678	0.3421	0.7564	0.4452	0.2903	0.9479
	69	1.98	0.98681	0.2533	0.5307	0.5243	0.2576	0.6347	0.5004
	Mean	**1.978**	**0.9603**	**0.3852**	**0.4104**	**0.6593**	**0.3674**	**0.3635**	**0.8859**
	SD	0.0249	0.0399	0.0865	0.129	0.1367	0.0717	0.162	0.4203
	SEM	0.0111	0.0179	0.0387	0.0577	0.0611	0.0321	0.0724	0.188
ST	63	2.01	0.99333	0.2709	0.5621	0.587	0.2319	0.3679	1.001
	64	1.75	0.97879	0.2908	0.2915	0.9792	0.2894	0.2828	1.011
	65	1.98	0.95018	0.3414	0.2966	0.6485	0.3405	0.2935	0.6554
	66	1.98	0.96356	0.5251	0.5296	0.6867	0.4669	0.3472	0.9979
	69	2	0.96892	0.3131	0.9321	0.8453	0.3023	0.7874	0.9295
	Mean	**1.944**	**0.971**	**0.3483**	**0.5224**	**0.7493**	**0.3262**	**0.4158**	**0.919**
	SD	0.1092	0.0162	0.1023	0.2616	0.1601	0.0878	0.2108	0.1508
	SEM	0.0488	0.00725	0.0457	0.117	0.0716	0.0393	0.0943	0.0675

Table 35. Results of binding assay of [^{3}H]DPN binding on membranes of different regions of the castrated testosterone-substituted adult male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM0	nH
FC	106	1.98	0.96424	0.3947	0.3646	0.8477	0.3915	0.3406	0.9041
	107	1.98	0.9295	0.3943	0.4906	0.7997	0.3862	0.4382	0.8579
	108	1.73	0.9548	0.3325	0.4841	0.7857	0.3218	0.4062	0.8796
	109	1.99	0.92482	0.2756	0.3853	0.9902	0.2785	0.4091	0.9368
	110	1.98	0.95501	0.1931	0.6523	0.8358	0.1803	0.4295	1.042
	111	2	0.9354	0.2992	0.5751	0.7766	0.276	0.3902	0.9643
	112	1.74	0.94455	0.3591	0.4911	0.9246	0.3554	0.4597	1.005
	Mean	**1.9143**	**0.944**	**0.3212**	**0.4919**	**0.8515**	**0.3128**	**0.4105**	**0.9414**
	SD	0.1227	0.0147	0.0722	0.1002	0.0792	0.0748	0.0384	0.0669
	SEM	0.0464	0.0055	0.0273	0.0379	0.0299	0.0283	0.0145	0.0253
LS	106	1.5	0.98594	0.2682	0.5547	0.9304	0.2957	0.6492	1.011
	107	1.99	0.97239	0.2123	0.2997	0.8399	0.2106	0.2834	0.8789
	108	1.73	0.97793	0.1838	0.3556	0.8024	0.1858	0.3058	0.7825
	109	2	0.95235	0.2098	0.3088	0.782	0.2055	0.2631	0.8445
	110	2.01	0.93057	0.1527	0.4316	0.9018	0.1452	0.3364	1.393
	111	1.98	0.91531	0.1815	0.3004	0.8199	0.1834	0.2643	0.7822
	112	1.98	0.9837	0.2654	0.3908	0.6656	0.25	0.3159	0.9323
	113	1.78	0.93791	0.1843	0.9326	0.9026	0.1654	0.7126	0.6947
	Mean	**1.87125**	**0.957**	**0.2073**	**0.4468**	**0.8306**	**0.2052**	**0.3913**	**0.9146**
	SD	0.185	0.0269	0.0411	0.2143	0.0852	0.0482	0.1813	0.2165
	SEM	0.0654	0.0094	0.0145	0.0758	0.0301	0.017	0.0641	0.0765
HYP	106	1.73	0.9754	0.1893	0.7061	1.004	0.1928	0.6337	0.9521
	107	1.99	0.93991	0.1999	0.4555	0.923	0.2005	0.4636	0.9094
	108	2	0.95952	0.1845	0.9347	0.8109	0.1707	0.7838	0.9711
	109	1.96	0.9604	0.166	0.4645	0.8566	0.1631	0.413	0.9053
	110	1.99	0.94531	0.1401	0.4653	0.7185	0.1451	0.5687	1.097
	111	1.97	0.94252	0.1272	0.2847	0.792	0.1259	0.2229	0.8482
	112	1.73	0.99467	0.1536	0.7314	1.088	0.1563	0.7928	1.016
	Mean	**1.91**	**0.9597**	**0.1658**	**0.5775**	**0.8847**	**0.1649**	**0.5541**	**0.957**
	SD	0.1237	0.0198	0.027	0.2213	0.1288	0.026	0.2058	0.0817
	SEM	0.0468	0.0075	0.0102	0.0836	0.0487	0.0098	0.0778	0.0309

Table 36. Results of binding assay of [^{3}H]DPN binding on membranes of different regions of the castrated testosterone-substituted adult male chicken brains (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM0	nH
AMY	106	1.75	0.99437	0.2756	0.9864	1.001	0.2711	0.8317	1.042
	107	1.77	0.97388	0.2344	0.7377	1.151	0.245	0.882	0.9797
	108	1.74	0.97633	0.1743	0.9083	1.037	0.1762	0.9523	1.004
	109	1.5	0.93501	0.1463	0.3817	0.9887	0.1466	0.4554	0.977
	110	1.51	0.98684	0.1515	0.6175	0.9467	0.1448	0.4226	1.178
	111	1.96	0.93484	0.1686	0.838	0.9463	0.1615	0.7098	1.085
	112	2	0.96068	0.2123	0.7881	0.956	0.2073	0.8049	1.029
	Mean	**1.7471**	**0.966**	**0.1947**	**0.7511**	**1.0038**	**0.1932**	**0.7227**	**1.0421**
	SD	0.1946	0.0237	0.0478	0.2014	0.0729	0.0496	0.2075	0.0708
	SEM	0.0735	0.0089	0.0181	0.0761	0.0275	0.0187	0.0784	0.0268
HPC	106	1.76	0.94112	0.2986	0.6702	0.8386	0.2818	0.5157	0.9969
	107	2.01	0.96731	0.2474	0.3742	0.9472	0.247	0.3719	0.9558
	108	1.99	0.94664	0.2576	0.6265	0.7765	0.2456	0.4977	0.885
	109	2	0.99494	0.1963	0.2398	1.101	0.1936	0.1629	0.8336
	110	2	0.94716	0.1803	0.3743	0.8804	0.1727	0.2966	1.21
	111	2	0.97617	0.2264	0.3576	0.8984	0.2246	0.3404	0.9291
	113	1.77	0.9612	0.2938	0.6979	0.8721	0.2786	0.4811	1.09
	Mean	**1.9329**	**0.9621**	**0.2429**	**0.4772**	**0.902**	**0.2348**	**0.3809**	**0.9858**
	SD	0.1148	0.0192	0.0453	0.1826	0.1023	0.0409	0.1279	0.1282
	SEM	0.0434	0.0071	0.0171	0.069	0.0387	0.0155	0.0484	0.0485
ST	106	2	0.9743	0.3573	0.5435	0.6847	0.3278	0.4117	0.8574
	107	1.99	0.99035	0.2529	0.689	0.981	0.2519	0.679	0.9931
	108	1.73	0.98225	0.18	0.623	0.547	0.1584	0.4031	0.8009
	109	1.74	0.96188	0.2525	0.422	0.686	0.2343	0.3334	1.038
	110	1.5	0.96634	0.2689	0.3517	0.8202	0.2619	0.3	0.9344
	111	1.97	0.94802	0.1947	0.4748	0.8518	0.1887	0.4006	0.9551
	112	1.99	0.96541	0.2749	0.5383	0.8453	0.2669	0.458	0.9455
	Mean	**1.8457**	**0.9698**	**0.25445**	**0.5203**	**0.7737**	**0.2414**	**0.4265**	**0.9321**
	SD	0.1936	0.0139	0.0582	0.1155	0.1434	0.0553	0.1231	0.08
	SEM	0.0732	0.0052	0.022	0.0437	0.0542	0.0209	0.0465	0.0303

Table 37. Results of binding assay of [^{3}H]DPN binding on membranes of different regions of the castrated testosterone substituted adult male chicken brains with naltrexone (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM0	nH
FC	71	1.99	0.96019	0.2998	4.056	1.033	0.4066	7.368	0.9028
	72	1.74	0.99974	0.1097	2.104	0.7821	0.1017	1.431	0.853
	73	1.98	0.96491	0.2302	4.094	1.042	0.2133	2.946	1.1
	74	1.77	0.94028	0.3796	1.736	0.9966	0.4103	2.274	0.9175
	75	2	0.95482	0.3508	3.177	1.012	0.3383	2.995	1.041
	76	2.01	0.81462	0.2941	1.524	1.038	0.4586	5.67	0.9016
	77	1.96	0.97253	0.4241	3.913	1.072	0.3685	2.857	1.166
	78	1.75	0.97809	0.4251	2.126	1.001	0.4287	2.177	0.991
	79	1.74	0.92667	0.3317	2.02	0.9912	0.3411	2.148	0.9608
	80	2	0.95882	0.3007	1.779	0.9971	0.311	1.954	0.9537
	Mean	**1.894**	**0.9471**	**0.3146**	**2.6529**	**0.9965**	**0.3378**	**3.182**	**0.9787**
	SD	0.1249	0.0506	0.0943	1.0417	0.0796	0.1084	1.8675	0.0978
	SEM	0.0395	0.016	0.0298	0.3294	0.0252	0.0343	0.5905	0.0309
LS	71	1.75	0.93731	0.2384	4.984	0.9935	0.2666	6.115	0.9463
	72	1.72	0.94458	0.1819	3.136	0.5553	0.1619	1.777	0.6383
	73	1.98	0.98254	0.3432	5.793	1.025	0.3767	7.454	0.9876
	74	1.75	0.9725	0.2742	1.819	1.008	0.2951	2.354	0.9334
	75	2	0.95309	0.16	1.744	0.937	0.1566	1.621	0.9643
	76	2	0.86425	0.204	1.515	1.034	0.2858	4.601	1.004
	77	1.74	0.93674	0.3473	3.008	1.059	0.3145	2.473	1.136
	78	2	0.97497	0.2806	2.313	0.946	0.2541	1.785	1.046
	79	1.73	0.93705	0.3078	2.129	0.9997	0.3226	2.352	0.9536
	80	1.75	0.9518	0.2307	1.241	0.8807	0.2211	1.052	0.9496
	Mean	**1.842**	**0.9455**	**0.2568**	**2.7682**	**0.9438**	**0.2655**	**3.1584**	**0.9559**
	SD	0.1321	0.0331	0.0649	1.5173	0.1463	0.0699	2.1509	0.1271
	SEM	0.0418	0.0105	0.0205	0.4798	0.0463	0.0221	0.6802	0.0402
HYP	71	1.76	0.90067	0.2056	2.471	0.959	0.1927	1.692	1.02
	72	1.75	0.98397	0.11	1.255	1.226	0.1135	0.8243	1.161
	73	1.99	0.90004	0.2578	3.811	1.043	0.3013	5.306	0.9459
	74	2	0.93247	0.3373	1.916	0.9298	0.3051	1.542	1.029
	75	2	0.94453	0.217	2.096	1.015	0.2213	2.256	0.9928
	76	1.98	0.89505	0.2334	1.906	0.9514	0.2582	3.365	1.084
	77	1.74	0.9779	0.3827	2.809	0.9777	0.3568	2.39	1.036
	78	1.97	0.92091	0.3197	2.046	0.9347	0.2997	1.719	1.006
	80	1.75	0.96918	0.2701	1.479	1.057	0.2862	1.765	0.9538
	81	1.75	0.9456	0.1908	1.202	1.072	0.2125	1.779	0.9047
	Mean	**1.869**	**0.937**	**0.2524**	**2.0991**	**1.0166**	**0.2547**	**2.2638**	**1.0133**
	SD	0.1258	0.0329	0.0795	0.7852	0.0898	0.0706	1.2554	0.073
	SEM	0.0398	0.0104	0.0252	0.2483	0.0284	0.0223	0.397	0.0231

Table 38. Results of binding assay of [^{3}H]DPN binding on membranes of different regions of the castrated testosterone substituted adult male chicken brains with naltrexone (Bmax = maximum binding capacity; Kd = dissociation constant; r = correlation coefficient of the Scatchard plot; nH = Hill coefficient)

Region	Chicken No.	Prot. Conc. (mg %)	% r Scatchard Analysis	Scatchard Analysis			Saturation Analysis		
				Bmax (pmol/mg)	Kd (nM)	nH	Bmax (pmol/mg)	Kd (nM0	nH
AMY	71	1.97	0.96824	0.1012	3.172	1.063	0.6619	39.83	0.8141
	72	1.5	0.68824	0	2.798		0.05353	0.2122	1.86
	73	1.75	0.9158	0.2173	4.331	1.066	0.3082	8.521	0.9006
	74	1.74	0.95228	0.2964	3.751	0.9524	0.2914	3.54	0.9624
	75	2	0.96387	0.24	4.36	1.044	0.2148	3.568	1.114
	76	1.76	0.70856	0.9357	24.23	1.029	0.4735	8.445	1.144
	77	1.75	0.96925	0.3349	5.561	1.054	0.2742	3.675	1.156
	78	1.72	0.97196	0.4032	3.321	0.9559	0.3871	3.207	0.9836
	79	1.5	0.98067	0.3069	3.277	0.9898	0.2955	3.016	1.018
	80	1.75	0.91134	0.2062	2.099	1.105	0.2786	4.519	0.801
	Mean	**1.744**	**0.903**	**0.3042**	**5.69**	**1.0288**	**0.3239**	**7.8533**	**1.0754**
	SD	0.1622	0.1104	0.2505	6.5841	0.0523	0.1606	11.5095	0.3031
	SEM	0.0513	0.0349	0.0792	2.0821	0.0174	0.0508	3.6396	0.0959
HPC	71	1.97	0.94898	0.1576	4.17	0.9954	0.1745	5.112	0.9431
	72	1.5	0.99378	0.1657	2.788	0.9895	0.1319	0.9158	1.277
	73	1.75	0.93942	0.4499	8.359	0.9747	0.5121	10.6	0.9384
	74	1.76	0.93891	0.3022	3.216	0.9652	0.2586	2.334	1.074
	75	2	0.95151	0.3288	3.912	0.9258	0.3005	3.577	0.9828
	76	1.74	0.95262	0.4144	5.93	1.032	0.3653	4.953	1.082
	77	1.75	0.98982	0.346	4.314	0.9666	0.3605	4.631	0.9453
	78	2	0.98661	0.2823	2.123	0.9803	0.2729	1.946	1.018
	79	1.5	0.96192	0.3014	2.712	1.011	0.3248	3.224	0.9473
	80	1.75	0.94005	0.276	2.342	0.9461	0.2537	1.871	1.034
	Mean	**1.772**	**0.9604**	**0.3024**	**3.9866**	**0.9787**	**0.2955**	**3.9164**	**1.0242**
	SD	0.1807	0.0217	0.0929	1.9112	0.0307	0.1066	2.7458	0.1042
	SEM	0.0571	0.0068	0.0294	0.6043	0.0097	0.0336	0.8682	0.0329
ST	71	2	0.94006	0.2032	5.13	0.9753	0.2786	9.127	0.8695
	72	1.73	0.99668	0.1362	2.83	0.9315	0.1153	1.41	1.13
	73	1.99	0.93781	0.2952	5.387	0.9917	0.292	5.318	0.9973
	74	1.78	0.96991	0.1983	1.664	1.128	0.2199	1.848	0.981
	75	2	0.92906	0.228	2.944	0.9944	0.2104	2.16	1.068
	76	2	0.95183	0.2349	2.344	1.032	0.2477	2.712	0.9874
	77	1.75	0.97692	0.2906	2.611	1.146	0.292	2.533	1.141
	78	1.74	0.96682	0.3029	1.949	0.9464	0.287	1.667	1.008
	79	1.51	0.98931	0.3425	3.169	1.001	0.3461	3.269	0.993
	80	1.78	0.9903	0.2381	1.393	0.9853	0.2392	1.407	0.9775
	Mean	**1.828**	**0.9649**	**0.247**	**2.9421**	**1.0132**	**0.2528**	**3.1451**	**1.0153**
	SD	0.1647	0.0241	0.0611	1.3464	0.0711	0.0629	2.4026	0.0798
	SEM	0.0521	0.0076	0.0193	0.4258	0.0225	0.0199	0.7598	0.0252

10.3 Chemical reagents, instruments and composition of solutions and buffers

10.3.1 Chemical reagents

Chemical Name	Company	Order No.
4-Androsten-17β-OL-3-ONE 3-O-Carboxymethyloxime Testosterone 3-CMO	Steraloids, Inc. Wilton, USA	Lot No. L-1195
Betaplate Scint	Perkin Elmer, Wallac, Finland	No. 1205-440 Batch 0270075
Betaplate Supermix	Perkin Elmer, Wallac, Finland	No. 1200-439 Batch 0126886022
Bovine Serum Albumin (BSA)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany	Sigma A-7888 Lot No. A-7888
Brilliant Blue G (Coomassie Brilliant Blue G)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany	Sigma B-1131 Lot No. 89C-0068
Chlorhexidine digluconate	Sigma-Aldrich Chemie GmbH, Steinheim, Germany	Sigma C-9394 Lot No. 64H0789
Citirc acid monohydrate	VWR International GmbH, Darmstadt, Germany	Merck -1.00244.0500
[15,16(n)-^{3}H]Diprenorphine	Amersham Pharmacia Biotech, England	Code TRK 730 Lot No. 63
Disodium hydrogenphosphate	VWR International GmbH, Darmstadt, Germany	Merck -1.06586.0500
Hydrogen peroxide urea	VWR International GmbH, Darmstadt, Germany	Merck -8.18356.0100
Iodine-125	Amersham Pharmacia Biotech, England	Code IMS.30 Lot No. 0004
Naloxone hydrochloride	Sigma-Aldrich Chemie GmbH, Steinheim, Germany	Sigma N-7758 Lot No. 48H1160
Naltrexone hydrochloride dihydrate	Sigma-Aldrich Chemie GmbH, Steinheim, Germany	Sigma N-3136
Neomycin sulphate	Sigma-Aldrich Chemie GmbH, Steinheim, Germany	Sigma N-1786
Orange G	Sigma-Aldrich Chemie GmbH, Steinheim, Germany	Sigma O-3756
Ortho-phosphoric acid 85%	VWR International GmbH, Darmstadt, Germany	Merck – 1.00563.1000 Lot No. K 29070673 117
Pentobarbitone sodium	Merial GmbH, Hallbergmoos Germany	Narcoren^{R}
Peroxidase acidic isoenzyme from horseradish	Sigma-Aldrich Chemie GmbH, Steinheim, Germany	Sigma P-6782 Lot No. 62H95051

Chemical Name	Company	Order No.
Potassium chloride	VWR International GmbH, Darmstadt, Germany	Merck -1.04934.9025
Potassium chloride	VWR International GmbH, Darmstadt, Germany	Merck -1.04934.9025
Potassium dihydrogenphosphate	VWR International GmbH, Darmstadt, Germany	Merck -1.04873.0250
Sesame oil	Sigma-Aldrich Chemie GmbH, Steinheim, Germany	Sigma S-3547 Lot No. 90K0870
Sodiumcarbonate decahydrate	VWR International GmbH, Darmstadt, Germany	Merck -1.06391.1000
Sodium chloride	VWR International GmbH, Darmstadt, Germany	Merck -1.06406.0050
Testosterone proprionate	Feinbiochemica, Heidelberg	Lot No. 35800
Tetramethylbenzidine	Sigma-Aldrich Chemie GmbH, Steinheim, Germany	Sigma T-2885 Lot No. 12158/1 34401
Tris(hydroxymethyl)-aminomethan	VWR International GmbH, Darmstadt, Germany	Merck -1.08382.2500
Tris or Trizma-Base	Sigma-Aldrich Chemie GmbH, Steinheim, Germany	Sigma T-1503 Lot No. 062K5429
Trizma-Hydrochlorid	Sigma-Aldrich Chemie GmbH, Steinheim, Germany	Sigma T-3253 Lot No. 112H5602
Tris[hydroxymethyl]aminomethane	VWR International GmbH, Darmstadt, Germany	Merck – 1.08382.0500
Tween^{R} 20 (Polysorbat)	VWR International GmbH, Darmstadt, Germany	Merck – 8.17072.1000
[Arg^{8}]-Vasotocin	Sigma-Aldrich Chemie GmbH, Steinheim, Germany	Sigma V-0130 Lot No. 114H5722

10.3.2 Instruments

Cell disruptor Brason Sonifier B-15	Branson Sonic Power Co., Germany
Automatic Gamma Master (1277 GammaMaster)	Pharmacia-LKB, Wallac, Sweden
CCD Blutgas System	Ciba Corning Diagnostics, England
Incubator	Neuberger, Germany
Liquid Scintillation and Luminescent Counter (1450 Microbeta Trilux)	Perkin Elmer Wallac, Sweden
Magnetic stirrer	Heidolph, Germany
Macrocuvette 4 ml for Photometer	Nerbe Plus GmbH, Germany
Microwave	Sharp, Japan
pH meter	Knick, Germany
Cryostat Fringocut model 2700	Reichert Jung GmbH, Germany
Spectophotometer UV/visible Ultrospec 2000 (Ultra Turpax)	Pharmacia Biotech, Germany
Spectophotometer SLT-Spectra	SLT Labinstrument Deutschland GmBH
Vacuum manifold and pump	Millipore GmbH, Germany
Vapour pressure osmometry	Wescor Inc., Utah, USA
Vacuum centrifuge (SpeedVac SCV 200H)	Savant, New York, USA
Vortex	Heidolph, Germany
96-well filtration plate	Millipore GmbH, Germany

10.3.3 Composition of solutions and buffers

5x PBS buffer

Composition:

NaCl	39.74 g
Na_2HPO_4	5.745 g
KCl	1.005 g
KH_2PO_4	1.02 g
Aq. Dest. To	1000.0 ml

pH 6.8

Washing buffer for testosterone assay

Composition:	
5x PBS buffer	100 ml
Tween^{R} 20	2.5 g

Testosterone assay buffer

Composition:	
0.1% BSA	1.0 g
Na_2HPO_4	5.7 g
NaCl	1.005 g
Na_2HPO_4	5.7 g
Orange G	0.02 g
0.005% Chlorhexidine	50 µl
Aq. Dest. To	1000.0 ml
pH 7.2	

Substrate buffer for testosterone

Composition:	
Citric acid	10.5 g
Na_2HPO_4	9.78 g
Hydrogen peroxide urea	0.5 g
TMB	250 mg
DMSO	20 ml
Aq. Dest. To	1000.0 ml
pH 4.05	

Coating buffer for testosterone

Composition:	
$Na_2CO_3.10H_2O$	4.29 g/l
$NaHCO_3.2H_2O$	2.93 g/l
Aq. Dest. To	1000.0 ml
pH 9.6	

Tris buffer for opioid binding analysis

Composition:	
Tris-HCl	6.06 g
Tris-Base	1.39 g
Aq. Dest. To	1000.0 ml
pH 7.8	

Tris buffer for AVT analysis

Composition:	
Tris	12.11 g
Neomycin sulfate	2 g
Bovine serum Albumin	3 g
Aq. Dest. To	1000.0 ml
pH 7.4	

Bradford solution

Composition:	
Coomassie brilliant blue G	0.1 g
In 50 ml 95% Ethanol	
85% Phosphoric acid	100 ml
Aq. Dest. To	1000.0 ml

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