THE ROLE OF CATECHOLAMINERGIC AND SEROTONINERGIC SYSTEMS IN SEXUAL DIFFERENTIATION OF THE MAMMALIAN BRAIN

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IN THE NAME OF ALLAH
THE MOST BENEFICENT
AND MERCIFUL

DEDICATIONS

This thesis is dedicated to the memory of my (late) dear brother-in-law

Ghias uddin Siddiqui

and to my parents

Mr & Mrs Altaf-ur-Rahman Siddiqui

Between 12 and 60 days, the biogenic amine content in almost all the brain regions increased more in control males and androgenised females than in control females and castrated males. Between 60 and 180 days there were variations with no clear pattern; at 180 days the amine contents were similar in control females and castrated males, and higher than those in control males and androgenised females, which were also similar to each other.

Summary

The neurotransmitters have an organizational role in the sexual differentiation of the mammalian brain. In rodents sex differences in brain amine concentrations appear to be dependent upon the levels of androgens circulating during the perinatal period. To examine the role of biogenic amines in sexual differentiation of the brain, newborn male rats were castrated, or sham-operated upon within 24 hours of birth. Newborn female rats received either 5 daily sc injections of 100 µg testosterone (in oil) or were administered oil vehicle only. Groups of animals were decapitated at the following assigned ages, 12, 60, 75, 90, 120 and 180 days. The brains were removed and separated into hypothalamus, amygdala, cerebral cortex, cerebellum, corpus callosum, hippocampus, corpus striatum and corpora quadrigemina. Circulating LH concentrations were measured by radioimmunoassay from blood samples collected from the above animals. Monoamine levels in each region of the brain were determined by high performance liquid chromatography coupled with electrochemical detection.

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Neonatal castration of male rats resulted in a significant depletion of the brain catecholaminergic content in the

younger animals, after which a pronounced rise occurred by four to six months of age. This observation was generally true for all the regions examined. The rise in catecholamine content in the androgenized females observed at 60 days of age was however short-lived, and a gradual diminution then followed which continued through to 180 days of age.

The brain 5-HT and 5-HIAA content of the neonatally castrated males was also reduced in the younger animals. However the situation was completely reversed by the time these rats reached 120-180 days of age. The 5-HT and 5-HIAA brain content of the androgenized females was higher than that of the controls at 60 days, but in the older experimental females the 5-HT content was greatly reduced compared to that of the oil-treated animals.

These marked alterations in the central neurotransmitter systems are the results of a long-lasting generalized effect on the cell bodies of different neuronal groups. These modifications of the development of the brain biogenic amine systems may be a result of changes not only in mechanisms regulating the hypothalamic-pituitary-gonadal axis, but also of changes in certain non-reproductive functions sensitive to the neonatal androgen environment.

An attempt was made to investigate the sex differences in the biogenic amine content of the hypothalamus, cerebral cortex and cerebellum of 17 human fetal brains obtained from prostaglandin-terminated mid-term pregnancies. There was a considerable variation in the amine levels of the tissues obtained from this source. This finding prompted a study of

the post-mortem stability of monoamines in the rat brain and also an investigation of the effect of prostaglandins on neurotransmitter levels in fetal rabbit brain tissue. The stability of the monoamines varied differentially in various regions of the brain, leading to the suggestion that a correction factor be used to compensate for the changes seen. The experiments on the fetal rabbit brains confirmed that tissue obtained from prostaglandin-terminated pregnancies is not an ideal source for studying the androgen-dependent sex differences in the biogenic amine concentrations. This is because the prostaglandin itself interferes with the detection of the neurotransmitters.

In the present study the results obtained are discussed in the light of previous work.

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COMMUNICATIONS AND PAPERS SUBMITTED

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   Annual Conference of the Society for the Study of Fertility, Reading (July, 1984).
2. Siddiqui, A. and Gilmore, D.P.
   Do biogenic amines play a role in bringing about sexual differentiation of the rat brain?
   Inaugural Meeting of Scottish HPLC Users Group, Glasgow (February 1985).
3. Siddiqui, A., Gilmore, D.P. and Clark, J.S.
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Post-pubertal catecholaminergic changes induced by neonatal androgens in female rats.
XXX Congress of the International Union of Physiological Sciences, Vancouver (July, 1986).

DECLARATION

I hereby declare that this thesis embodies the results of my own special work, that was carried out in the Reproductive Physiology Laboratory within the Institute of Physiology, The University of Glasgow, between February, 1983 and February, 1986.

This thesis does not include work forming part of a thesis presented successfully for a degree in this or another University.

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HPLC High performance liquid chromatography with electrochemical detection

PVN Paraventricular nucleus

List of Abbreviations

ACTH	Adrenocorticotrophic hormone
ADR	Adrenaline
AFP	Alpha Fetoprotein
AM	Amygdala
ARC	Arcuate nucleus
C	Cerebellum
C.C.	Corpus callosum
C.ct.	Cerebral cortex
CNS	Central nervous system
C.q.	Corpora quadrigemina
CRL	Crown rump length
C.S.	Corpus striatum
DA	Dopamine
DAS	Delayed anovulatory syndrome
DHT	Dihydroxytestosterone
FSH	Follicle stimulating hormone
GnRH	Gonadotrophin releasing hormone
HCl	Hydrochloric acid
5-HIAA	5-hydroxy indoleacetic acid
5-HT	5-hydroxy tryptamine
5-HTP	5-hydroxytryptophan
HIP	Hippocampus
HPLC-ECD	High performance liquid chromatography coupled with electrochemical detection
HYP	Hypothalamus

LC	Locus coeruleus
LH	Luteinizing hormone
LHRH	Luteinizing hormone releasing hormone
LTA	Lateral tegmental area
MAO	Monoamine oxidase
mRNA	Messenger Ribonucleic acid
MSG	Medullary serotonin group
NA	Noradrenaline
N Ac	Nucleus accumbens
OB	Olfactory bulb
OT	Olfactory tubercle
PAS	Permanent anovulatory syndrome
PC	Pyriform cortex
pCPA	Para-chlorophenyl alanine
PG	Prostaglandin
POA	Preoptic area
RIA	Radioimmunoassay
Sc	Superior colliculus
SDN-POA	Sexually dimorphic preoptic area
SEP	Septum
SN	Substantia nigra
SOS	Sodium octyl sulphate
STR	Striatum
THAL	Thalamus
VTA	Ventral tegmental area

GENERAL INTRODUCTION

Aims of the study

This study was specifically designed to further our knowledge about the involvement of the biogenic amine systems in brain sexual differentiation.

In particular it was hoped to learn:

a) How do changes in circulating testosterone levels affect brain amine concentrations and do the latter in turn influence LH production by the anterior pituitary of the adult offspring?

b) What are the implications for hypothalamic sexual differentiation of the changes that take place in brain amine concentrations?

c) Whether gonadotrophin feedback is programmed separately from other sexually dimorphic functions.

d) Are the concentrations of the different catecholamines and indoleamines inter-related?

e) Are brain amine concentrations directly related to the sex and/or the age of the human fetus?

f) If human fetal brain tissue obtained from PGE_2-terminated mid-trimester pregnancies was suitable for investigating brain sexual differentiation.

Introduction

Human embryos of both sexes develop in a similar fashion for the first two months of gestation. Anatomical and physiological divergence thereafter results in the formation of male and female phenotypes. Male development is induced only in the presence of specific hormonal signals arising from the embryonic testis (Jost 1953, 1983). According to the Jost formulation - now the central dogma of sexual development - sexual differentiation is a sequential, ordered and relatively simple process (Fig. 1). Chromosomal sex, established at the time of conception, directs the development of either ovaries or testes. Hormonal secretions from the testis elicit the development of the male secondary sex characteristics, collectively known as the male phenotype. If an ovary is present or in fact no gonad at all, anatomical development is female in character. Thus, whatever the mechanisms by which chromosomal or genetic sex is translated into gonadal sex, it is the action of the gonads as endocrine organs that is responsible for development of the sexual phenotypes (Fig. 2).

The following section reviews the current concepts of the processes by which the embryonic gonads acquire the capacity to function as an endocrine organ and of the mechanisms by which the endocrine secretions of the testis modulate male phenotypic development. The anatomical events involved in the formation of the sexual phenotypes and then the factors that mediate this development are summarised first.

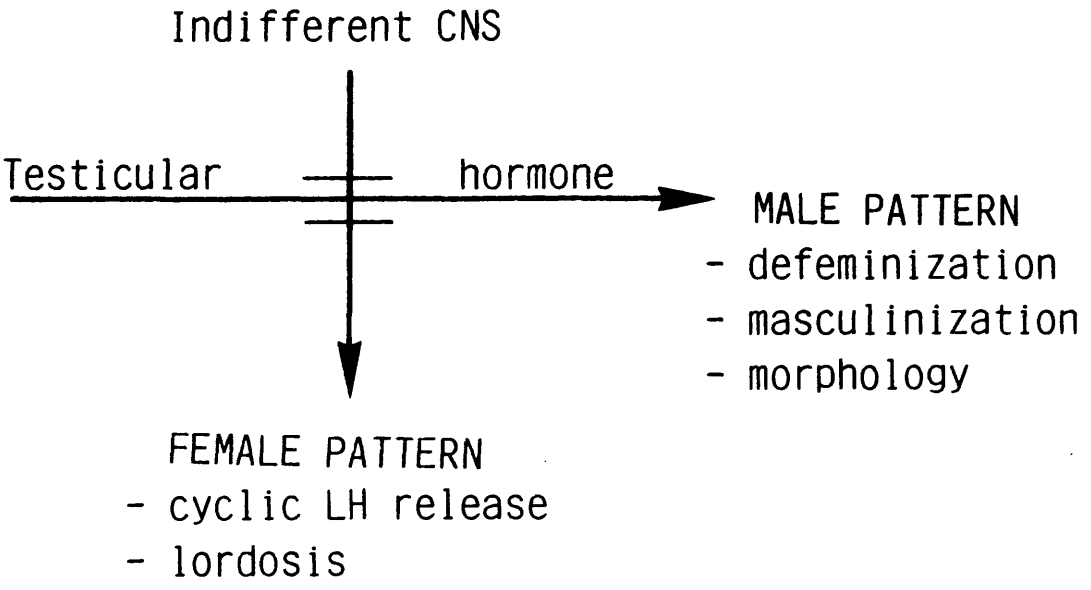


Fig. 1 Scheme summarizing permanent effects of functional pattern of neural structures involved in reproduction and sexual behaviour in the rat.

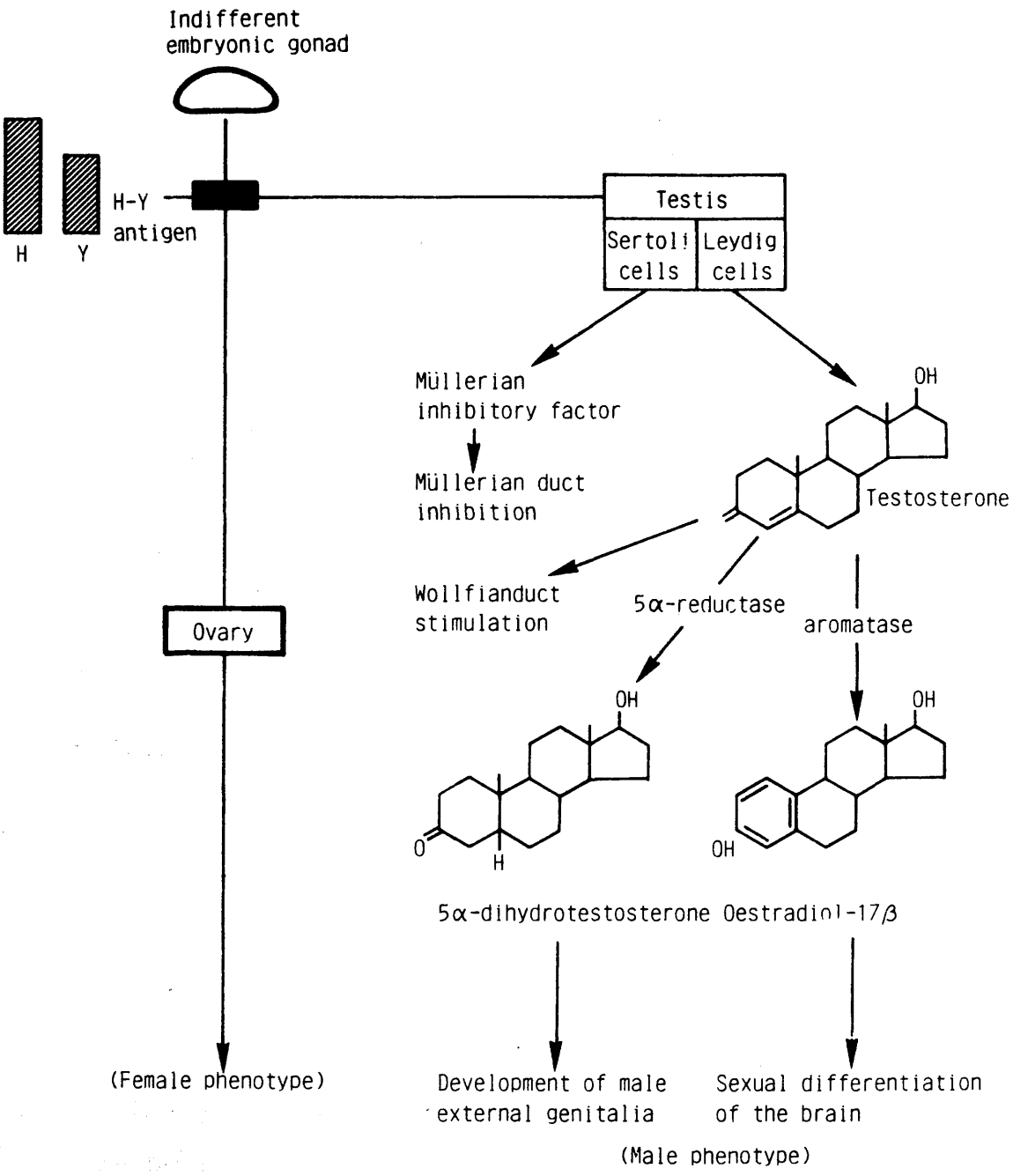


Fig. 2 Hormonal activities of the mammalian embryonic gonad

Formation of the sexual phenotypes

The Y chromosome carries genetic determinants that induce the indifferent gonad to develop into a testis. These determinants are either identical to or closely linked to the gene specifying a cell-surface antigen termed the H-Y antigen (Ohno, 1978; Watchel, 1980).

The emergence from the primitive undifferentiated gonad of a testis or ovary depends on the outcome of a competition for dominance between the medullary and cortical components of the bi-potential organ. Testes are derived from the medullary or mesonephric portion of the primitive gonad and ovaries from the thickened coelomic epithelium which comprises the cortex. Which component emerges as dominant is usually controlled by the sex-determining genes on the X and Y chromosomes. The resultant development of other parts of the reproductive apparatus is largely dependent on the nature of the gonadal development.

Prenatal differentiation

The internal genital ducts are derived from either the Müllerian or Wolffian primordia. The fetal Müllerian inhibitory substance, apparently produced by the Sertoli cells, is responsible for the regression of the female duct systems (Josso, Picard and Tran, 1977). Testosterone produced by the Leydig cells before spilling out into the systemic circulation masculinizes the Wolffian duct derivates at a critical stage in their development, producing male internal and external genitalia (Jost, Vigier, Prépin and Perchellet, 1973). In the absence of such a secretion,

the genital ducts will inevitably be feminine, whether or not ovaries are present. This suggests that the fetal ovaries themselves play no role in sexual differentiation. However, the recent discovery that the fetal ovaries contain large amounts of oestrogen suggests that this steroid might perhaps be important for ovarian development and the initiation of meiosis (George, Milewich and Wilson, 1978b).

Postnatal differentiation

Species vary in their maturity at birth e.g., the testes of neonatal rats contain supporting cells and gonocytes. Many of the gonocytes disappear during the first week of life, but sufficient numbers are maintained to give rise to the future spermatogonia. By 18 days the supporting cells have differentiated into Sertoli cells, and two generations of spermatogonia are present. By 26 days secondary spermatocytes are evident and a few tubules containing spermatids, fully differentiated Sertoli cells and spermatozoa are also present. In the rat interstitial cells do not regress after birth as is the case in some species of mammals (Harris, 1964).

The brain, like the reproductive system, is inherently female; if not exposed to gonadal hormones at a critical period in its development, it remains so regardless of genetic sex. Fortunately for investigators working in this field, in the rat differentiation of the reproductive system occurs before birth, while that of the brain takes place perinatally. Thus one can manipulate brain sexual different-

iation after differentiation of the genitalia has already taken place. In a number of other species differentiation of the brain, occurs before birth and experimental manipulation of this process may also affect the differentiation of the genitalia. Moreover, in the human, where brain sexual differentiation occurs prenatally, one can only make inferences from observations of certain types of pathology. However, the lessons that can be learned from the phenomena observed in experiments with rats are in some respects applicable to the human, as well. Thus the various components which go into determining an individual's sex are: genetic or chromosomal sex; gonadal sex; hormonal sex; and brain sex; all of which contribute to the phenotype.

This investigation is limited to changes in 'brain sex' brought about by the hormonal milieu present during differentiation of the various gonadotrophin-regulating mechanisms.

Sexual differentiation of the Central Nervous System

The sexual differentiation of the brain is more subtle than that of the urogenital system, but equally important in reproductive physiology. It may be regarded as involving the same four processes as the morphological differentiation of the urinogenital system. These processes represent the outcome of interactions between several different factors, amongst which the hormones secreted by the gonads are of paramount importance. Sex hormones seem to exert a dual action on the central nervous system (CNS). In the male,

during fetal or neonatal life the androgens (if present) act in an inductive capacity on the undifferentiated brain to bring about its masculinization. Congenital abnormalities or experimental manipulations can disrupt the normal process of sexual differentiation both in the urinogenital system and in the brain. Later, during adult life the gonadal hormones act on the CNS in an excitatory or inhibitory manner. In this fashion they influence gonadotrophic secretion and the expression of sexual behaviour.

The developmental pattern of the central neuronal systems during the prenatal and neonatal period has been widely investigated in mammals with the aid of histofluorescent, immunohistochemical, electrophysiological and spectrophotofluorescent techniques (Azmitia, 1978; Lindvall and Björklund, 1978; Dyer, 1984). The noradrenergic, adrenergic, dopaminergic and serotoninergic systems play an important role in the control and regulation of many brain functions. There is strong evidence that the neurotransmitters of these pathways, noradrenaline (NA), adrenaline (ADR), dopamine (DA) and serotonin(5-HT) exert a vital role in the regulation of hypothalamic stimulatory and inhibitory hormone production and secretion both in fetal and adult life (Gaziri and Ladosky, 1973; Dörner, Hecht and Hinz, 1976; Reznikov, 1978). These hypothalamic neurotransmitters in turn control the synthesis and release of the anterior pituitary hormones (Barraclough and Wise, 1982; Masudi and Gilmore, 1983).

The concept that there are specific 'sex centres' within the brain is derived from evidence that certain neural

areas are responsive to gonadal hormones and indispensable for the cyclic release of ovulating hormones in the female and for the expression of sexual behaviour in both the male and female. Sex differences in other parameters of brain function, such as the regulation of food intake and body weight, learning and cognitive function, and social behaviour, have also been ascribed to sexual differentiation of the brain. However, most experimental data pertain to reproductive differences and, this review will focus primarily on this subject.

The female rat exhibits a four- or five-day oestrous cycle, during which, rising plasma titres of oestrogen trigger a cyclic neural stimulus that activates the release of gonadotrophin-releasing hormone (GnRH) from the hypothalamus. In the controlled environment of the laboratory, the critical period during which this release occurs begins approximately two hours after noon on the day of vaginal pro-oestrus. GnRH in turn triggers the secretion of luteinizing hormone (LH) by the pituitary, which results in the release of the ova shortly after midnight (approximately 12 hours later). The LH surge is followed by an increase in progesterone secretion and, a few hours later, by expression of a characteristic sexual behaviour, the adoption of lordosis posture when approached by a male. It is likely that the sequence of high levels of oestrogen followed by progesterone facilitates lordosis because this behaviour is normally not exhibited

at any other time in the oestrous cycle. In the case of a female rat, therefore, cyclic fluctuations in hormone levels culminate in ovulation and the expression of sexual behaviour. In contrast, the male rat exhibits no comparable cyclic changes in hormone levels or sexual behaviour and shows mounting behaviour essentially whenever he has the opportunity.

That sexual differentiation in the rat is determined by the hormonal environment at a specific developmental stage was first demonstrated in 1936 by Pfeiffer. This investigator established the following:

i) Male rats castrated at birth and into which an ovary was transplanted, show the capacity when adult, to form corpora lutea.

ii) Female rats ovariectomized at birth and implanted with an ovary when adult, exhibit normal oestrous cycles and corpora lutea formation.

iii) Male rats which have their testes surgically transferred into the neck region at birth, and into which ovaries are implanted when adult, show no capacity to form corpora lutea in this ovarian tissue.

iv) Many female rats into which testes are transplanted at birth, when adult fail to show any sign of oestrous cycles. They enter a state of constant vaginal oestrus and fail to show the formation of corpora lutea in the ovaries.

Pfeiffer concluded that the anterior pituitary gland of the male rat becomes differentiated during development by exposure to testicular hormones; and it therefore cannot

support cyclic gonadotrophin secretion. This possible role of the pituitary in regulating reproductive function was disputed by Harris and Jacobson (1952). They transplanted the pituitary of a male rat to a new position under the hypothalamus of a hypophysectomised female, where it could be re-vascularised by hypophysial portal vessels. The female remained capable of maintaining normal oestrous cycles, mating, pregnancy and milk secretion. It was therefore concluded that the cyclic release of LH in the normal female and acyclic secretion in the normal male were not dependent upon sexual differentiation of the pituitary, but of the CNS. This led to the conclusion that the testes must influence the development of centres located within the brain.

It could be argued that sexual dimorphism is the consequence of differential neuronal gene expression in males and females, but as mentioned previously, the functional capacity of the adult brain depends on the steroid hormone environment during the period of sexual differentiation. The period of maximal susceptibility to androgens depends on the maturity of the CNS and is independent of the parturition. It is generally believed that testicular androgens exert an inductive, or "organizational", influence on the developing CNS during a 'critical' period restricted to a fetal or early postnatal period of neuronal differentiation. During this time the neuronal tissue is sufficiently plastic to respond permanently and irreversibly to these hormones

(Döhler, Coquelin, Davis, Hines, Shryne and Gorski, 1982). This phenomenon is well illustrated by the male rat which is born with this potential to support cyclic ovarian function. If the male rat is castrated before three days of age (the precise age is strain specific), it will as an adult support cyclic corpora lutea formation in ovarian grafts and display the female pattern of sexual behaviour. In many developing mammals, plasma concentrations of androgens are higher in males than females only during such 'critical' periods (Weisz and Ward, 1980). These observations support the view stated at the outset that sexually dimorphic functions are not determined by the neural genome, but are established by the hormonal environment during the perinatal development of an inherently female brain. This is in accordance with the concept proposed by Phoenix and his associates in 1959, that during development the steroid hormones have the capacity to organize or establish, at least in functional terms, the neural circuits that will regulate neuroendocrine function in the adult. In the adult the steroid hormones exert transitory, activational or inhibitory effects that modulate the function of these neural circuits.

In the female rat the critical period for androgen sterilization occurs neonatally, and covers approximately the first 10 postnatal days, when maximum growth of brain cells is taking place and their axonic and dendritic connections are proliferating (Donovan, 1973).

This period has been delineated in placental mammals (rat, guinea pig, rabbit, sheep, rhesus monkey and human) by examination of the effects of timed hormone treatments on sexually dependent neuroendocrine and behaviour patterns.

The critical period is an empirical concept, and does not represent a clearly defined stage of development. It cannot be assumed that all sexually differentiated CNS functions are maximally sensitive to gonadal hormones within the same period. Critical 'sensitive' periods differ temporally for different sexually dimorphic traits.

Developmental Patterns of Pituitary-Gonadal and Central Catecholamine Systems

The main testicular factor responsible for sexual differentiation of the mammalian CNS is testosterone. Administration of this androgen can essentially substitute for the testis in bringing about masculine patterns of gonadotrophin release and behaviour (Söderston, 1984; Wilson, George and Griffin, 1981). Radioimmunoassay (RIA) measurements have shown that during the period when sexual differentiation of the CNS is believed to occur, consistently higher plasma testosterone concentrations are present (Corbier, Kerdelhue, Picon and Roffi, 1978). In a detailed study of plasma testosterone concentrations in rats killed between day 17 and 23 after conception (birth on day 21) Weisz and Ward (1980) found that plasma testosterone levels increase to a peak at embryonic day 18. LH levels have been found to be elevated in males one day earlier, on embryonic

day 17 (Chowdhury and Steinberger, 1976), and an increase in pituitary LH and FSH as well as in hypothalamic LHRH has been reported during days 17-19 of embryonic life (Nemeskéri and Kurcz, 1980). It is interesting to note that during the same period, signs of increased activity are also seen in the adrenal axis (Kalavsky, 1971; Chatelain, Dupony and Dubois, 1979); which appears to be fully dependent on the CNS after embryonic day 19 (Lichtensteiger and Schlumpf, 1981). This early activational phase may be of general importance in the establishment of neuroendocrine organization (Jost, 1966). Furthermore, there exists a striking temporal coincidence between these developmental changes in pituitary axes and the time course of the development of ascending catecholamine (especially noradrenergic) projections: Central catecholaminergic nerve terminals have been detected in the hypothalamus as early as embryonic day 12 (See Schlumpf, Lichtensteiger, Shoemaker and Bloom, 1980a). These ascending projections, travelling in the median forebrain bundle, reach the anterior diencephalon by embryonic day 15. By embryonic day 16, shortly before the apparently sequential rise of plasma LH and testosterone, preoptic and other hypothalamic regions have become well innervated. The innervation then proceeds from the lateral preoptic region towards the periventricular area and is abundant in these areas by embryonic day 18. It seems possible, therefore, that ascending noradrenergic projections might be involved in the initiation of the events occurring in the male gonadal

axis around embryonic days 17/18. The effect might be facilitatory on LH secretion in this situation, as it is in adult animals also (Weiner and Ganong, 1978) or may however perform a different function altogether.

The manner in which dopaminergic innervation develops in the fetal brain is less well established and is not clearly correlated with its future physiological function. Although central catecholamine nerve cells have been detected in the hypothalamus as early as embryonic day 12 (Schlumpf et al., 1980a) it was later observed by Lichtensteiger and Schlumpf (1981) that catecholaminergic neurones in the rostral arcuate region were not detectable before embryonic day 18. At this age the basal part of the infundibulum was seen to contain a number of catecholaminergic fibres with apparent noradrenergic characteristics. Other investigators (Hyyppä, 1969; Smith and Simpson, 1970) have however reported that catecholaminergic innervation of the external layer of the median eminence is not evident at this age.

Probable Sites of Sexual Differentiation

The median eminence region in hypothalamus is the site of GnRH release. However, gonadotrophin secretion involves a complex interplay of regulatory systems extending beyond the hypothalamus, which does not function in isolation from the rest of the brain. Many experimental approaches have been used to identify these systems, including brain lesioning and the recording of brain electrical activity in

response to different hormonal situations. Implanting hormones directly into the brain has also helped to determine localized feedback effects (Christensen and Gorski, 1978), as has the labelling of hormones to identify their site of uptake in the brain. The sum of the available evidence from many investigators, indicates that the sexually dimorphic nucleus in the preoptic area of the hypothalamus is an extremely important morphological example of the organizing action of androgen in the perinatal rat brain (Jacobson, Davis and Gorski, 1985) and is involved in the control of gonadotrophin release and masculine sexual behaviour. The medial preoptic area of the neonatally-castrated male has been demonstrated as clearly feminine in its functional activity (See Gorski, 1984). Recently Swaab and Fliers (1985) described a sexually dimorphic cell group in the preoptic area of the human hypothalamus. Morphometric analysis has revealed that the volume of this nucleus is 2.5 ± 0.6 times as large in men as in women, and contains 2.2 ± 0.5 times as many cells. The medial preoptic area is a relatively large and complex region in the hypothalamus and is implicated in the control of many functional processes. However, it is not likely that this area is the only locus of hormone action during development. The ventromedial arcuate region (Hayashi and Gorski, 1974), amygdala (Staudt and Dörner, 1976; Nishizuka and Arai, 1981), hippocampus (Pfaff, 1966) and the spinal nucleus of the bulbocavernosus of the rat spinal cord (Breedlove and Arnold, 1980; 1981)

have also been suggested as possible loci.

Structural Sex Differences in the Rat Brain

The cellular basis for the permanent differentiating effects of steroids on the developing CNS is poorly understood. Testicular androgens, acting during perinatal period, may alter functional neuronal development in three different ways:
a) by changing the number of receptors for the various neurotransmitters (Arimatsu, Seto and Amano, 1981), or by affecting the enzymes controlling synaptic transmission (McEwen, 1983);
b) by interference with maturational or metabolic aspects of the receptor system (Vertés and King, 1971);
c) by alteration in membrane properties (Toran-Allerand, 1984).

Gonadal hormones have been shown to influence cellular differentiation in the CNS (Christensen and Gorski, 1978). Hormone-dependent structural dimorphism has been reported in such steroid receptor-containing regions as the hypothalamus, amygdala, cerebral cortex, hippocampus, habenula and spinal cord in many mammals including the human (Barraclough and Gorski, 1961; Nishizuka and Arai, 1981; Breedlove, 1984; Spencer, Saper, Joh, Reis, Goldstein and Raese, 1985). Such dimorphism is also found in the vocal control centres of the frog (Xenopus laevis) and songbirds such as the canary and Zebra finch (Nottebohm and Arnold, 1976; Gurney, 1981; Kelly and Fenstemaker, 1983). The morphological consequences of perinatal exposure of the CNS to endogenous or exogenous hormones are numerous. They have been summarized in Table I

Table 1

Steroid-Dependent Structural Dimorphism in the Vertebrate (Modified from Toran-Allerand, 1984).

Cytological Difference	Region	Animal
Neuronal numbers	POA, amygdala, spinal cord, vocal centres	Rat, mouse, songbird, frog
Neuronal size	POA, ventromedial area, amygdala, habenula, hippocampus cerebral cortex, vocal centres.	Rat, mouse, monkey, songbird, frog
Dendritic length/ branching	POA, suprachiasmatic, vocal centres	Rat, hamster, monkey, frog, songbird
Dendritic spines	POA, hippocampus, vocal centres	Rat, monkey, songbird
Numbers of synapses	Arcuate and suprachiasmatic nuclei	Rat
Type of synapses	Suprachiasmatic nucleus	Rat
Synaptic organization	POA, arcuate nucleus, amygdala	Rat
Synaptic organelles	Arcuate and suprachiasmatic nuclei, amygdala	Rat
Axonal density	Hippocampus (sympathetic); septum and habenula (vasopressinergic)	Rat
Regional nuclear volume	POA, spinal cord, amygdala, vocal centres	Rat, mouse, frog, songbird
Volume of neural structures	Cerebral cortex Corpus callosum ?	Rat Human

and are characterized by such cytological parameters as:

a) an increase in neuronal cell numbers (Staudt and Dörner, 1976; Breedlove and Arnold, 1980; Jordan, Breedlove and Arnold, 1982).

b) an increase in length and branching of dendrites and their fields (Greenough, Carter, Steerman and de Voogd, 1977; Ayoub, Greenough and Juraska, 1982).

c) a change in the topographic distribution and types of synapses and their organelles (Raisman and Field, 1973; Nichizuka and Arai, 1981; LeBlond, Morris, Karakiulakis, Powell and Thomas, 1982).

d) an increase in the regional density of axonal innervation, volume of neuronal groups and of certain neuronal structures including the corpus callosum and the cerebral cortex (Pappas, Diamond and Johnson, 1978; Breedlove and Arnold, 1980; De Lacoste-Utamsing and Holloway, 1982; De Vries, Best and Sluiter, 1983).

Early reports of possible structural sex differences in the rat brain involved variations in the size of individual cell nuclei and nucleoli (Dörner and Staudt, 1969). However, such size differences may merely reflect different metabolic demands due to the size and configuration of the dendritic fields or perhaps just the metabolic state in general.

Aromatization Hypothesis:

There is now overwhelming evidence that the masculinisation of neural functions generally involves intraneuronal conversion of the hormone, testosterone to its metabolite oestradiol 17β

(Naftolin and Ryan, 1975; MacLusky and Naftolin, 1981). McDonald and Doughty (1974) were the first to propose that sex behaviour was dependent on the conversion of androgens to oestrogens. Aromatization requires specific enzymes which are only present in certain brain areas including the anterior and medial basal hypothalamus and amygdala. Neurones in these regions contain high affinity oestrogen-binding macromolecules or "receptors" which bind the locally produced oestradiol. This cytoplasmic receptor complex is translocated to the cell nucleus where it alters the expression of the genome (McEwen, Lieberburg, Chaptal and Krey, 1977). Aromatization of androgens to oestrogens appears to be crucial for sexual differentiation of neuroendocrine function and behaviour in many species. However, there is a great deal of species variability and differences exist even between regions and between types of sexually differentiated functions within a given species with respect to the extent to which oestrogen is required for sexual differentiation of the CNS. Behavioural masculinization in the ferret, and monkey, and to a much lesser extent in the rodent is generally mediated by both testosterone and 5α-dihydrotestosterone (DHT). These act directly by binding to intraneuronal androgen receptors which may or may not be localized to many of the same loci as those for oestrogen (Toran-Allerand, 1984; Swanson, 1985). Even in the same species, however, certain sexually dimorphic functions may be differentiated by oestrogens and others directly by androgens (Jost, 1983). In rats and mice, for which brain sexual differentiation depends on aromatization,

intrahypothalamic implants of testosterone or oestradiol 17β have been found to be equally effective in eliciting masculinization of reproductive function and sexual behaviour (Christensen and Gorski, 1978). Anti-oestrogens have been shown to block both testosterone- and oestradiol-induced masculinization, and aromatase inhibitors to attenuate the masculinizing effects of both endogenous and exogenous testosterone. Non-aromatizable androgens such as dihydrotestosterone (DHT) appear to be largely ineffective (McDonald and Doughty, 1974) in bringing about brain sexual differentiation.

Another indication that endogenous androgens act to bring about brain sexual differentiation by their conversion to oestrogens is indicated by the observation that early exposure to oestrogen affects mammalian feminine sexual development in much the same way as does early exposure to testosterone. It has been widely reported (Palpinger and McEwen, 1978) that treatment of female rats with oestrogen during gestation or shortly after birth will result in a pattern of anovulatory sterility in adulthood which resembles that observed after perinatal testosterone administration. Several findings however seem inconsistent with the idea that androgen acts entirely via conversion to oestrogen. Testosterone-induced sexual differentiation is inhibited by androgen antagonists as well as by oestrogen antagonists (Gladue and Clemens, 1978). In male rats castrated at birth the developmental effects of systematic low dose oestrogen treatment seem to be potentiated by the simultaneous administration of 5α-DHT (Booth, 1979).

α-Fetoprotein and the Protection Hypothesis

In placental mammals, the fetus is continually exposed to endogenous oestrogen from the placenta and maternal circulation. If oestrogen formation within the brain plays a vital role in sexual differentiation, then it follows that the female fetus must somehow be protected from the effects of circulating oestrogen. It is assumed that the developing rodent brain (particularly that of the female) is completely protected from excessive exposure to oestrogen, which could occur when levels are high during the critical period of sexual differentiation. This protection in rodents is brought about by the binding of these oestrogens to α-fetoprotein (AFP) (McEwen, Palpinger, Chaptel, Gerlach and Wallach, 1975; Vannier and Raynaud, 1975). Significantly, however AFP does not bind testosterone, hence this hormone is free to enter the brain where it can be converted to oestrogen and interact with cellular oestrogen receptors (Palpinger & McEwen, 1973; Ali, Balapure, Singh, Shukla and Sahib, 1981). The administration of antibodies to AFP in newborn female rats produces effects on sexual development that resemble those of oestradiol injections (Mizejewski, Vonnegut and Simon, 1980).

It is not yet possible to extend the feto-neonatal oestrogen-binding protein protection hypothesis to species other than the rat and mouse. The limited data published in other species such as the guinea pig and human only partially agrees with this hypothesis (Ali *et al.*, 1981).

Possible Modes of Steroid Action

Putative cytoplasmic receptors for androgens, oestrogens, and progestins have now been identified in brain extracts of the newborn rat (Vito and Fox, 1982; McEwen, 1983). Physicochemically, the neonatal receptors appear similar to the homologous receptors from mature brain tissue. Two factors, however, set the neonatal systems apart from those present in adulthood.

a) The tissue concentrations of receptor sites are not static, but change rapidly both during and after the perinatal critical period;

b) the regional distribution of oestrogen receptors within the brain changes during development.

In adulthood, these receptors are concentrated in structures within the cortico-medial amygdala, preoptic area, and medio-basal hypothalamus. In the neonatal rat, however, a diffuse population of cells containing oestrogen receptors is also found extending through layers 5 and 6 of the cerebral cortex (Sheridan, 1984). These cortical oestrogen receptors decline to adult levels after postnatal day 10 (McEwen et al., 1975).

Autoradiographic and biochemical studies indicate that both androgens and oestrogens can bind within cell nuclei in the developing rat brain. Biochemical studies indicate that the oestrogen-binding systems are capable of interacting with oestradiol synthesized locally from androgen. After the administration of ^{3}H-labelled testosterone to newborn

rats, much of the radioactivity recovered from the amygdala and hypothalamic cell nuclei represents ^{3}H-labelled oestradiol, although some labelled testosterone and 5α-DHT is retained (Lieberburg, McLusky and McEwen, 1980).

Another mechanism of steroid action involves the brain neurotransmitters, particularly NA, DA and 5-HT. Since these biogenic amines are involved in normal neuroendocrine functions, a direct action of steroid hormones on the development of one or more of these neurotransmitter systems could be expected to have profound and permanent consequences. Some evidence for this possibility is available. For instance, it has been reported that androgenization of the female rat brain eliminates the cyclic turnover of dopamine that is characteristic of the adult female (Giulian, Pohorecky and McEwen, 1973) and that chemical destruction of serotoninergic neurones induces ovulation in androgenized female rats (Ladosky and Gaziri, 1972).

As described earlier, Raisman and Field (1973) have demonstrated that the spine synapses of non-strial origin in the medial preoptic area of the rat brain are sensitive to neonatal steroid hormone manipulation. This can lead to the experimental sex reversal of the synaptic pattern in the ventrolateral part of the nucleus where sex steroid receptors are abundant (Pfaff and Keiner, 1973; Sar and Stumpf, 1975). This provides clear evidence that synaptic sexual differentiation occurs specifically in the sex steroid-concentrating neuronal system. Neural components such as the dorsomedial

part of the ventromedial nucleus which lacks sex steroid receptors cannot be modified by the action of these steroids during the neonatal period.

It is now evident that the gonadal steroids alone can completely determine the volume of the sexually dimorphic nuclear-preoptic area. For example, the sexually dimorphic nuclear-preoptic area volume (SDN-POA) in the androgen-insensitive male rat with the feminizing testis mutation (Naess, Hang, Attramadal, Aadvaaq, Hansen and French, 1976) is equivalent to that of the normal male (Gorski, Gordon, Shryne and Southam, 1978). However, the spinal nucleus of the bulbo-cavernosus is completely feminine in the same animals (Breedlove and Arnold, 1981). This observation that the morphological differentiation of the CNS is determined in the same animal apparently by oestrogen in one locus (the sexually dimorphic nucleus-preoptic area) and androgens in anothers (the spinal cord) strongly supports the earlier mentioned concept of independence among the various neural parameters, presumably both structural and functional, which undergo sexual differentiation.

The interaction between gonadal steroids and those processes which lead to the formation of the SDN-POA in the rat may be quite complex even for the small cluster of neurones which comprise the SDN-POA. Five possible mechanisms can be postulated which can modify if not determine the final number of neurones, comprising the SDN-POA:

a, steroids stimulating neurogenesis,

b, steroids influencing the migration of neurones from

their origin in the ependymal lining of the third ventricle to the region of the SDN-POA,

c, steroids in the vicinity of the SDN-POA promoting the aggregation of these neurones into the distinct nucleus, perhaps by altering a cell-surface recognition process,

d, oestradiol promoting neuronal survival during the phase of neuronal death, and

e, oestradiol influencing the specification of neurones destined to form the SDN-POA; i.e. by activating or suppressing certain genes, oestradiol determines the functional specificity and thus perhaps the migration, aggregation and even survival of more neurones in the male (Gorski, 1984).

The mechanism elucidating the time course of neurogenesis for the sexually dimorphic-preoptic area in the rat has been well established by thymidine autoradiography (Sidman, 1970). The neurones of the medial preoptic area are reported to have become post-mitotic by about day 16 of gestation (Altman and Bayer, 1978; Anderson, 1978). However, Jacobson and Gorski (1981) revealed that mitotic activity in those cells which eventually form the neurones of the sexually dimorphic-preoptic area is still occurring on day 18, almost two days after these neurones theoretically stop dividing. The mechanism by which the hormone environment accomplishes this is unknown, but is of considerable conceptual significance. It is possible that the steroid environment actually prolongs the period of mitotic activity, or stimulates the aggregation of neurones into the region recognized as the SDN or, perhaps

most likely, prevents neuronal death during early development (Gorski, 1984). Moreover, this intensely dense medial preoptic area, which is 5-7 fold larger in volume in the male, is modified by the hormonal environment postnatally (Fig. 3). Castration of the neonatal male significantly reduces its volume in the adult, whereas exposure of the neonatal female rat to a single injection of testosterone propionate causes a significant increase in the volume of this region in the adult (Gorski et al., 1978). However, it should be noted that the two treatments do not completely sex-reverse the brain in terms of the sexually dimorphic and the preoptic area volumes. This could mean that there is in fact a genomic component to the sex difference in SDN-POA volume, and/or that the prenatal or perinatal hormonal environment is also important. However, the prolonged treatment with high dose of testosterone propionate from day 16 of pregnancy till postnatal day 10 completely sex-reverses the SDN-POA in terms of volume and apparently neuronal number (Döhler et al., 1982). Sex-reversal with such pharmacological treatments does not however prove that in the normal male genomic factors have no role.

The Ontogeny of Steroid Responsiveness

The discussion in the preceding section is based on the fundamental assumption that the development of the sexually dimorphic preoptic area is modified by hormone action. There is, however, no proof that any of the proposed actions of oestrogen are direct. Although it is known that

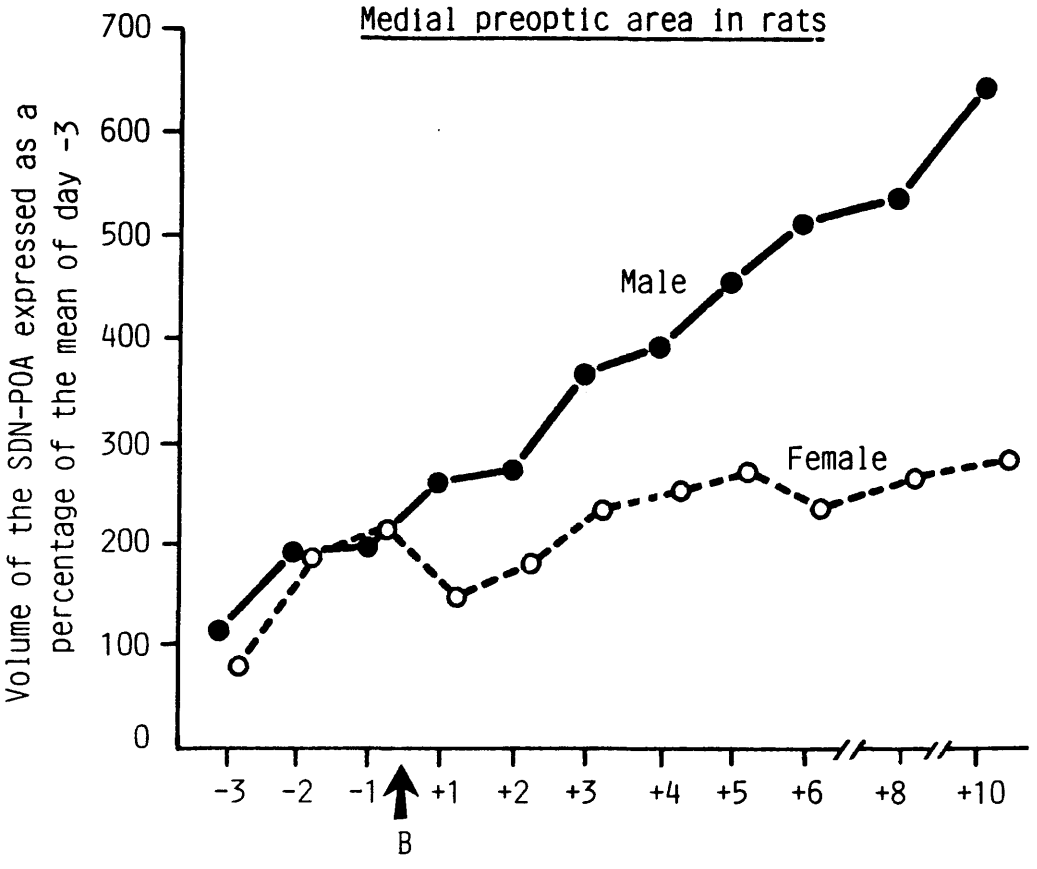


Fig. 3 Changes in the volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) related to the day of birth.

Serio, M., Motta, M., Zanisi, M. Adapted from Martin, L. (Eds). Sexual differentiation Basic & Clinical Aspects, Raven Press (1983).

a high percentage of the cells of the SDN-POA of the adult rat take up and retain radiolabelled steroids (Jacobson, Arnold and Gorski, 1982), this may not also be true during development. It is unclear as to exactly when the neurones of the SDN-POA acquire the ability to take up and retain steroids - presumptive evidence of hormone responsiveness. It is also unknown if migrating cells and presumptive neuroblasts take up steroids. Literature is very sparse in relation to these points.

The Function of the SDN-POA

Although the SDN-POA has been put forth as a model system for the study of sexual differentiation, there is, however, no clear knowledge available about its function. The absolute volume of the nucleus is not correlated with ovulatory function, nor with lordosis responsiveness (Gorski et al., 1978). Lesions of the SDN-POA do not disrupt masculine copulatory behaviour, although small lesions just dorsal to the nucleus do cause its disruption (Arendash and Gorski, 1983a). When punched out tissue from the medial preoptic area, including the SDN of newborn male rats is transplanted stereotaxically into the medial preoptic area of female littermates, they display enhanced behavioural responsiveness, both in terms of masculine and feminine copulatory behaviour, at adulthood (Arendash and Gorski, 1982). Recent studies have shown that transplant volume is increased by treatment of the

recipient with exogenous testosterone propionate (Arendash and Gorski, 1983b). Thus, a trophic influence of gonadal hormones on neuronal tissue has been demonstrated.

Androgen sterilization of female rats

In 1959, Phoenix and his co-workers distinguished between a neonatal differentiation period and a post-pubertal activation period in the guinea pig, with respect to sexual behaviour. In the first phase, differentiation of the hypothalamus is dependent upon androgens, while in the second the hypothalamus is activated either by androgens or oestrogens. Similar findings have since been found in other species, e.g. rats (Barraclough and Gorski, 1962), golden hamsters (Swanson, Brayshaw and Payne, 1974), and rhesus monkeys (Eaton, Goy and Phoenix, 1973).

On the other hand, Harris (1964) reported that male rats castrated neonatally demonstrated strong female sexual behaviour when primed with oestrogens and progesterone after puberty. Genetic males exposed to a temporary androgen deficiency during the period of brain differentiation, but normal or approximately normal androgen levels in adulthood, exhibited a preference of sexual responsiveness to partners of the same sex (Dörner, 1967). This neuroendocrine conditioned male homosexuality could be prevented by androgen administration during the first days of life (Dörner and Hinz, 1968).

Oestrogen administration to intact females results in

an increased secretion of LH. This positive oestrogen feedback plays a decisive role in the regulation of cyclical gonadotrophin secretion and ovarian function in women, and the evocability of the positive feedback action of oestrogen on LH secretion can be regarded as a criterion for a female-differentiated brain. If androgen levels are low during the differentiation period, a relatively strong oestrogen feedback action on LH secretion can be elicited after oestrogen-priming during the functional period (Dörner, Götz and Rohde, 1975). In homosexual men a delayed but significant surge of LH secretion after oestrogen administration has been claimed by Dörner and his associates (1975) but this has been disputed by others.

Clemens (1974) suggested that masculinization of a fetal female could be mediated via exposure to diffused testicular androgens from proximal male littermates. Since longer anogenital distance is a sensitive index of androgenic exposure, such a phenomenon in newborn females has been interpreted as evidence for the transfer of androgen from male littermates. It is anticipated that if females developing between two males in utero are exposed to more androgen than other females, they might therefore become anovulatory earlier than other littermates when given a single exposure to a small amount of neonatal testosterone propionate (Tobet, Dunlop and Gerall, 1982).

Neonatally androgenized intact female rats display some weak male sexual behaviour in adulthood, but female

behaviour prevails (Harris, 1964). However, following ovariectomy and androgen treatment, predominantly male behaviour is observed (Harris, 1964). These neonatally androgenized females lose the capability of cyclic gonadotrophin secretion and exhibit persistent oestrus, polycystic ovaries without corpora lutea and infertility (Götz and Dörner, 1978). It is now recognised that the developing pre-optico-hypophysiotrophic system is sexually dimorphic and can be differentiated by exposure to sex steroids (Barraclough and Gorski, 1961). An exposure of at least 48 hours is required. Even a small dose of testosterone propionate (10 μg) will permanently androgenize the brain (Hayashi and Gorski, 1974). Thus, the latency of onset of the sterility is determined by the dosage and age of androgenic exposure (Sheridan, Zarrow and Denenberg, 1973a). If the dose is below threshold there will be no apparent effect from the perinatal treatment. If an adequate amount of hormone is administered however, the female will be anovulatory from the time of vaginal opening. If the dose is intermediate, the partially-androgenized animal will exhibit delayed anovulation syndrome (Barraclough and Gorski, 1961; Pang and Tang, 1984). After showing four or five apparently normal ovulatory cycles, the animal will spontaneously become anovulatory at about 70 days of age. It will then exhibit reproductive failure typical of the fully androgenized rat, characterized by persistent vaginal oestrus and polycystic ovaries (Chappel and Barraclough, 1976). Experimental

evidence suggests that the early onset of sterility in this rat model is due to failure to achieve the LH surge that triggers ovulation (Chappel and Barraclough, 1976). Priming with oestrogen and progesterone is not effective in this situation, as it is in the ovariectomized, but otherwise normal female rat (Lookingland, Wise and Barraclough, 1982). The delayed anovulation syndrome (DAS) rat ovariectomized at 30 days of age exhibits a surge of LH release upon appropriate hormonal treatment at an age when identical treatment is effective in similar animals castrated at 95 days of age (Barraclough, 1979). In addition, prolonged administration of either oestradiol benzoate, a testosterone propionate to the ovariectomized, lightly androgenized female rat seems to hasten the onset of the delayed anovulatory syndrome (Barraclough, 1979). These experimental observations suggest that postpubertal exposure to either endogenous or exogenous steroids has a permanent effect on the cyclic gonadotrophin release system in the lightly androgenized female rat.

Since the organizational and activational effects of the gonadal steroids appear to interact in the DAS rat, it provides a useful model for the study of hormone action.

Recently several facts regarding the function of the suprachiasmatic tuberoinfundibular system in androgen sterilized rats have been established:

a, GnRH perikarya and their distribution in the hypothalamus is not changed when compared to normal female rats (King, Tobet, Snavely and Arimura, 1980);

b, stimulation of the medial POA still produces enough GnRH to cause pre-ovulatory-like LH and FSH surges (Kubo, Mennin and Gorski, 1975; Chappel and Barraclough, 1976);
c, the pituitary still response to GnRH (Castro-Vazquez and McCann, 1975);
d, progesterone priming of androgen sterilized rats may increase the releasable pool size of GnRH and LH (Chappel and Barraclough, 1976) and oestrogen receptor binding appears to remain normal (Etgen, 1981).

In spite of apparent normal physiological processes occurring in the preoptico suprachiasmatic tuberoinfundibular system, the positive feedback induction of LH and FSH surges by steroids (Harlan and Gorski, 1977a) and the spontaneous preovulatory release of these gonadotrophins do not occur in androgen sterilized rats. Apparently what is lacking is the neural signal responsible for the discharge of GnRH from the median eminence terminals (Lookingland et al., 1982).

Considerable evidence suggests that the neural trigger for GnRH release in normal cyclic pro-oestrous rats, or in oestrogen-treated ovariectomized rats, is NA (Rance, Wise, Selmanoff and Barraclough, 1981; Barraclough and Wise, 1982). These observations suggest that perhaps the early neonatal effects of androgen (or oestrogen) in the undifferentiated endocrine brain might involve the catecholamine system.

In other species, pre- and early postnatal exposure to steroids of the developing reproductive neuroendocrine system also permanently alters adult reproductive performance. In female hamsters, injection of testosterone propionate

is only effective in preventing ovulation when administered within 2 days of birth (Alleva, Alleva and Umberger, 1969). However these animals still mate. Similarly, adult cyclic hamsters exposed to androgens at 4 days of age still exhibit mating activity. However, it is unlikely that this late treatment would disrupt 4-day cyclicity although such hamsters do not become pregnant. If testosterone propionate treatment is delayed until the 12th day of life, these animals, when adult, mate and deliver normal young. Thus two critical periods seem to exist in the female hamster, one which establishes the eventual regulation of cyclic gonadotrophic secretion and the second which seems essential for maintenance of pregnancy.

In female guinea pigs, androgens must be administered prenatally to produce alterations in sexual behaviour and ovulation. Androgen injections have to be initiated between days 30-35 of the 68-day gestation period to be effective (Goy, Bridson and Young, 1964), since this species is developmentally much more mature than the rat at birth. As a consequence of such prenatal androgen treatment, the external genitalia are masculinized and the newborn animals are pseudohermaphrodites. Similar observations have been made in sheep (Clarke and Scaramuzzi, 1978).

In the rhesus monkey, prenatal androgen administration also masculinizes the genitalia and produces pseudohermaphrodites. Such animals, when adult, display increased mounting behaviour and exhibit a pattern of play behaviour

more like that of the genetic male. Furthermore in these animals menarche is significantly delayed but menstrual cycles when established are regular and ovulation occurs.

The inhibitory effects of oestradiol-17β on peripheral plasma LH levels have been studied in gonadectomized females, males and androgenized female rhesus monkeys (Steiner, Clifton, Spies and Resko, 1976). Low doses of oestradiol-17β suppress LH levels in normal females but not in males or androgenized females. After long term oestradiol-17β exposure, more oestrogen is required to suppress plasma LH in males and androgenized females than in females. In contrast, the positive feedback action of oestrogen in facilitating LH-like surges after oestrogen priming occurs in females, males and androgenized females (Steiner et al., 1976). Seemingly, negative feedback control mechanisms are differentiated as a function of hormonal pre-conditioning in fetal life whereas the positive feedback action of oestradiol is unaltered.

Studies in humans have utilized patients inadvertently exposed to androgenic influences. Female pseudohermaphrodites, produced secondary to the adrenogenital syndrome (or exogenous prenatal progesterone), show normal endocrine sex functions as adults although they have aberrant behavioural patterns. Females with a history of prenatal androgen exposure are claimed to exhibit tomboy behaviour. It has also been reported that women who experienced marked virilization early in life reveal, on or after puberty, profound psycho-

social disturbances (Money, 1973).

Dose Responses to Neonatal Androgens

When androgens are administered within the competent period for bringing about sexual differentiation the eventual concentration which reaches the various regions of the brain is the function of three factors:

a, Dose: A dose-dependent response to androgens injected during the critical period has been demonstrated in rats. A dose smaller than 10 µg of testosterone propionate administered on the 5th day of life to female rats has no effect on the oestrous cycle. As previously mentioned, a dose of 10 µg of this androgen results in early onset of sterility marked by constant vaginal oestrus from about 70 days, after several normal cycles. A dose of 1 mg testosterone propionate on day 4 results in an acyclic, sterile adult.

b, Duration: A single high dose of androgen does not parallel the effect of continuous secretion of low levels of androgen found in normal male rats during the first five postnatal days (Resko, Feder and Goy, 1968). Small doses of testosterone propionate, administered daily over a period of 10 days, give more consistent results than a high dose because the steroid is present long enough to allow androgenization to occur (Sheridan, Zarrow and Denenberg, 1973a).

c, Site of application: The route of administration also affects the extent of the response to exogenous androgen. In comparison to the efficacy of subcutaneous testosterone propionate injection in oil, intra-hypothalamic implants are effective for a much longer period of neonatal life

(Loble and Gorski, 1974).

Sites of action of androgens in the developing brain

Three basic methods have been used to study the site of action of androgens in sexually differentiating the brain.

a. Experiments involving hormone implantation

Attempts have been made to localize the site of action of androgens in the brains of new-born female rats by implanting testosterone directly into the brain instead of administering it by subcutaneous injections. Intracranial implantation of steroids is not a very satisfactory experimental technique because it is impossible to tell how far a hormone has diffused from its site of insertion. The presence of solid implants can also cause considerable physical damage in developing brains. Hayashi and Gorski (1974) reported that implants containing 0.25 or 0.5 µg testosterone propionate were equally effective whether they were in the ventromedial hypothalamus or in the ventral preoptic area. Loble and Gorski (1974) reported a high incidence of sterility in female rats implanted as late as day 11 after birth with 60 µg testosterone propionate at the level of the anterior commissure in the preoptic area. This dose was ineffective elsewhere in the brain and when given systemically Christensen and Gorski (1978), using 2 µg testosterone bilaterally implanted into the brains of two or five-day-old females, found that testosterone was only effective in preventing cyclic gonadotrophin release when implanted into the ventromedial hypothalamus. It is therefore suggested

that in the new-born rat, androgen acts upon the ventromedial hypothalamus to cause anovulatory infertility, but its action on the preoptic area may still be an important factor in the process of sexual differentiation.

b. Structural and functional studies

Further evidence for the action of testosterone on the developing brain comes from intensive studies of the anatomy of the hypothalamus and limbic system in newborn and adult rats. However, it must be pointed out that the differences which have been described between the brains of male and female have not necessarily been shown to be related to differences in the patterns of gonadotrophin secretion in adult rats. It must be remembered that the hypothalamus controls many functions besides gonadotrophin secretion; and sex differences in the structure of the POA may be related to other functional differences between the sexes, such as behaviour as well.

The POA in rats undergoes rapid synaptic development during the first two weeks of life (Reier, Cullen, Froelich and Rothchild, 1977). Raisman and Field (1971) observed sexual dimorphism in the types of synapse present in the preoptic areas of adult male and female rats. They studied synapses involving certain axons originating from the POA of the brain. In the male, these axons terminated mostly on the dendritic shafts, but in the female, they were mostly on dendritic spines. This sex difference was found to depend on exposure to androgens during infancy: neonatally

castrated male rats showed the feminine pattern of synaptic contacts and androgenized females the masculine pattern (Raisman and Field, 1973). In addition to this sexual dimorphism in the synaptic connections within the POA, there appear to be sexual dimorphisms in the appearance of the preoptic neurones themselves. Dörner and Staudt (1969a) reported that during the last two days of fetal life - perhaps corresponding to the start of the period of androgen sensitivity - the nuclei of neurones in the POA and ventromedial nuclei (VMN) enlarge in the female rat, so that at birth they are larger than in the male. A sex difference in the volume of this darkly staining cell group in the medial preoptic area of gonadectomized adult rats has previously been described (Gorski et al., 1978). The volume of this area is greater in males than in females, and the differences persist during hormonal treatments known to induce sexual behaviour in adult rats.

The mere existence of sex differences in the structure of the POA does not prove that this region of the brain is solely responsible for the differences in the control of gonadotrophin secretion and behaviour. However, functional differences have also been noted between the preoptic neurones of adult male and female rats, and between those of androgenized females and neonatally castrated males. Raisman and Field (1973), studying the response of neurones in the POA to electrical stimulation of the amygdala, found no sexual differentiation of the afferents from the amygdala to the medial preoptic area. However, Dyer, MacLeod and Ellendorf (1976) demonstrated that there were fewer neurones

which changed their firing rate in the female and neonatally castrated male than in males and androgenized females. It was explained that the net effect of the stimulation of a group of afferents to a neurone depends on the balance of all the inputs to the cell.

In addition to this sexual dimorphism in the POA of the rat, there also appear to be sex differences in the structure of the amygdala itself. Staudt and Dörner (1976) found that the volume of the amygdala was increased in male rats castrated at birth, compared with normal males, and was similar in size to that in normal females. An injection of testosterone propionate on day three restored the normal male pattern.

c. Studies with radioactively labelled hormones

The sites of action of androgens in the brain of newborn rats have been demonstrated rather more convincingly by means of autoradiography as compared to biochemical techniques (Sheridan, Sar and Stumpf, 1974a). These investigators described autoradiograms in which radioactivity from ^{3}H -testosterone had accumulated in the nuclei of neurones in the POA, and in the periventricular, arcuate, and part of the ventromedial nuclei of the hypothalamus of two-day-old female rats.

Neural Activity, Neurotransmitters, and Sexual Differentiation of the Brain

After stimulating protein synthesis in the developing brain, steroid hormones might be expected to act by altering the metabolism of neurotransmitters within it. This could then lead to changes in the electrical activity of the developing neurones. Many studies have been carried out to investigate these possibilities. They have mainly involved the use of drugs which change the rate of synthesis or degradation of specific neurotransmitters, or which have generalized effects on neural activity. The difficulty in interpreting the results of these studies is that the drugs very rarely have completely specific actions. The reported effects could be due to changes other than those claimed by the authors: for example, a drug may affect the peripheral metabolism of a steroid as well as neural activity within the brain, or it may affect the metabolism of several neurotransmitters. Another problem is that it is rarely possible to tell whether a drug has been enhancing or inhibiting the effects of testosterone or whether it has been having direct effects of its own on the developing brain.

Although the influence of gonadal steroids on sexual differentiation of the brain has been extensively studied (Jarzab and Döhler, 1984; Gorski, 1984), very little is known yet about the detailed physiological and molecular mechanisms of steroid-induced sexual differentiation. There are some indications that biogenic amines may be involved in this

process since a number of tranquillizers and psychotrophic drugs known to influence biogenic amine activity have been shown to influence sexual differentiation of the brain (Booth, 1979; Dörner, 1981). These drugs when administered postnatally, permanently alter, not only the pattern of gonadotrophic hormone release and sexual behaviour, but also the structure and chemistry of specific brain areas (Dörner, Staudt, Wenzel, Kvetnanisky and Murgas, 1977).

Androgenization by testosterone propionate can be prevented by concurrent administration of various agents: Barbiturates, tranquillizers, steroids and the anti-androgen cyproterone acetate.

a. Barbiturates

Several workers have reported that the barbiturates pentobarbital and phenobarbital prevent the neonatal effects of testosterone propionate on the brains of female rats and mice (Arai and Gorski, 1968b; Nishizuka, 1976). However Brown-Grant, Munck, Naftolin and Sherwani (1971) disputed these findings. Gorski (1974) explained that it is possible that the barbiturates provide a protective effect by stimulating the liver enzymes which inactivate testosterone as well as having a direct effect on the central nervous system.

b. Tranquillisers

The CNS depressant reserpine depletes the storage capacity of nerve terminals for DA, NA and 5-HT, whereas chlorpromazine affects neural transmission and blocks the action of testosterone on the CNS (Ladosky and Gaziri, 1970).

Both drugs have been reported to protect neonatal female rats from the effects of testosterone propionate or oestrone on cyclic gonadotrophin secretion in adulthood (KiKuyama, 1962); and also prevent the normal differentiation of gonadotrophin secretion in intact newborn male rats (Ladosky, Kesikowski and Gaziri, 1970). Administration of reserpine during infancy leads to a reduction in the amount of monoamines present in the hypothalamus of male rats prepubertally and to permanent changes in the amygdala and hippocampus of adult rats (Hyyppä and Rinne, 1971; Dörner et al., 1977). This suggests that reserpine may protect the developing female brain against the effect of testosterone by its action on nerves containing monoamines, but in the males, this drug prevents testosterone secretion by inhibiting gonadotrophin release (Booth, 1979). A prime candidate for mediation of sex hormone activity during sexual differentiation of the brain seems to be the serotoninergic system. Serotonin levels are the same in both sexes of rats up to 8 days, after which they rise significantly in females on day 12 (Ladosky and Gaziri, 1972; Giulian et al., 1973). This rise can be prevented if testosterone propionate is injected on the day of birth. Conversely castration of males at birth leads to serotonin levels similar to those of females (Ladosky and Gaziri, 1972), whereas treatment of newborn females with androgens resulted in reduced brain serotonin content on day 12 and 14 (Giulian et al., 1973).

These studies suggest a relationship between masculinization of the brain and reduced brain serotonin levels during development. However, they fail to indicate whether the reduced serotonin levels are a cause of, or a consequence

of masculinisation. The fact that the critical period for steroid-induced masculinization of the brain is limited to the first 10 days of life, whereas the sex differences in brain serotonin levels were observed only after this period, favours the latter assumption. On the other hand, there are a variety of experimental data which suggest the active participation of the serotoninergic system in sexual differentiation of the brain immediately after birth. Shirama et al. (1975) observed that postnatal treatment of female rats with the serotonin precursor 5-hydroxytryptophan delayed the permanent anovulatory sterility (PAS)-inducing effect of postnatal TP. An even stronger protective effect on androgen-induced PAS was observed when the rats were treated postnatally with the serotonin synthesis inhibitor para-chlorophenylalanine (pCPA) (Reznikov, Nosenko and Demkiv, 1979). Hyyppä, Lempinen and Lethenin (1972), however, reported that postnatal treatment of rats with pCPA had no influence on cyclicity, but that it reduced ear-wiggling behaviour in females and stimulated male sexual behaviour in males.

Acetylcholinesterase inhibitor pyridostigmine, which prevents breakdown of acetylcholine, when given to newborn rats increases masculine behaviour in both males and females (Dörner et al., 1976; Dörner and Hinz, 1978) and leads to a permanent decrease in the hypothalamic content of noradrenaline (Dörner et al., 1977). Pyridostigmine was not however tested for a direct effect on the sexual differentiation of the

brain. Nevertheless, Libertun, Timiras and Kragt (1973) have shown testosterone-dependent sex differences in the synthesis and breakdown of acetylcholine in the POA of the rat.

c. Steroids

Development of steroid-induced anovulatory infertility in new-born rats can be prevented by concurrent administration of progesterone or cyproterone acetate. Simultaneous administration of testosterone propionate and progesterone to female rats on day 5 did not result in brain masculinization (Kinal and Maqueo, 1965; Arai and Gorski, 1968b). Similarly progesterone prevented the sterilising action of ethynyl-oestradiol 3 methyl ether in male rats injected on day 5. Shapiro, Bongiovanni and Marino (1976) observed that normal new-born female rats have higher levels of progesterone in their circulation than do males.

Cyproterone acetate (1,2α, methylene-6-chloro-6-17α hydroxy progesterone), a potent anti-androgen antagonizes the action on accessory glands and other tissues, and suppresses morphogenesis of male characteristics in fetal development. It would seem that both progesterone and cyproterone acetate compete with the testosterone propionate for available receptor sites and thus prevent androgenization from occurring at all.

The mechanisms of action of androgens in causing differentiation of the CNS would seem to follow the pattern set out below:

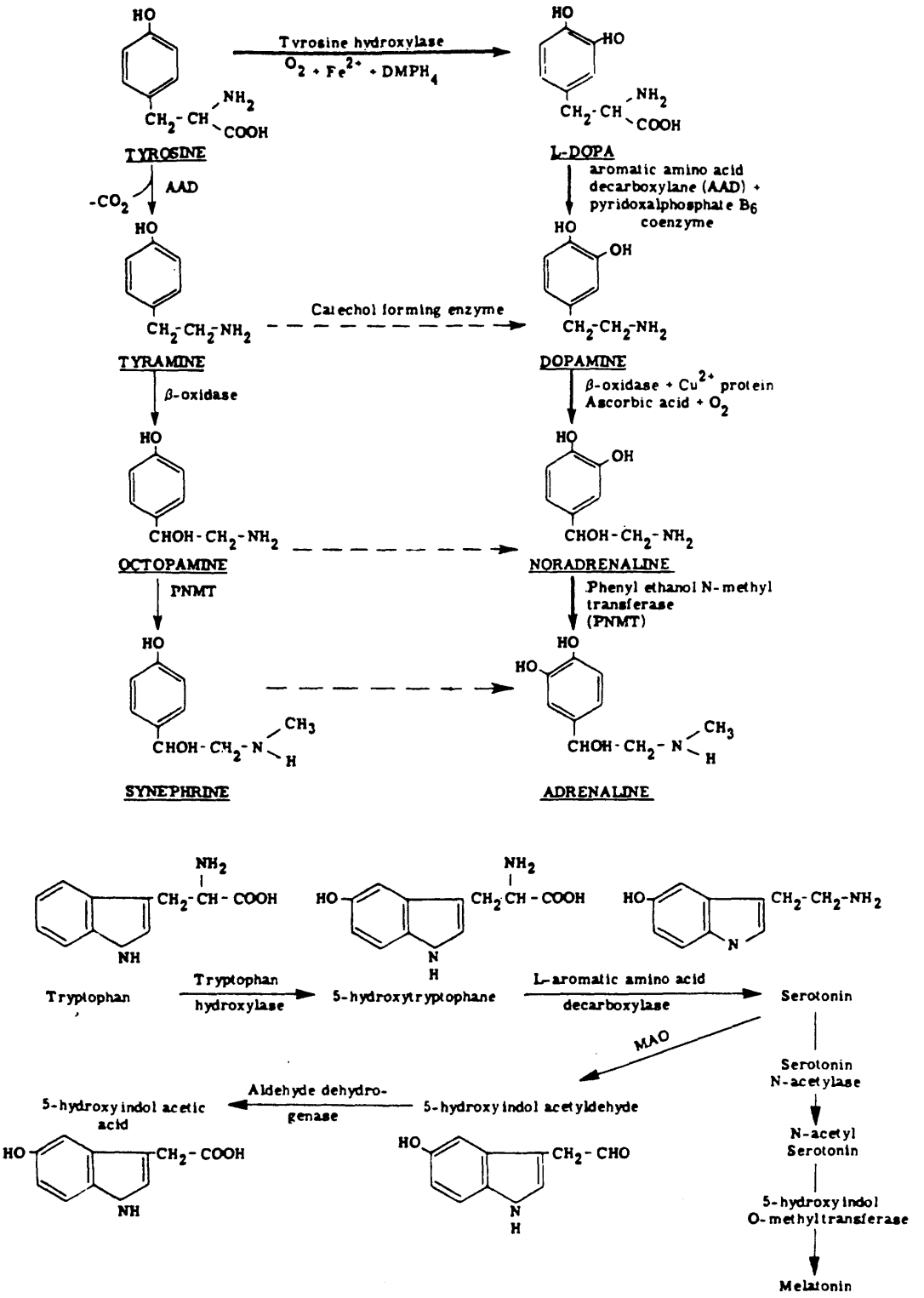


Fig. 4 Biosynthetic and metabolic pathways of biogenic amines

Testosterone competes for uptake into the cell, probably at oestrogen receptor sites.

If the testosterone is taken up it has several effects:

a. alters the ability of the cell to bind oestrogen
b. causes changes in the pattern of serotonin release
c. alters enzyme systems associated with luteinizing hormone releasing factor.

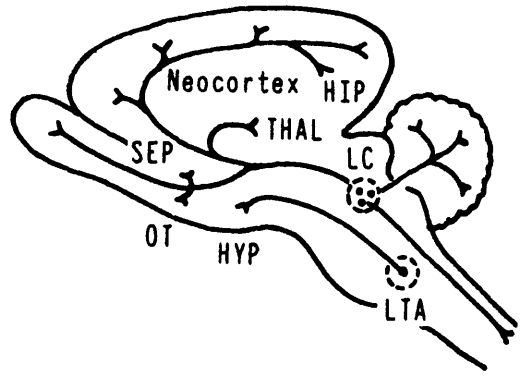
Monoaminergic Neurones in the Embryonic Brain

The monoaminergic neurones possess the enzymatic machinery to synthesize DA, NA, ADR and 5-HT (Fig. 4). Histochemical and fluorescent studies upon these cells in young rats show that their early maturation (consisting of the enlargement of the perikarya and the growth of the axon cylinder) is followed by the appearance of a characteristic fluorescence indicative of the presence of monoamines. These monoamines are produced largely before the development of any synapses. This is suggestive of a non-neurotransmitter role for the monoamines during the early organogenesis of the CNS. Also, the fact that monoamine-containing axons reach their target regions at a time when only the very first signs of differentiation are present may point to a trophic or inductive influence by them upon the axon nerve terminals. The absence of any synaptic connections at this time indicates that this influence cannot be mediated by a depolarization-type of activity (Keyser, 1983) but may be related to their monoamine content.

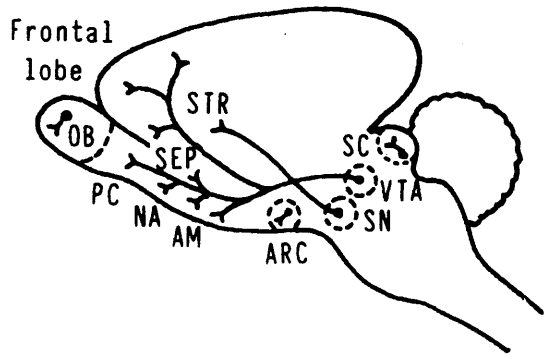
Fig. 5

Summary diagrams showing locations of neurones containing different neurotransmitter substances in the mammalian brain. AM, amygdala; ARC, arcuate nucleus; HIP, hippocampus; HYP, hypothalamus, LC, locus coeruleus; LTA, lateral tegmental area; MSG, medullary serotonin group; NA, nucleus accumbens; OB, olfactory bulb; OT, olfactory tubercle; PC, pyriform cortex; SC, superior colliculus; SEP, septum; SN, substantia nigra; STR, striatum; THAL, thalamus; VTA, ventral tegmental area. (Adapted from Angevine, J.B.Jr. and Cotman, C.W., In Principals of Neuroanatomy, 1981).

A. Noradrenaline



B. Dopamine



C. Serotonin

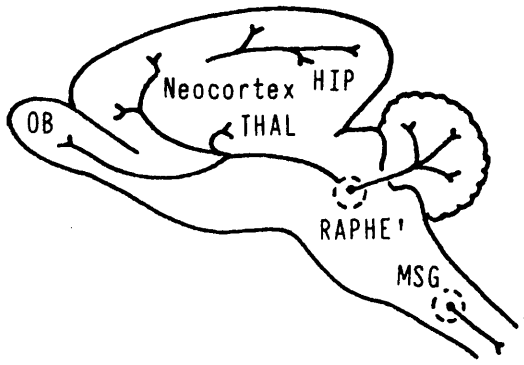


Fig. 5 Summary diagrams showing locations of neurones containing different neurotransmitter substances in the mammalian brain.

For key to abbreviations, see facing page.

The Development of Brain Steroid Receptors and their Role in the Sexual dimorphic Differentiation of the Brain

Steroid hormones play a role in normal brain development. Autoradiographic studies by Stumpf and Sar (1978a) have demonstrated a widespread presence of steroid-binding sites within the adult nervous system. However, steroid receptor mapping in the adult rodent brain is insufficient to evaluate the role played by the steroids during neurogenesis and sexual differentiation. It is feasible to think of steroid receptors being temporary functional properties of the brain nerve cells that can be lost during development and ageing. The affinity between receptors and ligands may also be modified by the interference of other hormones.

The selective stimulation of the nuclear or cytoplasmic steroid receptors by the steroid may trigger a process which gives rise to protein synthesis and eventually to the production of neurotransmitters in those cells that are sensitive to the effect of circulating androgens (Thomas and Knight, 1978). By the induction of this sex difference in neurochemistry a secondary dimorphism of synaptogenesis may also be generated in the target areas of these cells (Raisman and Field, 1973).

Dörner (1981) collected evidence for the mediation of sexual differentiation of the brain by both systemic hormones and neurotransmitters. In his opinion, the eventual results of both sex hormone-mediated differentiation and psycho-socially-induced differentiation are effected by neuro-

transmitters. Thus, neurotransmitters may be regarded as the mediators of the sex hormone-induced sexual differentiation of the brain. Both sets of influences, i.e., the androgen-dependent alteration of neurotransmitter levels and the psychosocially-induced changes in turnover of neurotransmitters, may have a complementary impact on brain function.

Central Neurotransmitter Systems

Dopamine

DA is the first neuroactive substance in the pathway for catecholamine synthesis (see Fig. 4). It is present in biochemically measurable concentrations in all brain areas, but in this review, only the topography of DA-containing cell groups and pathways is summarized.

The locations of dopaminergic neurones are shown in Figure 5 B. There are two main populations of neurones, located within the midbrain (mesencephalon). One consists of the output cells of the substantia nigra, which project to the caudate striatum, the other is located in the ventral tegmental area (VTA) of the mesencephalon (Phillipson and Griffith, 1980). These cells project to a number of sites in the forebrain, including the amygdala, olfactory tubercle, septal area, nucleus accumbens, and frontal cortex. Dopaminergic afferents enter the hippocampus mainly through the dorsal route together with mesocortical dopaminergic afferent. The major input to the rostral and caudal hippocampus derives from the ventral tegmental area (Scatton, Simon, Le Moal and Bischoff, 1980). In addition to these

tracts, there are also short-axon dopaminergic cells in several other regions. In the hypothalamus DA cells send axons to the median eminence to modulate the output of releasing and inhibitory hormones from the neuroendocrine cells there. Few dopaminergic cells are present along the third ventricle in the hypothalamic and preoptic periventricular nuclei. DA containing cells have recently been described in the paraventricular nucleus (Swanson, Sawchenko, Berod, Hartman, Helle and Vanorden, 1981). Finally, there is a system of dopaminergic cells around the fourth ventricle, extending within the core of brainstem to the hypothalamus (Skagerberg, Björklund, Lindvall and Schmidt, 1982).

Noradrenaline

Detailed biochemical and histofluorescence techniques have now mapped the widespread distribution of NA throughout the CNS (Hökfelt, Elde, Fuxe et al., 1978a; Moore and Bloom, 1979; Palkovits, 1978; 1980a). (Fig. 5A).

NA is synthesized from DA by the enzyme dopamine-β-hydroxylase (Fig. 4). Like DA, NA is found in clusters of cells in the midbrain. One of these is called locus coeruleus. Approximately one-half of all noradrenergic cells in the brain are located in this area, mainly ventral to the locus coeruleus (sub-coeruleus area). Axons from this area branch repeatedly and extend to almost every region in the CNS. There are relatively few terminals within any region, but the noradrenergic branches and terminals achieve their effects by secreting NA diffusely on to synaptic terminals in the

surrounding neurophils. As mentioned earlier, these actions are believed to be neuromodulatory in nature. Because of their widespread ramifications and diffuse actions, the noradrenergic cells are well-suited for setting levels of central neural activity underlying different behavioural states.

Complementing the locus coeruleus is a nearby cluster of noradrenergic cells in the lateral tegmental area. Projections from the lateral tegmental area overlap those of the locus coeruleus, but are directed mainly to the hypothalamus, where they are believed to participate in the regulation of releasing hormone secretion. Noradrenergic innervation of the hippocampus arises exclusively in the locus coeruleus. The axons course into the hippocampus by three routes: fasciculus cinguli, fornix and ventral amygdaloid bundle (Loy, Koziell, Lindsey and Moore, 1980).

Adrenaline

Distribution of ADR in the CNS has been mapped using biochemical and immunohistochemical techniques (Elde and Hökfelt, 1978; Moore and Bloom, 1979; Palkovits, 1978, 1980a). ADR is synthesized from NA in the catecholamine pathways (Fig. 4). ADR-containing perikarya have been identified in two lower brainstem areas, which project anteriorly as far as the diencephalon (Palkovits, Mezey, Zaborszky, Feminger, Versteeg, Wijnen, De Jong, Fekete, Herman and Kanjicska, 1980c), and posteriorly to the spinal cord (Ross, Armstrong, Ruggiero, Pickel, Joh and Reis, 1981). Terminals are found

particularly in the dorsal motor nucleus of the vagus (cranial nerve X) and the nuclei of the tractus solitarius, where adrenaline may have a role in modulating motor control of the viscera and taste information from the tongue, respectively (Chamban and Renaud, 1983; Shepherd, 1983).

Serotonin

Serotoninergic immunoreactive cells are first detectable in the rat brain early on the 13th embryonic day (Wallace and Lauder, 1983). In adult rats, several serotoninergic cell groups and scattered cells can be visualized (Steinbusch, 1981), including those in the dorsal raphe nucleus, central superior nucleus, and in the medulla. As shown in Figure 5C the fibres of these cells project widely to the forebrain, cerebellum, and spinal cord in a pattern that resembles that of the noradrenergic fibres. Beside the rostral raphe cells, a few serotoninergic cells have been found in the ventral tegmental area and in the interpeduncular nucleus (Palkovits, 1984). Immunoreactive serotoninergic cells have been reported in the hypothalamic dorsomedial nucleus by Frankfurt, Lauder and Azmitia (1981) and in the ventrolateral hypothalamus by Sakumoto, Sakai and Jouvet et al., 1982).

Medial 5-HT-containing pathways, originating in the midbrain raphe cells, pass the ventral tegmental area and ascend to the forebrain bundle. Lateral 5HT-containing pathways arise together with medial 5HT pathway, but run more laterally throughout the lateral hypothalamus.

A widespread and dense 5HT network has been demonstrated in the hypothalamus (suprachiasmatic nucleus, medial subdivision of the ventromedial nucleus, the lateral hypothalamic area), several thalamic nuclei, basal amygdaloid nucleus and substantia nigra (Steinbusch, 1981).

In the cerebral cortex, serotoninergic innervation is relatively dense and uniform across all layers (Lidov, Grzanna and Molliver, 1980). Fine serotoninergic fibres are distributed throughout the entire cerebellum (Takeuchi, Kimura and Sano, 1982).

ACTIVATION

The Control of Sexual Development and Puberty

The ovaries of immature rats when successfully transplanted into adults at once begin to function i.e. undergo cyclic changes leading to ovulation (Harris, 1964). This would indicate in the young animal the absence of some mechanism necessary for the development of puberty or the presence of some restraining factor (Frank, Kingenz and Gustavson, 1925).

Several reviews of neuroendocrine reproductive development have been published in recent years (Odell and Swerdloff, 1976; Winter, Fairman, Reyes and Hobson, 1978a; Styne and Kaplan, 1979; Ryan and Foster, 1980; Ojeda, Andrews, Advis and Smith White, 1980a; Ojeda, Aguado and Smith White, 1983). The mechanism regulating puberty have been mainly examined in four species: human, rhesus monkey, sheep and laboratory rat. It is realized that each species differs with regard to the control of puberty, but certain basic mechanisms operate in them all.

It is now generally agreed that puberty is not the result of a unique 'trigger', but is rather the culmination of a series of developmental processes initiated very early in life that progress in a synchronized manner throughout maturation. These changes affect all components of the reproductive neuroendocrine system (such as establishment of sexual behaviour and full fertility) and occur at different but concerted paces. The term 'puberty' is used to describe

the period during which the individual becomes sexually mature due to the interaction of many factors. Early events that occur during fetal life in primates and perinatally in the rat may be of particular importance for subsequent neuroendocrine maturation. As discussed later, the importance of certain events may be more easily demonstrated in the rat in which puberty is attained shortly (approximately 5 weeks) after the initiation of gonadotrophin release.

For the purpose of the present study, puberty in female rats was taken as being the age (around day 35-40 of life) at which the first post-oestrous discharge occurs. Puberty in the male is a much more gradual and less well-defined process, taking place between days 40-50. When considering how the onset of puberty is controlled it is possible to divide the various influencing factors into three main groups, social, metabolic and endocrinological, the effects of all being mediated by way of the CNS.

1. Social

Female mice reach puberty sooner if caged with a male than if kept in an all female group (Vanderberg, 1967). This social influence on puberty is not due to more rapid growth because the first oestrus is achieved not only at an earlier age, but at a lower body weight. It is possible that the presence of an olfactory cue from the male affects the female gonadal function and thus induces oestrus.

2. Metabolic

The possibility that the onset of puberty is influenced by metabolic cues has been considered for many years (Frisch, 1974, 1980; Steiner, Cameron, McNeill, Clifton and Bremner, 1983). Experiments in rats have demonstrated the importance of nutrition in reproductive function and puberty (Frisch, 1974, 1980). Female rats fed a high-fat diet have been observed to experience their first oestrus earlier than rats fed a lower fat diet, but in both cases the caloric intake at vaginal opening or first oestrus is similar (Frisch, Hegsted and Yoshinaga, 1975). Availability of essential fatty acids is another metabolic factor which greatly influences the initiation of puberty by delaying the vaginal opening and first ovulation. The mechanisms underlying this effect appear to reside at the hypothalamic and ovarian levels (Smith White and Ojeda, 1983b).

3. Endocrinological

It is possible that the mechanism which controls sexual function in the adult is also involved in the timing of puberty. At puberty something must upset the delicate balance between gonadal activity and hypothalamic function to cause the increased secretion of gonadotrophins. This could be due to a gradual lowering of the threshold sensitivity of target organs to their trophic hormones (Donovan and Harris, 1966).

Changes in the CNS induced by the presence of androgens during the 'critical' period gradually becomes apparent as the biogenic amine systems in the CNS mature. Different systems mature at different rates and puberty can only occur when all components are functional. Thus in the rat, the mean plasma gonadotrophin levels become raised during the second and third weeks of life. Short bursts of gonadotrophin release result in a wide variation in basal levels which only settle during the fourth week (Döhler and Wuttke, 1974; Kamberi, de Vellis, Bacleon and Inglish, 1980). Although total plasma oestradiol levels are also raised during this period, free levels are low due to their binding to circulating AFP, and do not rise until the fourth week of life (Puig-Duran, Greenstein and MacKinnon, 1979). Kamberi et al. (1980) observed a steady increase in hypothalamic GnRH levels over the first four weeks of life, and hypothalamic androgen receptors also increase in number during this time (Lieberburg, MacLusky and McEwen, 1980). However hypothalamic and pituitary oestrogen (MacLusky, Lieberburg and McEwen, 1979) and progestin (MacLusky and McEwen, 1980b) receptors and pituitary GnRH receptor (Dalkin, Bourne, Pieper, Regiani and Marshall, 1981) numbers reach a peak during the third week of life and then either decline or remain constant (Figs. 6 and 7).

In male rats, plasma LH levels remain low until puberty, while FSH, prolactin and testosterone levels gradually increase and hypothalamic GnRH levels also rise (Chiappa and Fink, 1977).

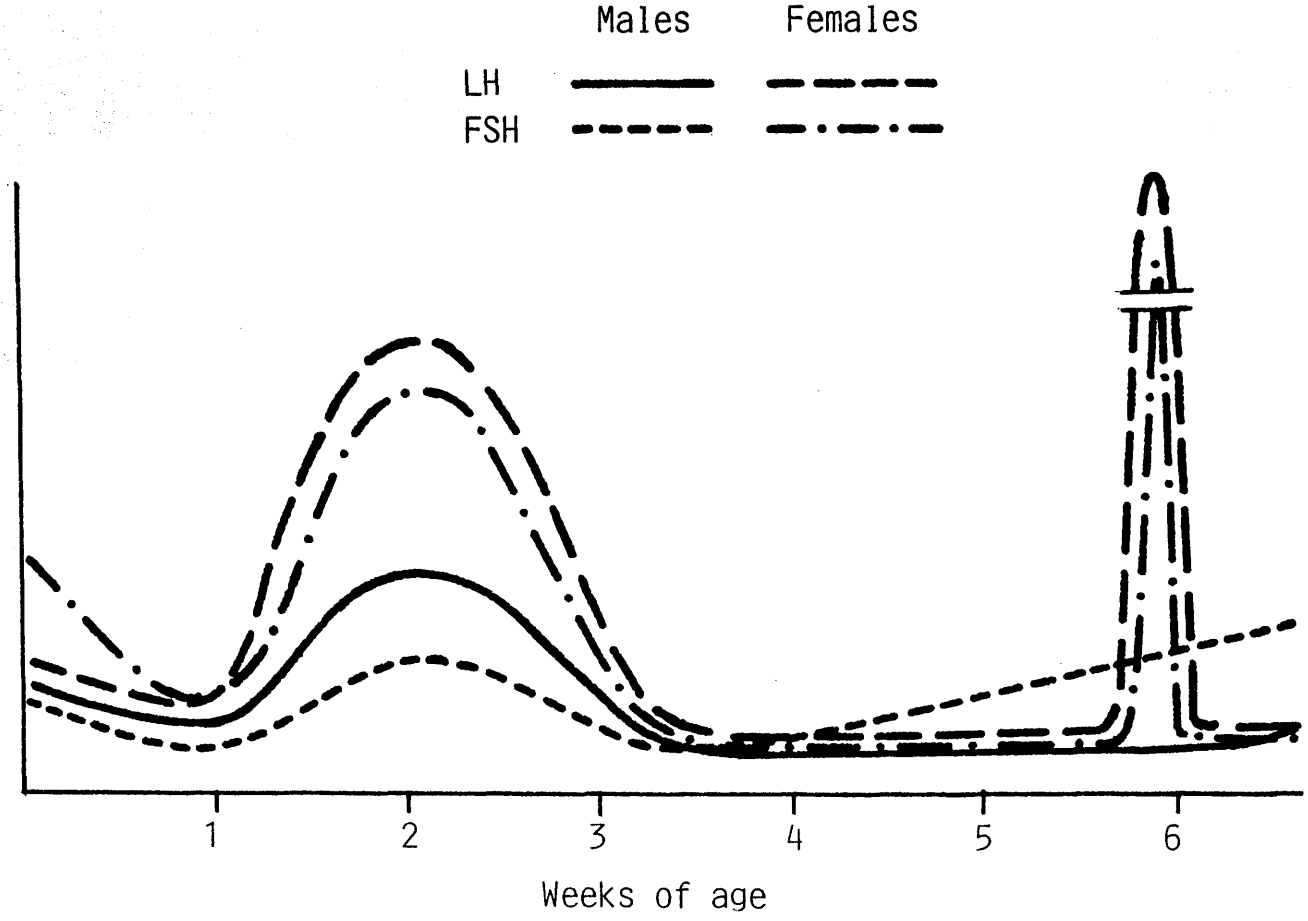


Fig. 6 Changes in plasma gonadotrophin levels during sexual development in the rat. Schematic diagram (not to scale) of relative changes Adopted from Döhler and Wuttke, (1974), Puig-Duran et al. - 1979.

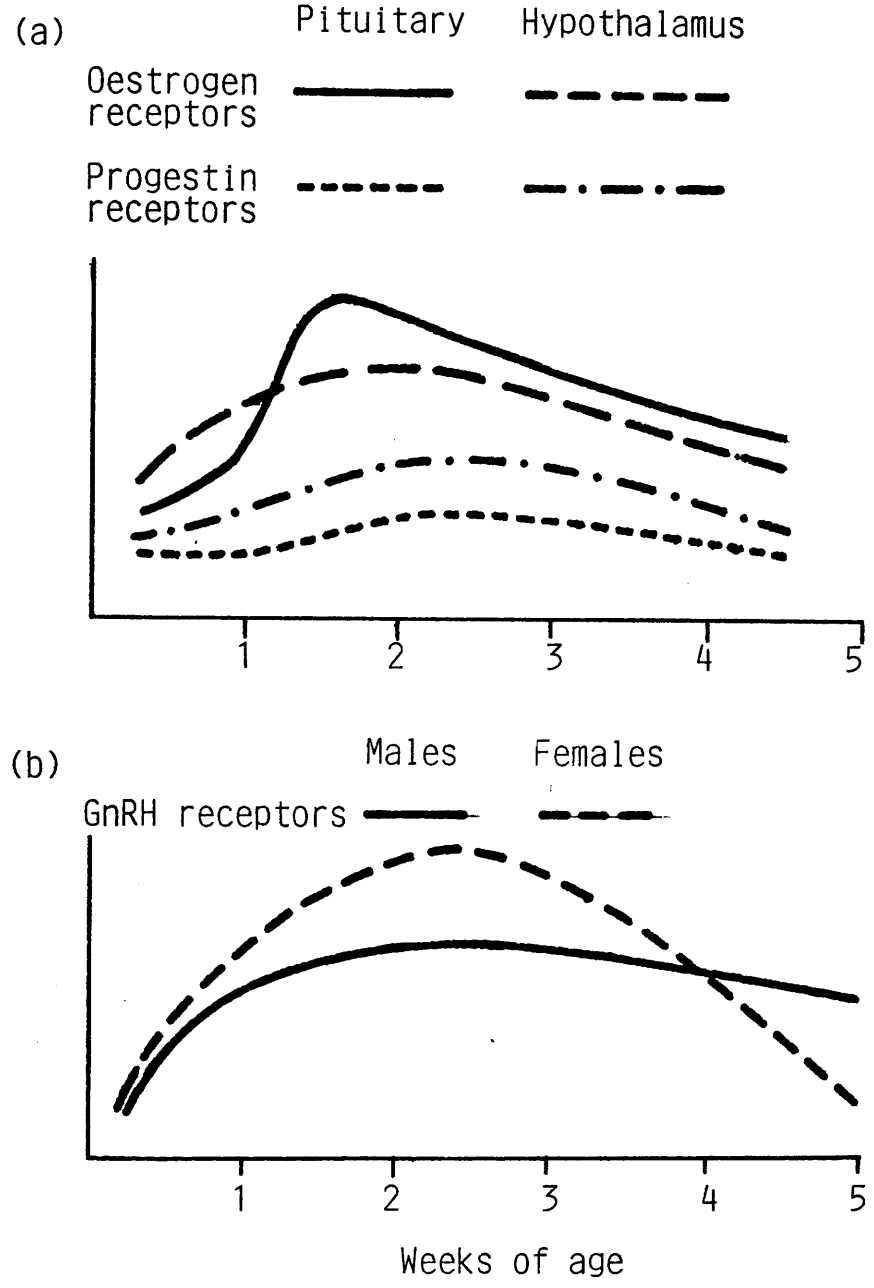


Figure 7 Changes in sex steroid and GnRH receptor levels during sexual development in the rat. Schematic diagram (not to scale) of relative changes in (a) hypothalamic and pituitary cytoplasmic oestrogen and progesterone receptor levels in female rat and (b) pituitary GnRH receptor levels in immature male and female rats. Drawn using data from MacLusky et al (1979), MacLuskey & McEwen (1980b), Dalkin et al (1981).

The Onset of Puberty

The onset of puberty can be considered as the transitory sequence of events that occur during reproductive immaturity. For many years the prevailing hypothesis to explain the onset of puberty has been that of gonadostat resetting (Andrews, Advis and Ojeda, 1981d; Ojeda, Smith White, Urbanski and Aguado, 1984). According to this hypothesis a decreasing central sensitivity to steroid negative feedback results in a gradual increase in gonadotrophin levels which in turn, stimulates further release of steroids until enough are produced to induce the first preovulatory gonadotrophin surge.

In the prepubertal female rat, the first developmental event of reproductive importance appears to be the infantile elevation in serum FSH levels (Döhler and Wuttke, 1974). This increase promotes follicular development and facilitates the subsequent acquisition by the ovary of a FSH and LH receptor population (Smith White and Ojeda, 1981a). Throughout the juvenile period (days 21 to approximately 32) the ovary develops under the control of low but pulsatile LH (and FSH?) titres and the influence of rising prolactin and growth hormone levels (Ojeda, Negro-Vilar and McCann, 1981b). Circulating ADR as well as direct adrenergic and vagal influences also appear to facilitate the ovary's maturation, through a mechanism involving augmentation of gonadotrophin effects (Aguado, Petrovic and Ojeda, 1982; Ojeda, Smith White, Aguado, Advis and Andersen, 1983a). Although mean levels of radioimmunoassayable serum gonadotrophin do not increase as

female rat matures at the end of the juvenile period, the pattern of pulsatile LH release changes (greater pulse amplitude). This perhaps reflects completion of interneuronal connections that permit a synchronized activation of GnRH-secreting neurones (Richard, 1980). At this time the pattern of elevated GH and prolactin levels also becomes established. Under these conditions the follicles destined to ovulate at puberty enter into their final maturational stage. Significant increases in LH and FSH secretion and in β-adrenergic receptors occur, while GnRH receptor numbers decline.

The factors determining the marked increase in oestradiol secretion which precedes the first surge of gonadotrophins are not clearly understood (Puig-Duran et al., 1979). Both a critical level of follicular development in the presence of maintained gonadotrophin stimulation, and perhaps CNS signals reaching the ovary via neural pathways, may be involved. Once serum levels achieve sufficient amplitude, and are maintained for an adequate length of time, the central component of positive feedback is activated. This occurs in the presence of moderately elevated serum progesterone levels, which facilitate the stimulatory effect of oestradiol. Activation of noradrenaline impulse flow accompanied by an increase in prostaglandin E_2 synthesis, in turn, elicit the first LH surge. Between this time and ovulation a further change occurs at the central level; the hypothalamic-pituitary unit becomes less responsive to oestradiol negative feedback. This change is not, however, completed until after puberty.

Throughout adult reproductive life oestradiol inhibitory effectiveness depends on the presence of progesterone. In addition, both steroids interact to maintain cyclic gonadotrophin release.

Monoamines in the Control of Puberty Onset in Female Rats

Changes in neurotransmitter metabolism by psychotrophic drugs given during brain differentiation can lead to structural and biochemical alterations in discrete regions of the brain (Dörner et al., 1977), permanent behavioural changes (Dörner, 1985) and to long-term alterations of gonadotrophic function (Dörner et al., 1977). Monoamines in the hypothalamus and amygdala have been implicated in the control of gonadotrophin secretion in adult female rats (McCann, Moss, Ojeda, Martinovic and Vijayan, 1977) and are also involved in the gonadotrophin-releasing mechanisms before puberty (Raum, Glass and Swerdloff, 1980).

As was first reported by Shirama, Anazawa, Takeo, Shimizu and Malkawa (1976) treatment with monoamine oxidase inhibitors during the first two or three weeks of life induce precocious puberty in female rats. The puberty advancing effect caused by the inhibition of monoamine degradation can also be demonstrated after postnatal psychosocial deprivation which will delay puberty. Treatment with pargyline from days 15 to 28 of life will normalize the onset of puberty (Dücke, Rohde, Smollich and Dörner, 1978). Administration of pargyline blocks the enzyme involved in the degradation of monoamines and thus increases the stores of biologically active compounds.

Inhibition of onset of puberty by higher centres of the brain

Damage to the hypothalamus, whether induced experimentally, accidentally or by tumour, often leads to precocious puberty. Because of this a gonadotrophin-inhibitory centre has been postulated to exist in the posterior hypothalamus of man.

Isolation of the medial basal hypothalamus in 3 week old rats will advance vaginal opening, presumably due to the severence of inhibitory pathways (Ramaley and Gorski, 1967). This inhibitory influence may come from the hippocampus and be antagonized by an effect arising in the amygdala, a situation which is already thought to feature in the control of ovulation in the adult (Kawakami, Seto, Terasawa and Yoshida, 1967). There is however, other evidence which would tend to contradict this idea. Lesions of the hippocampus delay vaginal opening (Bar-Sela and Critchlow, 1966), and hippocampal and pyriform lobe damage in infancy diminish gonadal development (Riss, Burstein and Johnson, 1967). Inhibition of pubertal development seems to come from the amygdala and/or stria terminalis. Evidence for this has been provided by Elwers and Critchlow (1961), who demonstrated advanced puberty in female rats with lesions in either of these two areas, and by Bar-sela and Critchlow (1966) who delayed vaginal opening in the rat by electrical stimulation of the amygdala.

Sex differences in Neurotransmitter Systems

The sex differences which are found in neuronal connectivity (for a review see Toran-Allerand, 1984) suggests that such differences may also be expected with respect to the neurotransmitter content measured within sexually dimorphic areas. The state of activity within a specific region can be ascertained from:

i) the concentrations of neurotransmitters, of their precursors and metabolites that are present,

ii) the activities of synthesizing and degrading enzymes within the regions and

iii) the receptor content.

The presumed involvement of monoaminergic systems in sexually dimorphic functions such as gonadotrophin release and sexual behaviour (Everitt, Fuxe, Hökfelt and Johnson, 1975; Héry, Laplante and Kordon, 1976; Meyerson, 1984) would suggest that sex differences in the activity of these systems could be expected.

Sex differences have indeed been found in neurotransmitter content and enzyme activities of the noradrenergic, dopaminergic and serotoninergic systems (Gordon and Shellenberger, 1974; Vaccari, 1980). Similar differences have been reported in monoaminergic receptors (Oren Sanz, Guillamon, Ambrosio, Segovia and Azuara, 1982; Fischette, Biegon and McEwen, 1983). Sex differences in neurotransmitter systems were in fact known even before any structural sex difference in the brain

had been demonstrated. In an earlier report, Kato (1960) demonstrated that from the 68th day of life onward the 5-HT content of the entire brain is higher in the female than in the male rat. Sex differences have been described in the content of 5-HT, of its precursors and metabolites, and in the activity of the enzymes implicated in its metabolic pathways in a variety of rat brain areas (Vaccari, 1980). Further, elevated 5-HT levels in the female brain have been reported to be present as early as the 12th postnatal day (Ladosky and Gaziri, 1970; Giulian et al., 1973). The early appearance of this sex difference is dependent on the neonatal presence of androgens in the male rat and, in turn, has been thought to be responsible for further sexual differentiation of the brain. This hypothesis would explain why it is that certain psychotrophic drugs which interfere with the serotoninergic activity of the brain, when given during pregnancy, can interfere with processes such as "defeminization" and "masculinization" of the brain (Jarzab and Döhler, 1984). A similar involvement in sexual differentiation has been claimed for the catecholaminergic system (Hyyppä, 1969; Hyyppä and Rinne, 1971; Reznikov, 1978).

In addition to its use for tracing sex differences in neurotransmitter systems, chemical assays may give an indication of the influence of gonadal hormones upon the activity of a certain neurotransmitter system. They therefore enable one to relate the neurotransmitter system in question

to sexually dimorphic functions which vary consistently. Although this technique does not allow a detailed morphological insight into the observed differences, it does however clarify the knowledge of a given morphological sex differences to a considerable extent.

Effects of Gonadotrophins and Gonadal Hormones on Brain Monoamines

A number of investigators have examined the relationships existing between circulating gonadal hormone levels and the metabolism of monoamine neurotransmitters (chiefly NA in the brain (Vaccari, A., 1980; Harris, 1964; Gorski et al., 1977)). Hormone levels have either been allowed to vary physiologically (i.e., with the oestrous cycle) or have been modified experimentally (i.e., by castration, with or without replacement therapy).

The specific aim of such studies - an estimation of whether more or less of a transmitter is being released at a particular synapse, causing greater or lesser activation of postsynaptic receptors - cannot really be attained using existing methods. Instead, investigators have obtained indirect evidence - e.g. that presynaptic neurones contain more of the transmitter or synthesize it faster after a particular endocrine manipulation - and have assumed that the transmission of information across the synapses is proportional to what is actually being accumulating pre-synaptically.

Gonadectomy appears to have been first shown to modify brain monoamine metabolism by Donoso and his associates (1967). These investigators reported that NA levels in the anterior hypothalamus were significantly elevated (and those of DA reduced) 10 days after rats were castrated. In a subsequent publication (1969), they showed that treatment of ovariectomized rats with oestradiol plus progesterone could restore the concentrations of both neurotransmitters to normal. Studies by Fernstrom and Wurtman (1977), Gaziri and Ladosky, 1973; Crowley, O'Donohue and Jacobwitz (1978) have provided evidence that removal of gonads also increases the synthesis and/or turnover of NA in whole brain or in particular regions.

Sex differences in the human brain

Development of catecholaminergic and serotoninergic systems in the human brain during the period of sexual differentiation has been sparsely investigated. DA and 5HT are present in the human brain as early as three to four months of gestation (Nobin and Björklund, 1973). In 1983 Gilmore and Wilson reported on sex differences in indoleamine and catecholamine concentrations in the brains of mid-term human fetuses obtained by hysterotomy. Masudi and Gilmore (1983) found significantly higher DA and 5-HT levels in the hypothalamus compared with the cortex although NA and 5HIAA levels were similar in both regions. However, since this previous study, almost all mid-term abortions are now performed by administering

prostaglandin E_2 (PGE_2), extra-amniotically.

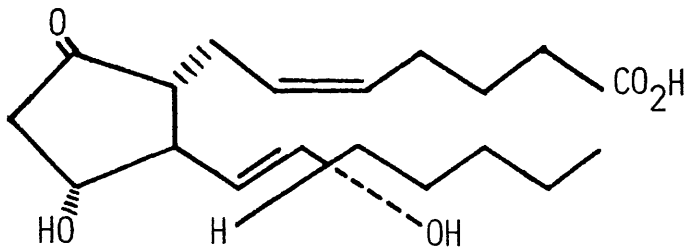
This method of mid-term pregnancy terminations introduces many factors such as a variable length of labour, stress, hypoxia and the presence of PGE_2 itself may affect neuro-transmitter levels in the fetus, thus rendering the suitability of this source of specimen for such studies doubtful.

Prostaglandin as an Abortifacient

There are several methods currently employed for therapeutic abortions. Early pregnancy is generally terminated by curretage or vacuum suction. After the 12th week of pregnancy, terminations, until recently, were sometimes brought about by abdominal hysterotomy. However, this procedure is associated with a slight operative and post-operative risk (Karim and Filschie, 1970). Also the presence of a scar in the uterus may in future pregnancies render caesarian section the only safe method of delivery. Another method of therapeutic termination of pregnancy is the intra-amniotic injection of hypertonic saline. This procedure is usually employed after 14 weeks and although generally successful requires skill and experience. Therapeutic abortion is therefore not a simple operation, and no method of terminating pregnancy before term is entirely devoid of risk.

The extra- and intra-amniotic injections and intravenous infusion of $PGE_2\alpha$ and PGE_2 have been investigated for use in first and second trimester pregnancy termination and are

considered to be comparatively effective and safe. PGE_2 has fewer side effects from $PGF_2\alpha$ and the dose required for complete abortion is much less.



Prostaglandin E_2 (dinoprostone)

Prostaglandins have been detected in the amniotic fluid during normal pregnancy, at full term parturition and during spontaneous abortion (Karim and Devlin, 1967). At mid-term gestation, although PGE_1 is present in small quantities (0.06 - 1.4 ng/ml), neither PGE_2 nor $PGF_2\alpha$ have been found in the amniotic fluid. The presence of both of these has however, been recorded in the fluid during labour ($PGF_2\alpha$ in much greater concentrations than PGE_2).

Prostaglandins are thought to affect the hormonal functioning of the feto-placental unit, causing a rapid fall in plasma progesterone. The removal of the progesterone block on inherent myometrial activity results in the initiation of labour. Prostaglandins also have a direct effect of themselves on the uterine myometrium.

Karim (1972) investigated in detail the effects of PGE_2, on the pregnant uterus. He observed that the uterine activity produced by PGE_2 in the first and second trimesters

of pregnancy is different from that observed near term. Intravenous infusion of PGE_2 near term produce normal labour like contractions with an increase in both frequency and amplitude, but no increase in tone. In the first and second trimesters PGE_2 infusions also increase uterine muscle tone. It is therefore likely that this form of pregnancy termination may cause a higher incidence of stress and hypoxia to the fetus as compared with hysterotomy and may, therefore affect neurotransmitter levels in the fetus.

Both PGE_1 and E_2 have been shown to inhibit the electrically and K^+-evoked release of NA from slices of rat brain cortex, and the K^+-evoked release of NA from rat hypothalamic synaptosomes (Hillier-Templeton, 1980; Reimann, Steimhauer, Hedler, Starke and Heriting, 1981). PGE_2 has also been shown to reduce the electrically-evoked release of DA from slices of rat corpus striatum, although Von Voigtlander's (1976) results showed that PGE_2 failed to affect the release of DA in the corpus striatum. PGE_2 may therefore play a role in presynaptic inhibition of cerebral catecholaminergic neuronal activity. However, the results obtained to date, are equivocal, as Reinman failed to demonstrate any inhibitory effect of PGE_2 on catecholamine release from slices of rabbit cortex and striatum.

Hypoxia, which could result from the PGE_2-induced pregnancy termination, may also bring about changes in neurotransmitter levels in various parts of the brain (Siddiqui, Clark and Gilmore, 1985). It has also been suggested that

different regions of the brain may be more susceptible to hypoxia than others. (Anisman, 1978). A stress too is known to cause changes in neurotransmitter output, which in turn modulates adrenocorticotrophic hormone (ACTH) secretion. The adrenocorticotrophic system plays an important role in full-term parturition when intra-uterine stress upon the fetus results in a release of ACTH from the fetal adeno-hypophysis, which in turn increases cortisol output from the adrenal cortex. The cortisol indirectly stimulates the maternal posterior pituitary to release oxytocin, which brings about increased uterine contractions.

In summary, extra-amniotic injections of PGE_2 may result in changes in monoamine transmitter levels in the fetal brain. These changes could make it difficult to determine exactly what is occurring under normal circumstances with regard to neurotransmitter turnover in brain regions.

Post-mortem effect on brain tissues

Human tissue cannot usually be analyzed until some time has elapsed after death. In our laboratory 2 to 5 hours usually elapse between delivery of a human fetus after prostaglandin-induced pregnancy termination and brain tissue processing (Siddiqui et al., 1985). Central monoamine levels in these fetuses show too great within-group variations to allow useful developmental observations to be made. Post-mortem degradation of enzymes, monoamines and metabolites depends on the time between death and autopsy, temperature

at which the body is maintained following death, and the interval between autopsy and freezing or assay of the tissue removed (Fahn and Côte, 1976; Grabarits, Chessik and Lal, 1966; Sloviter and Conner, 1977). It is therefore very important to be able to estimate the degree to which the above factors may alter differentially the concentrations of brain neurotransmitters present in the living tissue.

High Performance Liquid Chromatography coupled with Electrochemical Detection

In recent years the technology of high performance liquid chromatography (HPLC) has reached a degree of maturity which permits its routine use in most laboratories at a modest cost. HPLC usually involves ordinary column chromatography with the employment of stationery phases of a very small particle size so that excellent separations may be achieved with relatively short columns. However, because of the small particle size, the columns will not achieve adequate flow without operation under pressure. For separation of the biogenic amines and their metabolites, chromatography is usually carried out under pressures of 3000 psi or less.

The use of electrochemical detection systems for the measurement of biogenic amines and their metabolites is a recent development. These substances are oxidized at an inert electrode surface by an applied potential. Quantification is thus achieved by counting the electrons released in the oxidation process. This is accomplished by measuring the current. The sensitivity of this technique as applied to

catecholamines analysis is in the low picogram range and is thus comparable to the sensitivity achieved by routine gas chromatography - mass spectrometry.

Although the technique of HPLC with ECD only originated in 1973 (Kissinger, Refshauje, Dreiling and Adams) it is already well established and has replaced many of the classical biochemical procedures for measurement of monoamines and their metabolites. The rapid acceptance of this technique is in part explained by the fact that the basic equipment required for HPLC-ECD analysis of monoamines is relatively inexpensive. Furthermore, the sensitivity achieved in routine use is excellent, and it is possible to quantitate numerous amines and their metabolites in a single sample. Figure 8 illustrates the type of separation that can be achieved for monoamines and some metabolites employing HPLC-ECD.

The electrochemical oxidation of catecholamines has permitted development of the technique of voltammetry, whereby extraneuronal concentrations of catecholamines (an index of concentration in the synaptic cleft) can be measured directly in freely-moving animals (Adams, 1976). This technique, applied so far to DA, but not to the other catecholamines, still suffers from limitations of specificity; however it offers much promise. Histochemical, especially immunofluorescence, techniques have permitted mapping of neurones containing catecholamines and the enzymes involved in their formation in the brain (Palkovits, 1984).

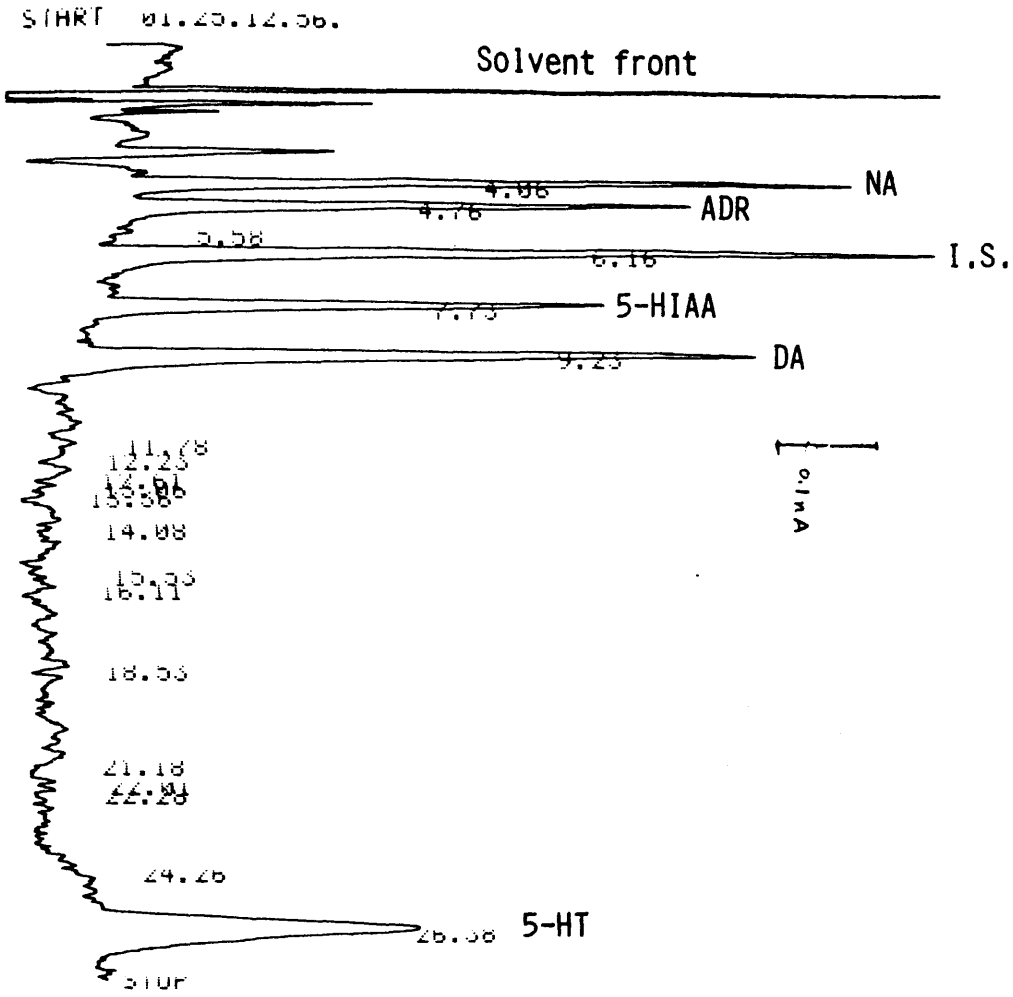


Fig. 8 Representative chromatogram showing separation of noradrenaline (NA), adrenaline (ADR), dihydroxybenzylamine (Internal standard), 5-hydroxyindolealetic acid (5-HIAA), dopamine (DA) and 5-hydroxytryptamine (5-HT); 2ng of each injected in a reference solution (20µl). Mobile phase: citrate-acetate buffer (pH5.2) containing SOS (51mmol/l), 10% methanol and 1.2% THF. Potential: +0.65V.

MATERIALS AND METHODS

Animals:

The Sprague-Dawley rats used in this study were a colony originating from Tuck and Sons, Battlesbridge, Essex. They were housed in the Animal Unit at the Institute of Physiology, University of Glasgow, under conditions of controlled temperature (21 $\pm$ 1°C) and 12 hours light: 12 hours darkness (lights on 06.00 - 18.00 hours). Food and water were available ad libitum.

All experimental animals were raised in groups of the same sex. Each group, originating from two litters, varied randomly in numbers from 2 to 5 animals per cage.

Cages were of transparent polycarbonate (macrolon) with a removable galvanised grill lid. The dimensions were approximately 42 x 25 x 20 cms.

Cages were cleaned each Monday when fresh sawdust was supplied. Females with litters were left undisturbed and the infantile rats housed with their mothers (10-15 pups per dam) till weaning.

Breeding:

Female rats of the strain used have regular four day oestrous cycles. Females were left with intact males for a period of one week. Pregnant rats were then transferred to fresh cages and when approaching term were checked every morning and evening for the presence of litters. Any new litters detected in the morning were presumed to have been born during the previous night. Litters varied from 8 to 15

in number. Cannibalism by the mother was the only real danger to survival of the young.

Newborn rats were collected and sexed. This was determined by measuring their anogenital distance. This distance in the male is about two-three times longer than that in the female. The infant male rats underwent castration or sham-castration within 24 hrs of their birth. Within the first five days of birth, female pups received either testosterone in corn oil, or corn oil only (controls). At 21-23 days post-partum the animals were weaned, ear-clipped for identification and rehoused according to sex and day of neonatal treatment.

Experimental Design:

Neonatal rats were assigned to one of the following treatment regimes:

Males
1. Castration
2. Sham-castration

Females
3. 500 µg testosterone in (50 µl) corn oil, subcutaneously for five days.
4. Corn oil (50 µl) subcutaneously for five days.

All treatments were given between 13.00 - 15.00 hours.

Records were kept of weight, onset of puberty and vaginal cyclicity. The latter was determined by taking daily vaginal smears.

From this point experimental methods are described in the order in which they were performed.

Operating procedures:

All male pups were initially anaesthetized by placing them in an ether chamber. Anaesthesia was maintained throughout the operation by means of an ether-soaked pad placed over the nostrils. Instruments used were sterilized with absolute alcohol. Fatalities were rare, and any animal which ceased breathing due to over exposure to ether, could invariably be resuscitated by squeezing the upper abdomen and diaphragm.

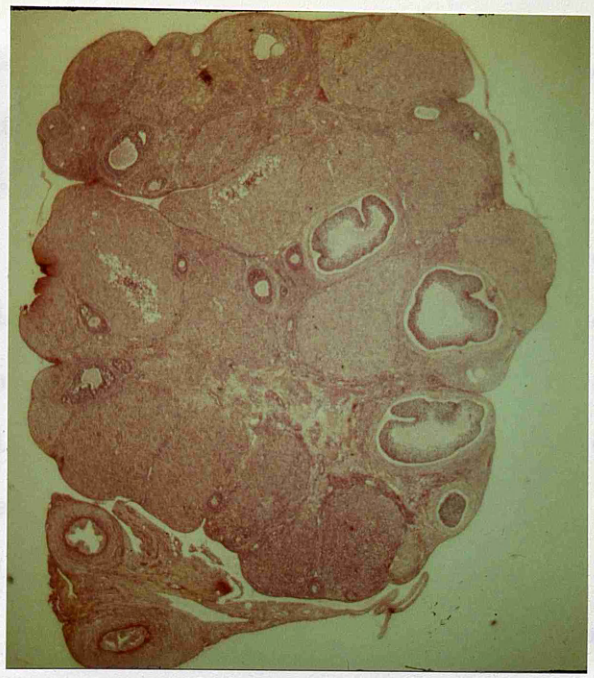
a) Castration:

While under anaesthesia the male pup was secured to the dissecting board in a supine position and a midline incision made in the lower abdominal cavity beside the anogenital sinus. The muscle of the abdominal cavity was cut on one side of the midline incision and the testes exposed by pressing downwards on the abdomen so that each testis was extruded in turn. The testes were surgically removed and the skin incision closed by a single mercerised cotton stitch. The incision was sprayed with antibacterial polybactrin powder.

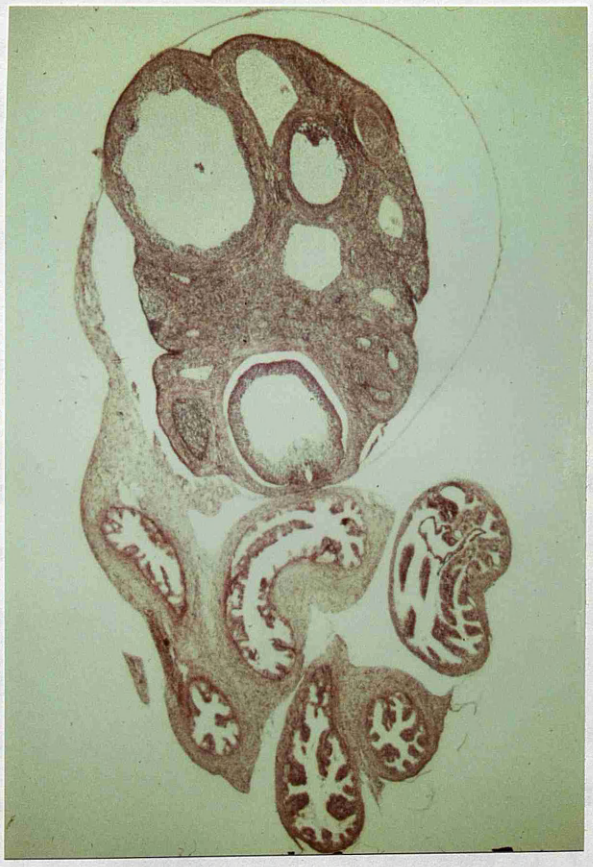
The same surgical procedure was repeated for sham-castrated animals except that their testes were not removed.

b) Hormone administration:

Testosterone in oil was administered to female pups by a subcutaneous injection under the loose skin at the back of the neck. Injections were made with 1 ml plastic disposable syringes (plastipak) with 0.01 ml graduations. The needles



(a) x 50



(b) x 50

Fig. 9

(a) Ovarian section of an oil-treated female rat.
(b) Ovarian section of an androgenized female rat.

used were 25 G (Microlance). A dose of 100 µg/50µl was administered daily for the first five days of life.

Testosterone (^{4}-Androsten-17β-ol-3-one) was supplied by Sigma in 5 gm bottles. The testosterone was dissolved in absolute alcohol. The appropriate volume of corn oil (Mazola) was added and the alcohol was evaporated by slowly heating the mixture over a gas flame. 50 µl of this solution was injected each day for five days.

To minimise the possibility of cannibalism the mother was separated from her litter just prior to any experimental manipulation. Pups destined to receive either surgery or injections were also removed from their home cage, but their littermates left in it. Immediately upon recovery from anaesthesia, the experimental pups were replaced in their cage and all pups, including untreated littermates, were sprayed with polybactrin powder before the mother was returned to them.

The relationship between brain monoamine levels and the manner of gonadotrophin release was investigated in the experimental groups at 60, 75, 90, 120 and 180 days. All control females were killed on the day of oestrus nearest to the assigned age group.

Vaginal opening:

The administration of testosterone to female rats results in the delayed anovulatory syndrome (DAS) and the animals exhibit persistent cornification of the vaginal mucosa (Fig. 9). In some animals vaginae do not open at all. Control

and androgenized rats were examined daily from 10 days after weaning to establish the age at which vaginal opening occurred.

Vaginal Cyclicity:

Vaginal lavages were performed daily from puberty up to the time at which animals were killed. The technique of smearing for assessment of changes in vaginal cytology is simple and rapid (Stockard and Papanicolaou, 1917). It consists of swabbing the vaginal lumen and examining the smears under the compound microscope. The stage of the cycle for each animal was determined by the following criteria:

Major cell-type present	Stages of the cycle
Cornified epithelial cells	Oestrus (O)
Leucocytes	Di-oestrus (D)
Nucleated epithelial cells	Pro-oestrus (P)

The vaginal smears were monitored each morning.

Autopsy:

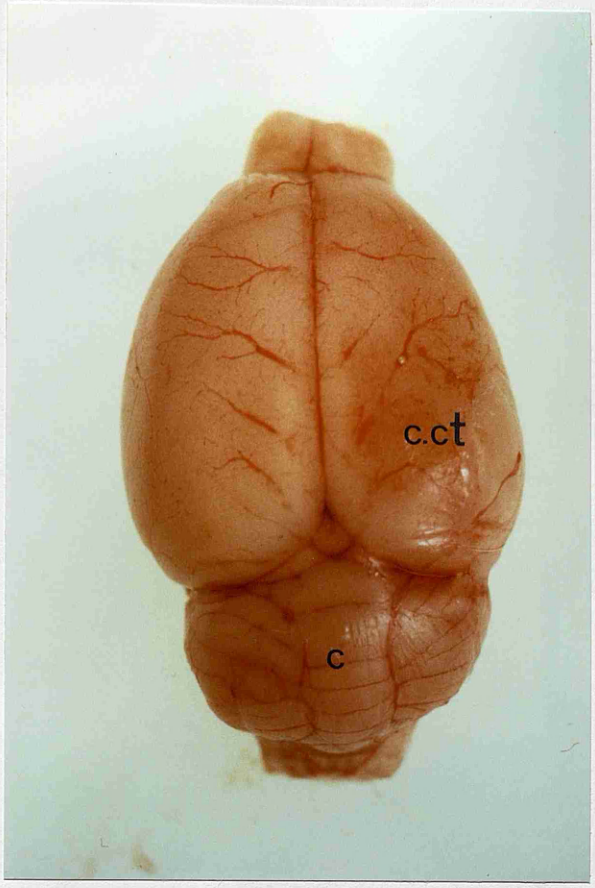
The animals were killed by decapitation using a guillotine. This procedure was considered to be relatively painless when performed very swiftly. The decapitated head was immediately placed on crushed ice, and trunk blood was collected, and centrifuged (Quickfit Instrumentation, England) for 5 minutes at 3000 r.p.m. at $4^{o}C$. The separated plasma was snap frozen in liquid nitrogen and stored at $-30^{o}C$ until assayed for its LH levels at St. George's Hospital Medical School, London. The abdominal cavity of the female was opened,

the two ovaries were excised so they could be checked for the presence of polycystic follicles before being fixed in Bouin's fluid for subsequent histologic examination.

Removal of the Brain:

The skin lying over the top of the neck and head was cut away and pointed scissors inserted into the foramen magnum so that the roof of the skull could be split along the midline. It was then cut laterally on each side from the posterior region to along near the olfactory bulbs. This enabled the complete skull to be removed, thus exposing the whole brain. The olfactory bulbs were cut and the brain eased upward from the anterior. This enabled the two optic nerves to be severed and the brain was then completely removed from the skull. Dissections were performed on an ice-cooled steel tray. Eight regions of the brain (as shown in Figs. 10,11 and 12) were surgically isolated using a sharp scalpel. These comprised:

1. Hypothalamus
2. Amygdala
3. Cerebellum
4. Cerebral cortex
5. Corpus callosum
6. Corpus striatum
7. Hippocampus
8. Corpora quadrigemina



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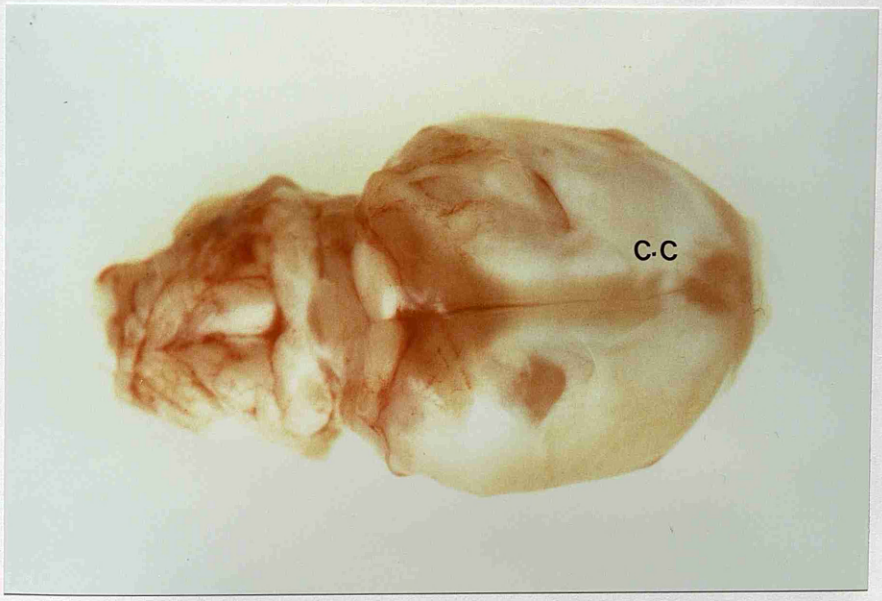
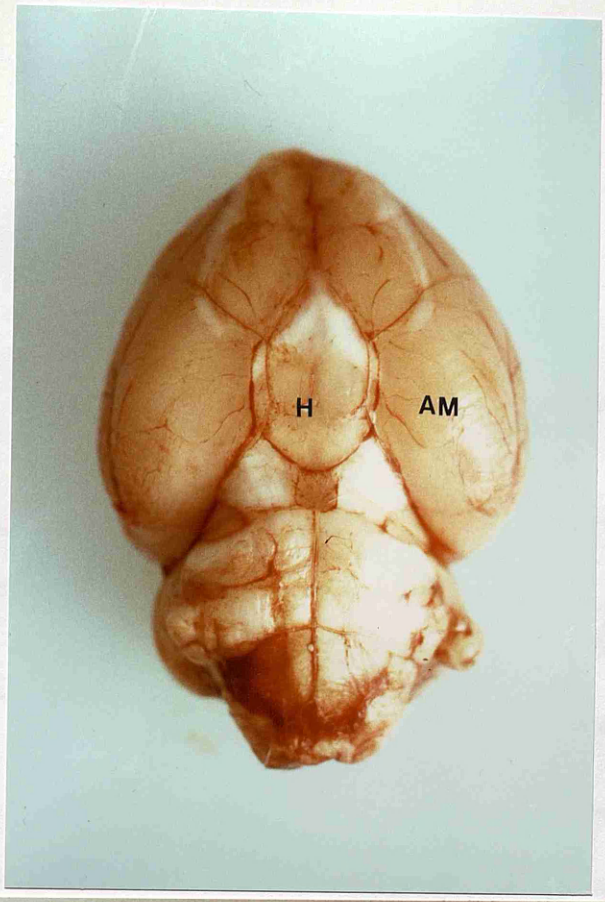


(b)

Fig.10

(a) Dorsal aspect of rat brain showing the cerebellum(c) and cerebral cortex(c.ct).

(b) Dorsal aspect of the rat brain showing the corpus callosum (c.c) after peeling back the cerbral cortex.



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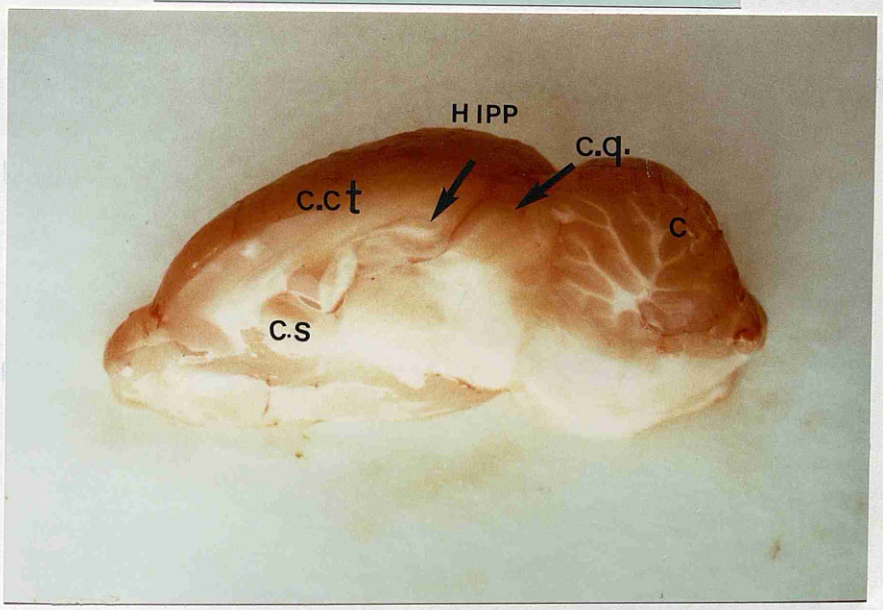


(b)

Fig.11

(a) Ventral aspect of the rat brain showing the hypothalamus(H) and amygdala(AM).

(b) Saggital aspect of the rat brain showing the cerebral cortex(c.ct), corpus striatium(c.s.), hippocampus(Hipp.), corpora quadrigemina(c.q.) and cerebellum(c).

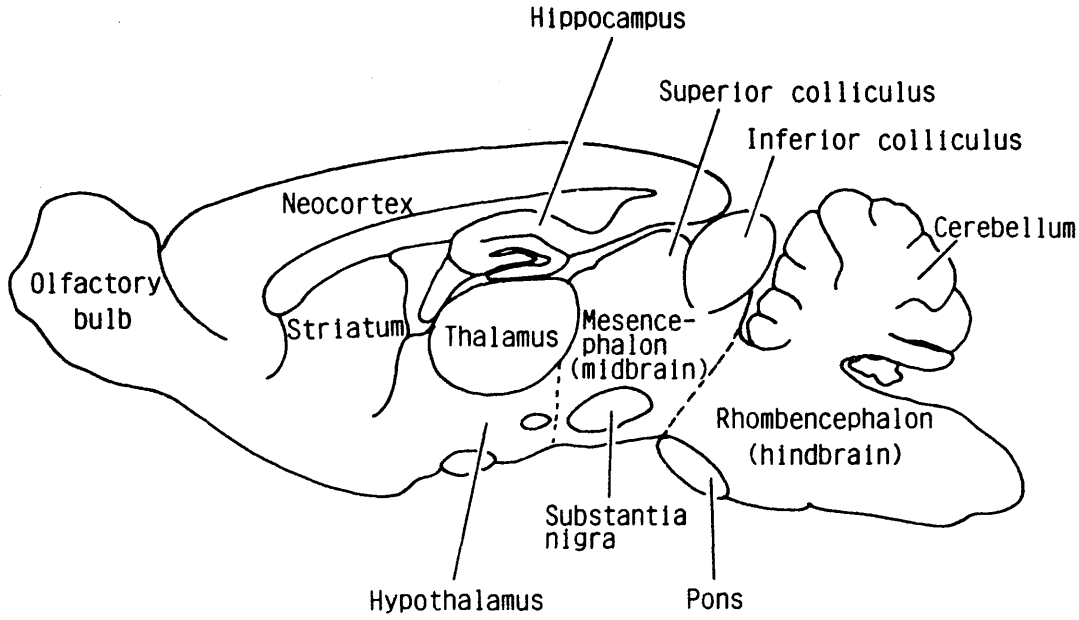


Fig. 12 Saggital map of anatomical structures in the rat brain. (Adapted from Nauta, W.J.H. and Feirtag, M. In 'the Brain' (1979) p.45).

Disposable Swann-Morton B.S.2982 scalpel blades were used for the procedures undertaken, as these had a curved end and were ideal for surgically isolating small regions of the brain.

The dissection of the brain was performed as follows: Proceeding from the ventral aspect, the hypothalamus was removed en bloc by a saggital cut medial to the temporal lobes and by a coronal cut rostral to both the optic chiasma and mamillary bodies. The amygdaloid complex, which extends between the pyriform cortex and the hypothalamus, is medially, dorsally and posteriorly bounded by the hippocampus. The amygdala was gently excised along the above borders.

Approaching from the dorsal aspect, the cerebellum was separated by cutting the three cerebellar peduncles. Fronto-parietal cerebral cortex was next removed by peeling it back along the roof of the partly exposed corpus callosum (a broad white band connecting the two hemispheres). By clearing the remainder of the white matter - the corpus callosum was separated by making a cut along its borders adjoining the hemisphere. The 'striatum' was dissected with the external walls of the lateral ventricles as latero-ventral limits and the corpus callosum as dorsal limits. The anatomically distinct structure of the hippocampus was then dissected. The protuberances of corpora quadrigemina were apparent following the removal of the cerebellum. A horizontal cut at the level of the anterior base of the cerebellum was made to dissect this out. The entire procedure

was completed within two minutes.

In 12-day old infant rats all regions were surgically isolated except the corpus callosum and hippocampus. These are not fully developed or distinct at this early age. The whole procedure of removing the brain and dissecting out its regions was carried out in exactly the same manner as it was performed in the adults.

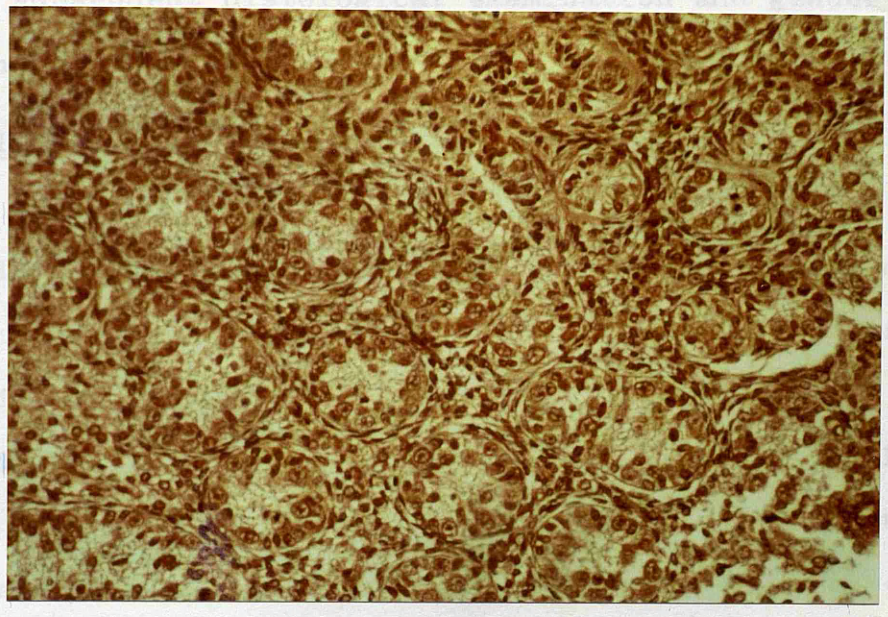
Sample Preparation:

The samples were immediately snap frozen in liquid nitrogen. They were then weighed and placed in a glass homogenizer, kept in ice. An appropriate volume of 0.1M Hydrochloric acid (along with the internal standard) was added and the tissues were homogenized gently. The homogenates were centrifuged for five minutes at 3000 r.p.m. using a microcentrifuge. The resulting supernatant was either injected immediately on to the reversed-phase High Performance Liquid Chromatography (HPLC) column or snap frozen in liquid nitrogen and stored at -30°C until assayed. However, all the samples were analyzed within one month of their preparation. 20 µl samples were injected on to the HPLC column and concentrations of noradrenaline (NA), adrenaline (ADR), dopamine (DA), serotonin (5-HT) and 5-hydroxy indoleacetic acid (5-HIAA) measured using an electrochemical detector.

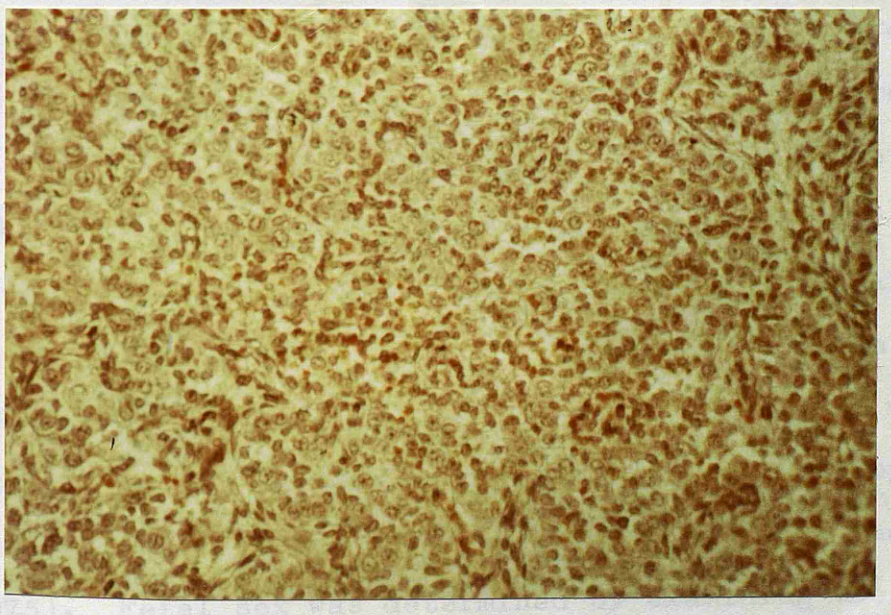
Collection of rabbit fetuses:

Rabbit fetuses used in this study were obtained from mature females (New Zealand White) which were mated in the Animal Unit of the Institute. Before mating, females were kept in isolation for at least 2 weeks (to avoid failure of conception due to pseudopregnancy). Pregnancies were terminated on the 28th day of gestation (term is 31 $\pm$ 1 day) either by extra-amniotic injection of prostaglandin E_2 or by hysterotomy immediately after the mother had been sacrificed by a sharp blow to the head. In the former case 1.1 mg of dinoprostone (Prostin E_2: UpJohn) in 3 mls of saline was injected intravenously (i.v.) into the marginal ear vein. If pregnancy was not terminated within 4-6 hours it was followed by administration of 1-2 1.U. of synthetic oxytocin Syntocinon - (Sandoz Pharmaceuticals) via the same route. In the latter animals the abdomen was opened via a mid-line incision and the entire uterus was removed and kept on ice. All the fetuses were removed individually within 30 minutes of the mother's death and weighed. Thereafter they were decapitated and the regions of hypothalamus, amygdala, cerebellum and fronto-parietal cortex surgically isolated. The method for the collection and storage of the brain tissues were the same as those used for the rats.

Fetal sex was initially determined by examination of the external genitalia. However, some difficulty was experienced in establishing the sex in this way. The fetal gonads were therefore removed and fixed in Bouin's solution



(a) x160



(b) x160

Fig.13
Sections of newborn fetal rabbit gonads,
(a) Testis showing presence of spermatogonia in the seminiferous tubules.
(b) Ovary.

for subsequent histological examination and gender identification (Fig. 13).

Collection of human fetuses:

This portion of the study was approved by the local ethical committee. Mid-term fetuses were collected from the Glasgow Royal Infirmary within 1 to 2 h of intra-vaginal delivery following extra-amniotic prostaglandin E_2 administration (Fig. 14). The 5 mg prostaglandin (Prostin E_2, UpJohn) was reconstituted in 50 ml of saline and was usually administered between 21.00 - 22.00 hr. The uterine contractions which followed culminated in the termination of pregnancy 7-9 hrs later. Fetuses were brought to the Institute of Physiology, where dissection was performed. The average time period between termination and freezing of the tissues in liquid nitrogen was 2-3 hrs. Details from each fetus was recorded on specially prepared sheets (Fig. 15). Crown rump length (CRL) was measured by Vernier calipers and the fetuses were weighed to their nearest gram on a Shandon balance. For assessing fetal age these measurements were compared with those in the tables published by Iffy, Jakobivits, Westlake, Wingate, Caterin, Kanafsky and Menduke (1975). Fetal sex was determined by examining the external genitalia. On the basis that the critical period for sexual differentiation of the brain occurs during mid-gestation when increased neuronal activity has been reported (Masudi and Gilmore, 1983) the fetuses were divided into two groups of 12-16 and 17-22 weeks.

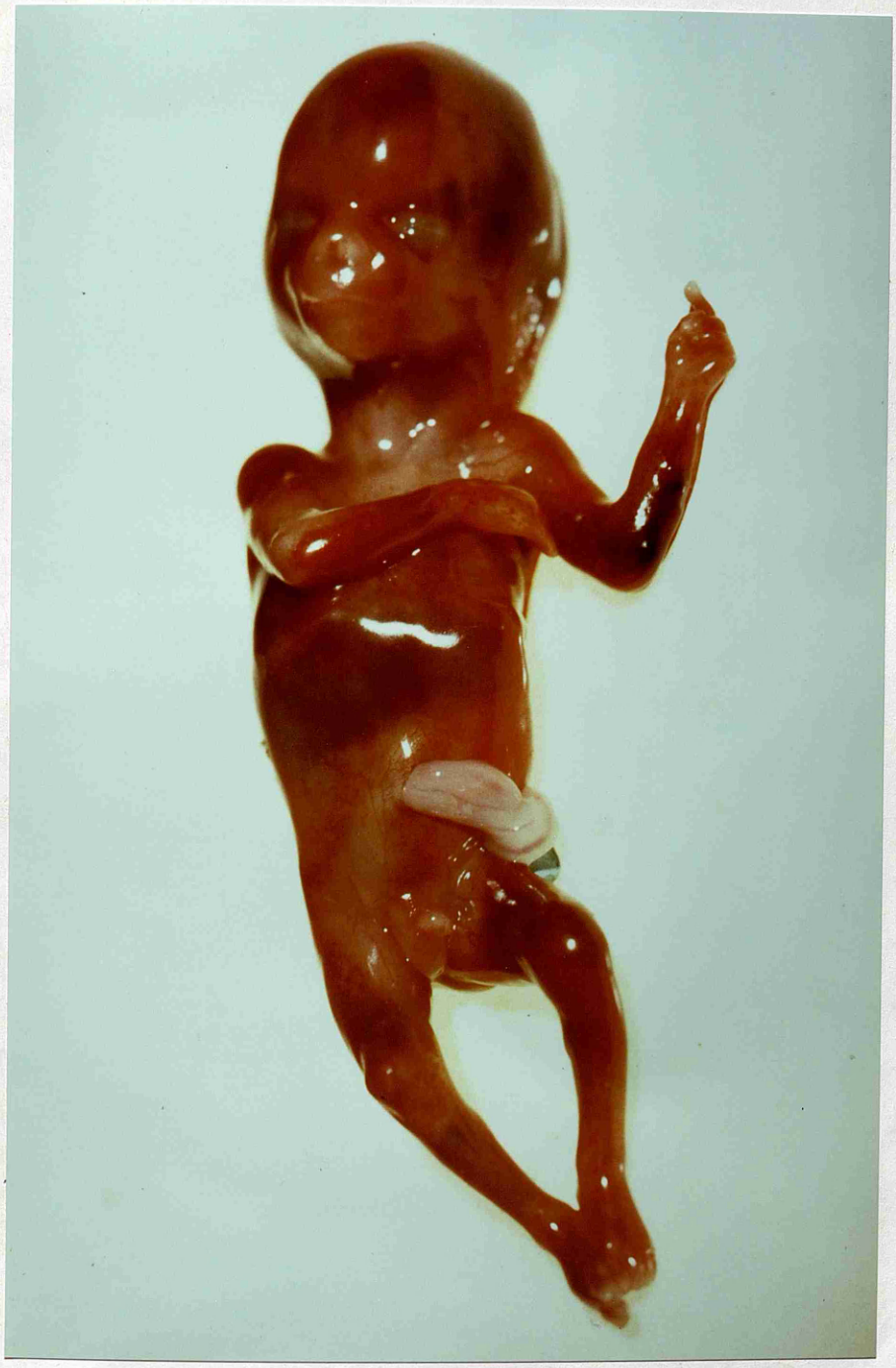


Fig.14
Human fetus of 18weeks gestation obtained from a PGE_2-terminated pregnancy.

UNIVERSITY OF GLASGOW

INSTITUTE OF PHYSIOLOGY

SPECIMENS OBTAINED FROM PROSTAGLANDIN TERMINATIONS

DATE..... NO. OF FETUS.....

CROWN-RUMP LENGTH..... WEIGHT.....SEX.....

PES LENGTH..... FETAL AGE.....

FETAL ABNORMALITIES..... DETAILS OF MOTHER.....

....

TIME INTRA-AMNIOTIC INJECTION GIVEN

.....

TIME FETUS DELIVERED..... TIME TISSUE REMOVED.....

TISSUE COLLECTED

PLACENTA..... AMNIOTIC FLUID.....

CSF..... CORD SERUM.....

PITUITARY..... HYPOTHALAMUS.....

CORTEX..... CEREBELLUM.....

OTHER BRAIN TISSUE..... OTHER ORGANS.....

....

REMARKS

Fig.15
Specimen sheet indicating data collected for each human fetal specimen

Dissection of the brain:

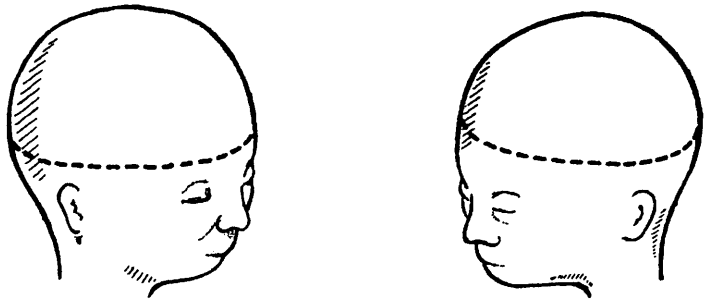
The brain was exposed by cutting across the frontal bones of the skull and down towards the ear to the angle of jaw on either side (Fig.16a,b). The whole of the front of the skull was lifted to expose the brain which was then tilted back out of the skull so that its ventral surface was exposed (Fig.16c,d). The hypothalamus, cerebellum and a piece of fronto-parietal cortical tissue were surgically isolated and frozen separately in liquid nitrogen. (Fig. 17).

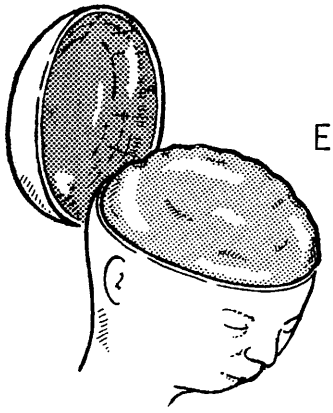
Catecholamine Analysis:

The technique of high performance liquid chromatography with electrochemical detection (HPLC-ECD) was employed throughout this project to measure the catecholamines and indoleamines present in the different brain tissue samples. The main components of the HPLC system are shown schematically in Figure 18 . This comprises:

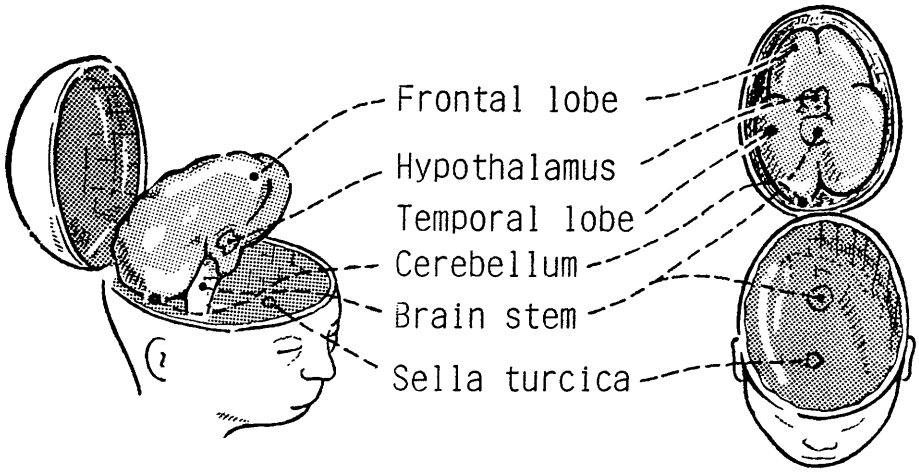
a. a microparticulate high resolution chromatographic column.
b. a pump capable of producing the high pressures necessary to force fluid through such a column and, after amine separation.
c. a detector able to detect and quantitatively measure the separated amines. In addition, the system includes an injection port, in-line filters, a solvent reservoir, a pressure gauge and a suitable recorder/data processor. Eluant from the solvent reservoir is filtered, pressurised and pumped through the chromatographic column.

a) Dissection of the skull, across frontal bones.





b) Exposed brain.



c) Tilted view of brain.

d) Ventral surface of the brain.

Fig.16 Dissection of the Human Fetel Brain, adapted from Masudi, 1981).

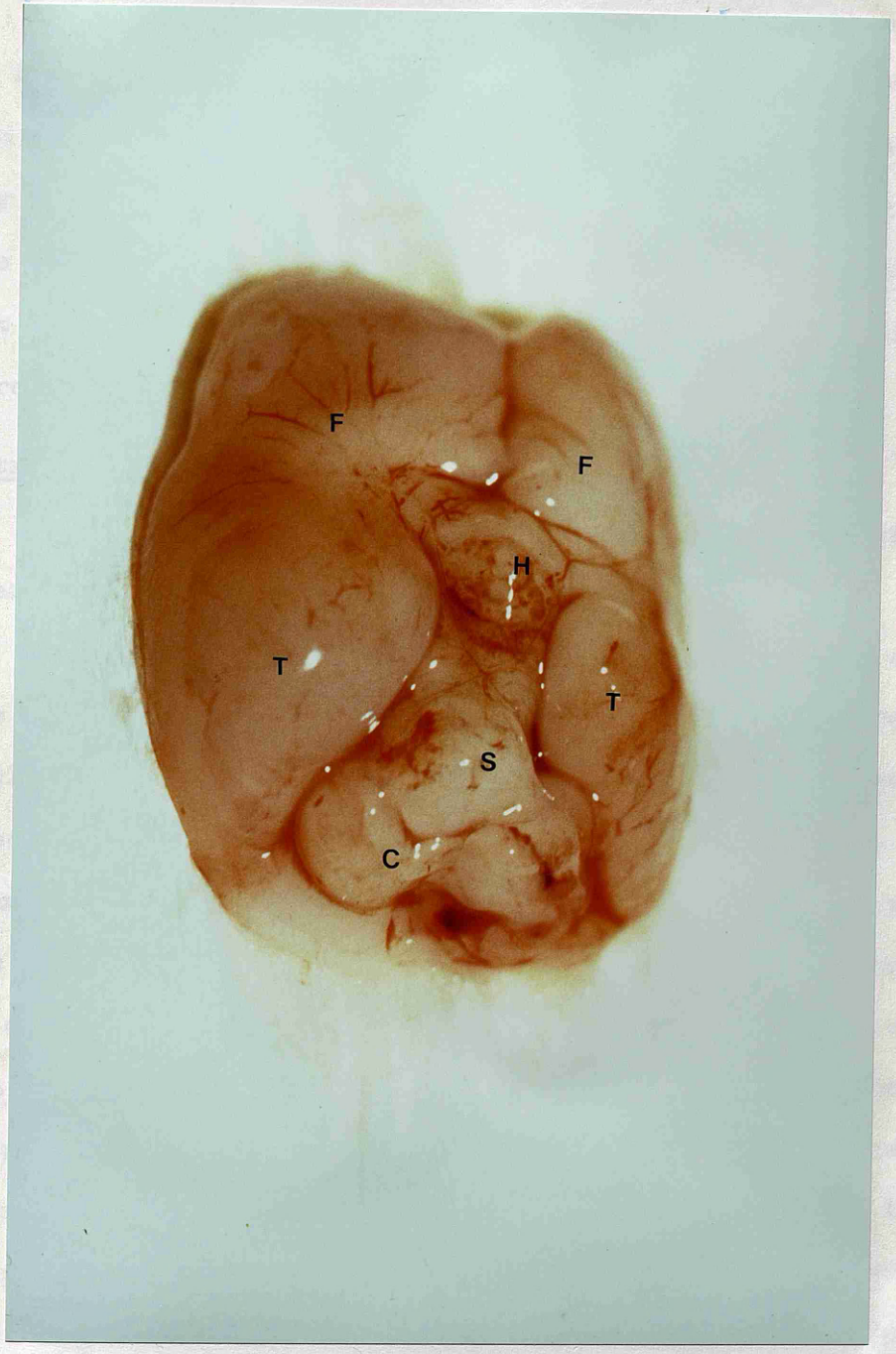


Fig.17
Human fetal brain of 18weeks gestation,

H = hypothalamus.
T = temporal lobe of cerebral cortex.
F = frontal lobe of cerebral cortex.
S = section of the brain stem.
C = cerebellum.

A mixture of solutes introduced into the system through the injection port is separated into components travelling down the column. The individual solutes are measured as they pass through the detector, the resultant voltage signal once amplified is recorded as peaks on a data processor. Peak heights/areas are proportional to quantity of the neurotransmitters present.

Instrumentation:

All equipment was purchased from Scotlab Instrument Sales Ltd. unless otherwise stated.

The mobile phase was pumped through the system at a rate of 1.5 ml/min using a Gilson 302 pump. Standards and samples were loaded onto the column through a Rheodyne 7125 injection valve, fitted with a 20 µl injection loop, using a Hamilton Syringe. The main analytical column was protected from deterioration by the samples first passing through the pre-column (Anachem). The analytical column itself consisting of a 25 cm x 0.46 cm stainless steel tube (Rainin Instruments), was purchased pre-packed with microsorb (5 µm) silica particles coated with hydrocarbon chains comprising 18 CH_2 groups in length. The column was frequently repacked(after about eight hundred injections), with Shandon ODS Hypersil (5 µm particle size) supplied by a Shandon Column Packing system. Compounds were detected by a Bioanalytical system (BAS Inc., Indiana, U.S.A.) LC-17 glassy carbon transducer cell (Fig.19).

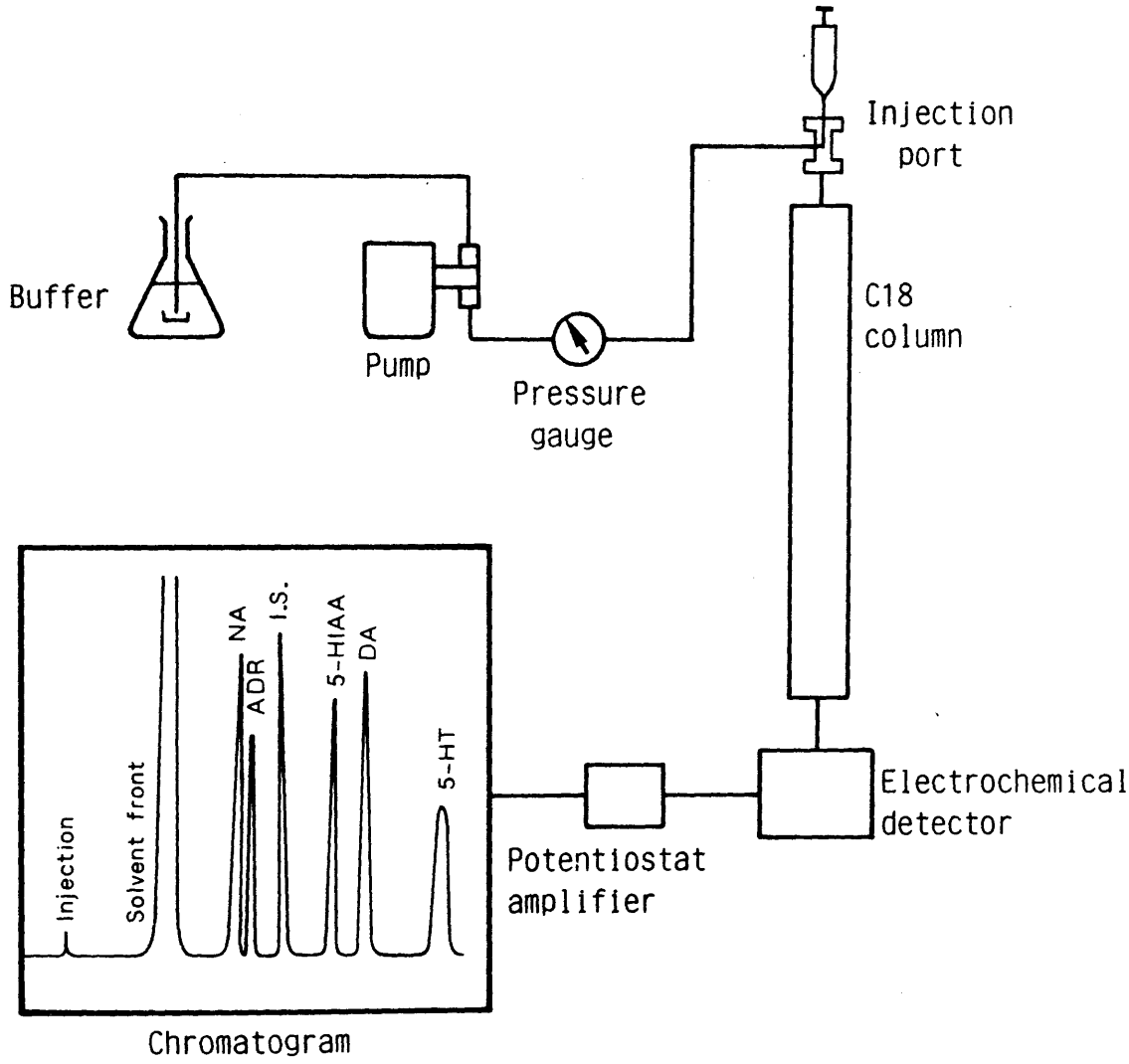


Fig. 18 Schematic diagram of HPLC apparatus.

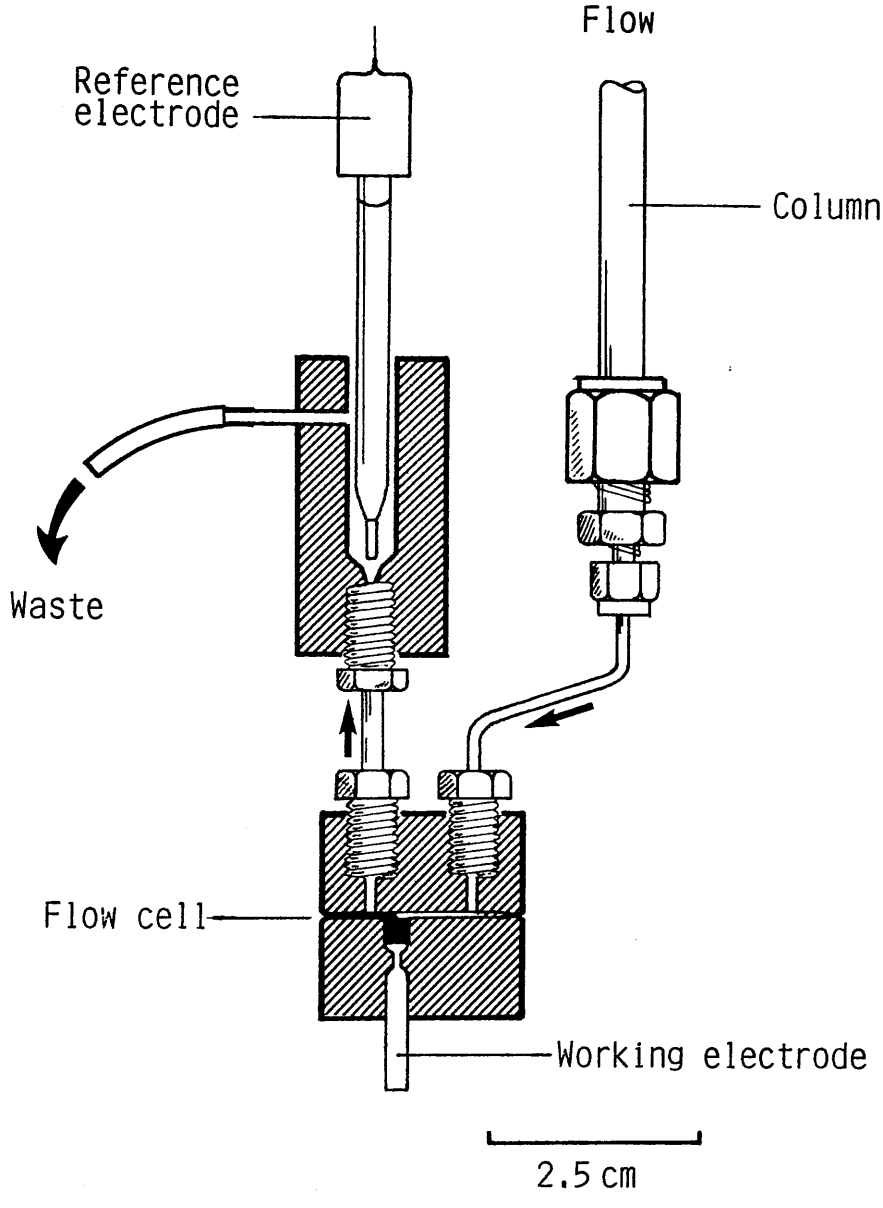


Fig. 19 Cross-section of the BAS LC-17 transducer cell

The cell consists of a Plexiglass block divided in half by a 0.2 mm polytetra fluoroethylene (teflon) gasket. A flow cell with a volume of <1 µl, containing the glassy carbon working electrode, is formed by a slit (1.6 cm x 0.5 cm) cut in the gasket. An electronic controller provides the operating potential (+0.65 V) with reference to an Ag/AgCl reference electrode (sensitivity 1 nA, time constant 5 sec. (ideal for noisy baselines)). The output signal from the working electrode is amplified and displayed on a chromatopac C-R1B data processor.

Mobile Phase:

An aqueous mobile phase (buffer) was used, consisting of 0.1 M sodium acetate, 0.1 M citric acid, 19 mM glacial acetic acid, 126 µm Na EDTA, 5% (v/v) methanol, 2% (v/v) tetrahydrofuran (THF) and 431 mM sodium octyl sulfate (Mayer and Shoup, 1983). This was adjusted to a pH of 4.9-5.1 with sodium hydroxide to ensure complete ionisation of biogenic amine molecules. Distilled, deionized water and Analar grade reagents were used throughout the course of study.

Solvent Pretreatment:

The buffer was filtered under pressure through a Millipore solvent clarification kit with 0.45 µm aqueous filters before use and then degassed with helium for 15 mins. Filtering prevents contamination of the pump and column by excluding microparticulate debris.

Degassing the mobile phase reduces the possibility of air bubbles forming in the pump or detector under the high pressure conditions; air bubbles can disrupt solvent flow through the column and cause severe baseline noise as they pass across the surface of the detector. THF was then added and the buffer and THF very gently mixed taking care not to introduce any air into the mixture.

Solvent Pumping System:

The solvent pumping system provides a constant and reproducible supply of mobile phase to the column. Because the small particles used to pack modern liquid chromatography columns offer substantial resistance to flow, a high-pressure pump is required to deliver solvent to the column. This pumping system can deliver solvent at precise flow rates with a relatively pulse-free output at pressures up to 5000 psi.

Sample Loading:

As the mobile phase was pumped through the system, the samples were introduced on to the top of the column via the injection valve. The Rheodyne valve was then turned from the 'load' position to the 'inject' position thereby diverting mobile phase through the loop to carry the sample on to the column. Sufficient time was left for all the sample to leave the loop before the valve was returned to the load position, ready for the next sample.

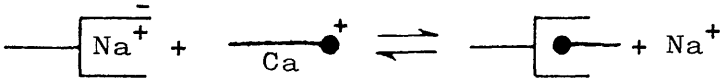
Liquid Chromatography:

High performance liquid chromatography is a sensitive and rapid technique for simultaneous determination of the concentrations of different amines in a sample. The method employs a rapid deproteinization in hydrochloric acid or perchloric acid as the sole extraction/purification step before application of the sample to the HPLC system.

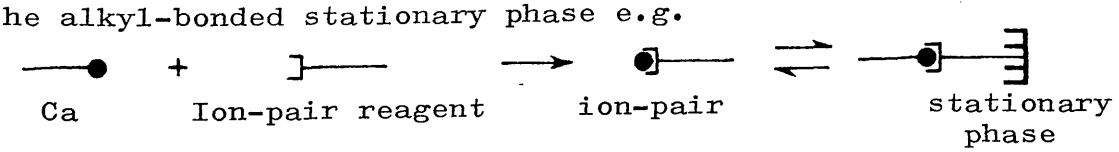
Ion-Pair Chromatography:

Sodium-ocytyle sulfonate was used as an ion-pairing reagent to improve resolution of all the compounds. This enters into an equilibrium complex with the ionised catecholamine molecule e.g.

$$[CH_3(CH_2)_7OSO_3^-Na^+]_{org} + R_3NH^+ \text{ aqu.} \rightleftharpoons [CH_3(CH_2)_7OSO_3^-R_3NH^+]_{org.} + Na^+$$

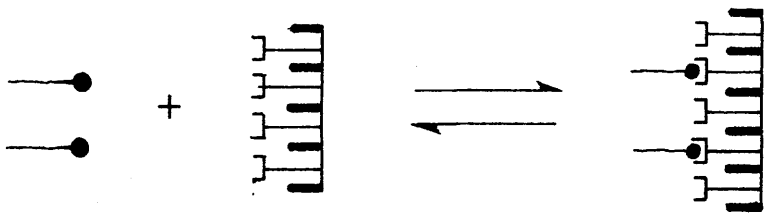


The 'ion-pair' then behave as an non-ionic species with some non-polar characteristics. Several suggestions for the separation of catecholamines in ion-pair chromatography have been put forward. In the original theory, the ion pair is formed in the mobile phase prior to partition into the alkyl-bonded stationary phase e.g.



The catecholamines only interact with the stationary phase when in an ion-pair form.

More recently, a second theory has been proposed. This suggests that the ion-pair reagent primarily attaches itself to the stationary phase by hydrophobic attraction, partitioning into it in a dynamic exchange process. The initial reverse-phase mode of the column has basically been changed to an ion-exchange mode. Ionised catecholamines then partition onto the newly-formed stationary phase by conventional ion-exchange processes.



CA's Ion-pair/stationary phase ion-exchanger

Both views are no doubt partially correct since the mechanism of actual separation is probably a mixture of both processes.

By employing an ionization control technique with citrate/acetate buffer at pH 4.9-5.1, catecholamines are eluted from the octadecyl silane (ODS, C18) column in the order of decreasing polarity of these compounds, that is, NA, ADR and DA. 5HT elutes following the less hydropholic catecholamine NA, ADR and DA (Fig. 20).

The addition of water-miscible organic solvents such as methanol and tetrahydrofuran to the mobile phase decreases solute retention in reversed-phase liquid chromatography.

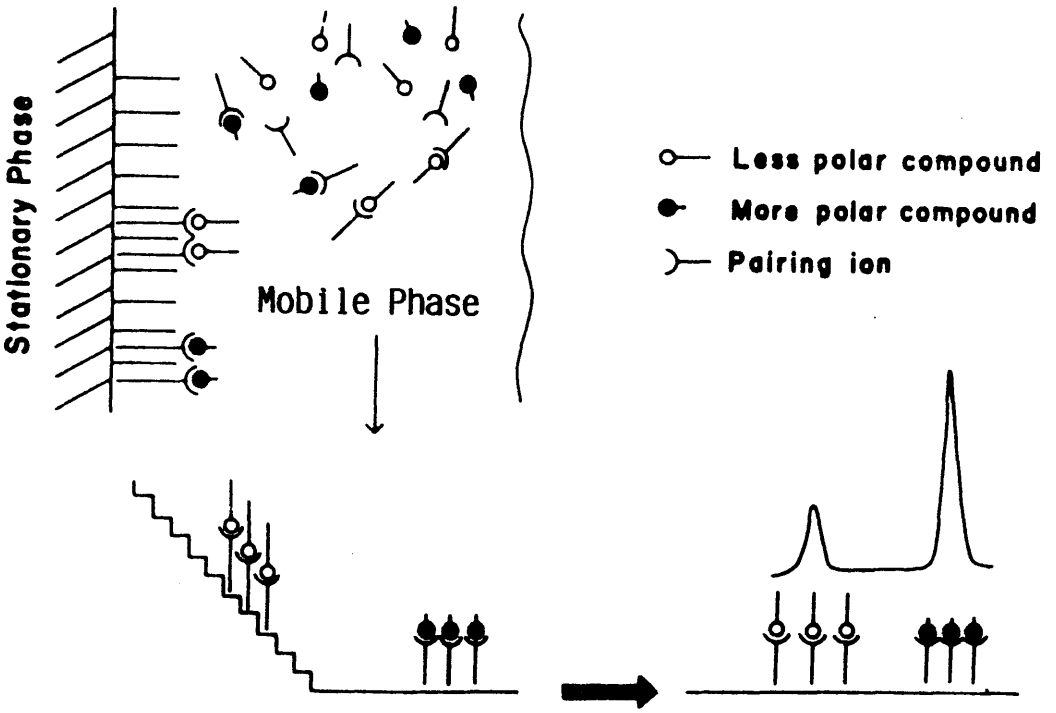


Fig. 20 Schematic illustrations of the primary mechanism of ion-pair reversed phase liquid chromatography.

These organic modifiers alter the solvation properties of the mobile phase for analytes through decreases in both the polarity and surface tension of the eluants.

Electrochemical Detection:

The catecholamines once separated on the chromatography column are detected and measured electrochemically. The amount of catecholamine in each band eluting from the column is measured using an electrochemical detector combined with a potentiostat amplifier, the output being displayed on recorder/data processor.

The potentionstat system consists of carbon working electrode, a platinum wire auxiliary electrode and a silver/silver chloride reference electrode, all contained in the detector. Electrochemical detection (ECD) is a form of polarography and operates on the principle that compounds capable of oxidation or reduction in an electrical field result in the passage of current and the magnitude of this current is a measure of the quantity of compound oxidised or reduced. Each chemical reaction has a threshold voltage related to its Redox potential and the potentiostat, a form of feedback voltage control, allows this voltage to be set for the particular compound to be measured. This introduces some measure of selectivity into the system. For catecholamines and indolamines a +ve potential of +0.65 V is used. When catecholamines pass across the surface of the working electrode they oxidise giving up electrons to it thereby generating an oxidation current.

Each catechol and indole derivative is readily oxidised to generate the corresponding ortho-quinone, 2 protons and 2 electrons. Provided flow rate, temperature and working electrode area remain constant the current detected is directly proportional to the concentration of amine passing the electrode.

The potentiostat amplifier has two functions in the system:

1. It maintains the constant preset potential across the electrochemical detector and
2. It amplifies the nanoampere oxidation current and provides a proportional output voltage which is displayed on the recording integrator. As oxidisable bands of each compound pass the detector, the current (and resultant voltage) rises and falls as a function of time to yield a liquid-chromatography, electrochemical chromatogram (Fig. 8). The first peak on the chromatogram, known as the solvent front, represents the oxidation of hydrochloric acid (perchloric acid in the case of the rabbit and human tissues) which has the shortest retention time on the column and is the first band to pass through the detector. This peak followed first by NA, then by ADR, DA, 5HIAA and finally by 5HT. Because 5-HT is retained longer on the column, it is susceptible to band broadening which results in a shorter and broader 5HT peak. In this system, the threshold amounts of NA, ADR, DA, 5HT and 5-HIAA which could be detected were 0.05 - 0.1 ng and within the range of amounts assayed (up to 100 ng) the

relationship between amount of amine and response amplitude is linear.

Calculation of results:

Biogenic amine content of experimental samples was derived from the peak heights using calibration curves constructed at least twice daily from chromatograms of standard amine solutions.

Since the same mass of internal standard (2 ng/20 ml) had been added to standard mixture and to all the samples, it was possible to calculate the concentration of each amine relative to the internal standard peak area.

Standards:

Stock solutions of noradrenaline bartrate, adrenaline bitartrate, dopamine hydrochloride, 5-hydroxy-tryptamine creatinine, sulphate complex, 5-hydroxy indoleacetic acid and of internal standard, 3,4-dihydroxy benzylamine hydrobromide were prepared in 0.1 M HCl. This stock solution was stable for up to six weeks when stored in the dark at $4^{o}C$. Every week a working standard solution was made by diluting an aliquot of the stock solution and it was used to calibrate the chromatography system for catecholamine analysis. The range of concentrations used routinely was 50 pg, 100 pg, 200 pg and 2 ng per 20 µl injection. The standard curves were linear over this range (Fig. 21). An internal standard of 2 ng/20 µl of DHBA was included in both the sample homogenizing medium and in the standard solutions.

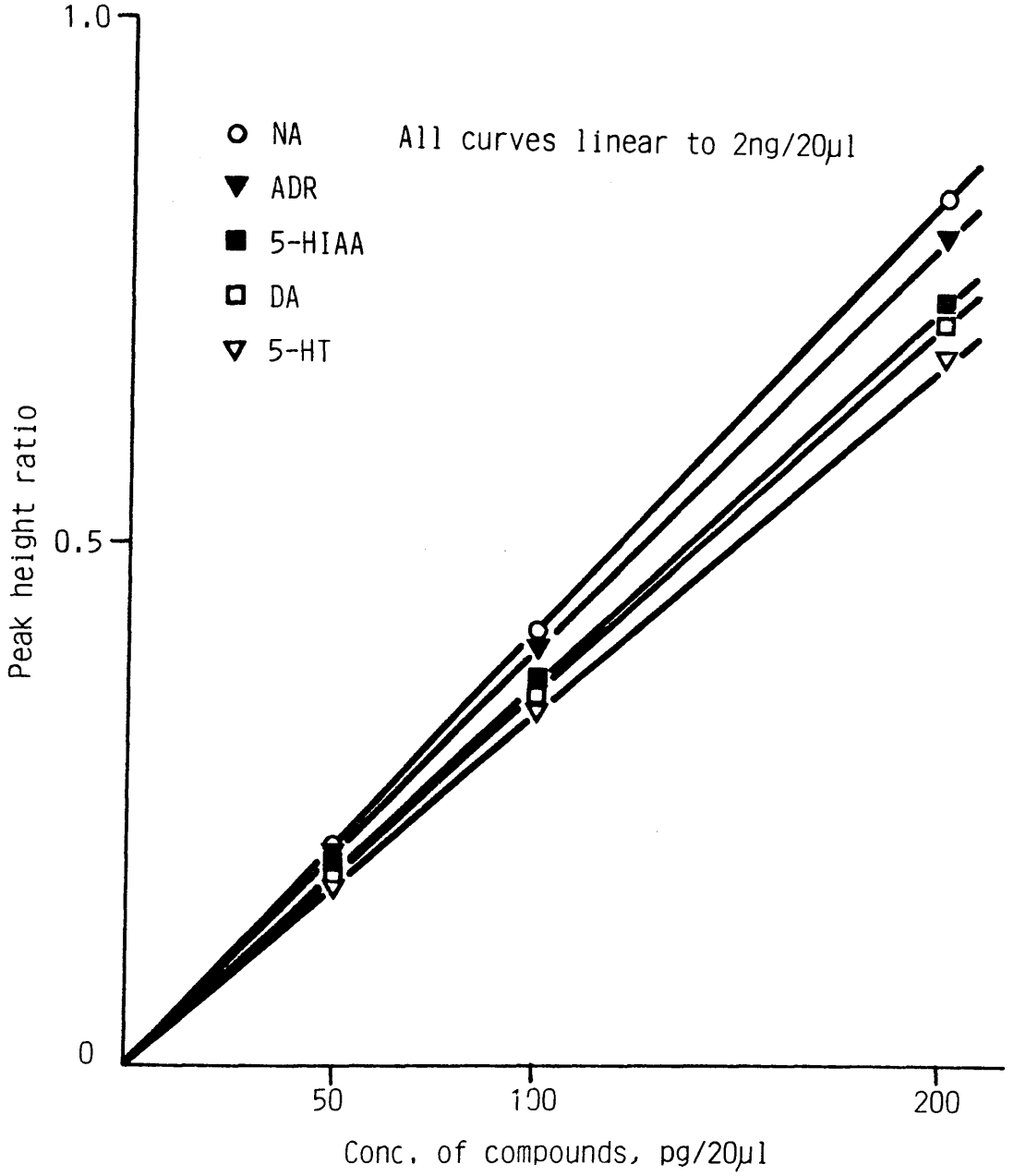


Fig. 21 Standard curves for noradrenaline (NA), adrenaline (ADR) 5-hydroxyindole acetic acid (5-HIAA), dopamine (DA) and 5-hydroxytryptamine (5-HT) using peak height ratios = $\frac{\text{Peak height of standard ('X' ng/20µl)}}{\text{Peak height of internal standards (2ng/20µl)}}$

Protein Estimation

The precipitate of the homogenate was resuspended in 2 ml of 1 M NaOH and shaken in an ultrasonic bath for at least 12 hrs. The protein content of the brain regions was determined by the Lowry method (Lowry, Roseborough, Farr and Randall, 1951) using 100 µl samples of the protein. The assay was carried out in duplicate on 5 tissues from each of the 4 brain regions.

Lowry Protein Estimation

Reagents

Solution A: 2% Na_2CO_3 in 0.1M NaOH

Solution B: 0.5% $CuSO_4$ in 1% Na Citrate

Solution C: 1 volume of solution B: 50 volumes of solution A (freshly made)

Solution D: Folin Ciocalteau Reagent diluted 1:1 with distilled water

Bovine serum albumin standards: 1 mg/ml, in distilled water. A range of concentrations were prepared 0 µg - 100 µg/ml.

Procedure

1) Glass tubes were set up with 100 µl of standards and samples.
2) To each tube was added 800 µl of solution C. This was allowed to stand at room temperature for at least 10 min.
3) 100 µl of solution D (Folin/ciocalteau) was added to each tube, vortexed immediately and allowed to stand

at room temperature for >30 min. <2 hr.

4) The optical density of each tube was read, using distilled water as a blank to Zero, at 750 nm (Tungsten Lamp).

A calibration curve was obtained for 0-100 µg of Bovine Serum Albumin. The protein content of the rabbit brain samples was calculated using the regression equation from the standard graph (Fig. 22).

Radioimmunoassay

LH was assayed according to a modified method of Naftolin and Corker (1971) as described by Kendle, Paterson and Wilson (1978). The LH standard was LER-C_2-1056 (potency 1.72 times LH-SI) obtained from Professor L.E. Reichert, Albany University, NY, U.S.A. and the antiserum Rabbit No.15 from Professor G.D. Niswender, Colorado University, Co. U.S.A. The intra- and inter-assay coefficients of variance were 10.3 and 8.7% respectively.

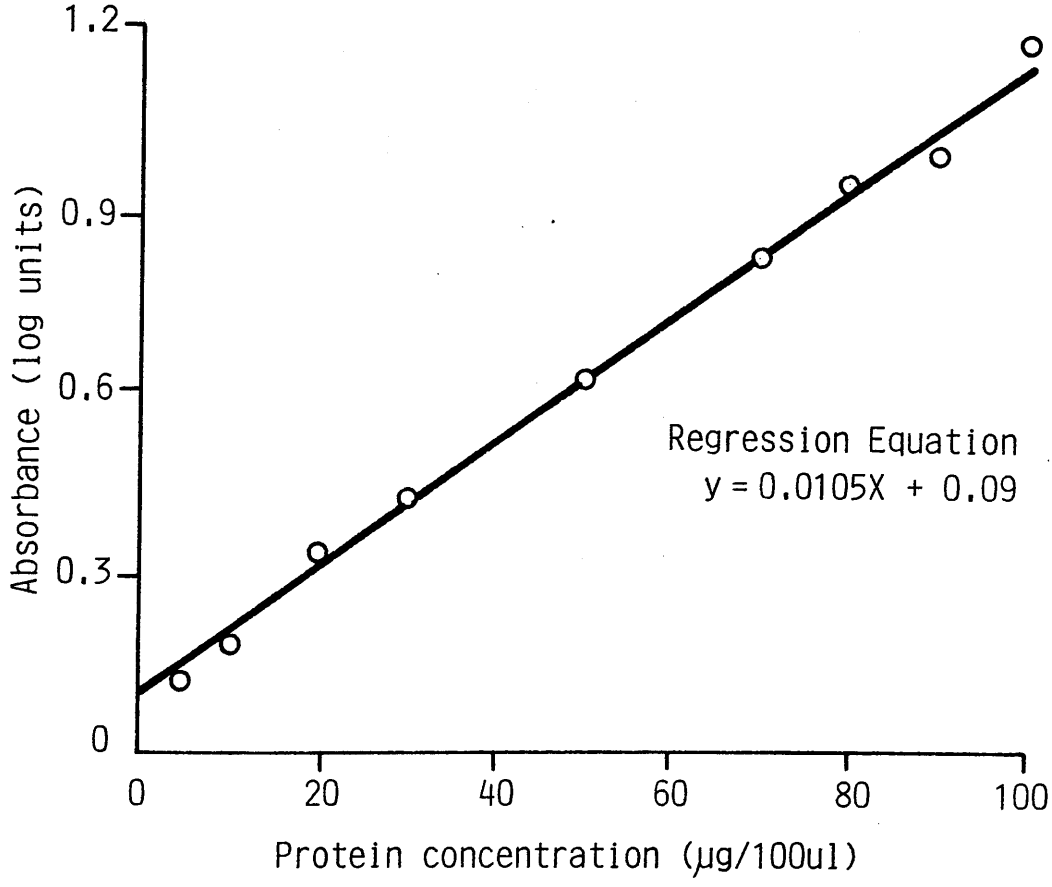


Fig.22 Calibration graph for 0-100µg. of Bovine Serum Albumin.

RESULTS

Results

Effects of neonatal androgen manipulation

In the present study all oil-treated females showed regular 4 day oestrous cycles in direct contrast to the androgenized rats. The latter exhibited either constantly cornified vaginal smears, or smears that alternated between days of predominantly nucleated cells and days of predominantly cornified cells. In some instances vaginal opening was even delayed until 60-75 days. The ovaries of the oil-treated females showed follicles at various stages of development and large corpora lutea were also evident. The ovaries of those sterilized by exposure to androgens during development were polyfollicular and lacked corpora lutea.

Plasma LH concentrations in the rat at various post-pubertal ages were measured by RIA, and are shown in Table 18.

Biogenic amine analysis

The method of HPLC coupled with ECD described in an earlier section was utilized to measure the distribution of biogenic amines in various rat brain regions, and in human fetal brain tissue.

It was observed that concentrations varied in a time-dependent manner which in most instances was affected by the presence or absence of androgens during the period of brain sexual differentiation.

These absolute changes in neurotransmitter content following neonatal manipulation of androgen milieu, are

presented in Tables 2-17 and in Figures 23-46.

Results were analyzed statistically using the student's t-test. In the following account the concentrations of each neurotransmitter in discrete brain regions are described.

Distribution of Noradrenaline in Various Regions of the Rat Brain

1. Hypothalamus (Fig. 23, Tables 2 and 3)

a. Males:

In sham-castrated rats concentrations of NA exhibited a gradual increase from day 12 leading to levels being quadrupled by the age of 90 days. However, by 120 days NA levels had begun to decline. By 180 days they had fallen back to the level that was observed at 12 days. However, deprivation of testosterone during the neonatal period resulted in a significant reduction ($p < 0.001$) in NA concentrations in the infantile and young rat (Fig. 25). Although a slight increase in hypothalamic NA concentrations was observed in the castrated animals it was significantly lower than that observed in the controls and the difference was still evident at 90 days. By 120 days NA concentrations had risen and at 180 days they reached a level that was significantly higher ($p < 0.001$) than in the controls. Thus, the castration effect observed in the younger age groups was completely reversed by 180 days of age.

b. Females:

In untreated rats the NA content of the hypothalamus rose steeply until 75 days. At 90 days the NA content had

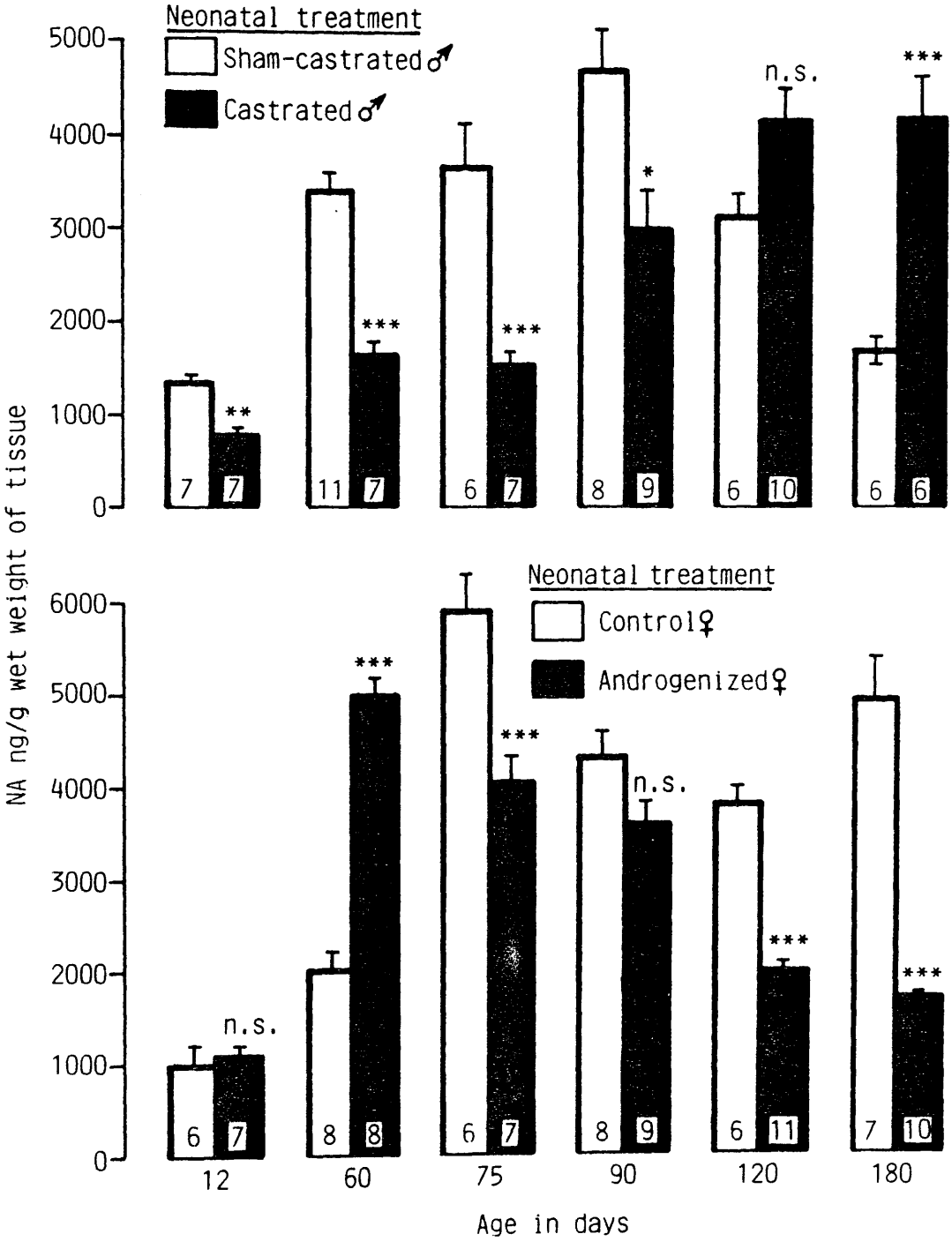


Fig. 23 Mean (± SEM) NA concentration in the hypothalamus at various post-natal ages in the control and experimental rats. *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

Table 2

Concentrations of biogenic amines in the hypothalamus of male rats at various postnatal ages.

A. Sham-castrated.

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5-HIAA
12	1352.2±112.3 (7)	287.3±124.7 (5)	455.2±39.1 (7)	377.8±57.5 (7)	1050.0±93.0 (7)
60	3416.9±188.8 (11)	32.2±3.4 (5)	651.4±68.0 (10)	1131.9±170.5 (7)	1553.5±259.3 (7)
75	3658.7±483.2 (6)	-	496.9±36.4 (6)	956.6±160.2 (6)	940.8±225.2 (5)
90	4710.0±430.3 (8)	-	896.0±58.7 (8)	2270.0±383.9 (6)	1472.0±126.5 (7)
120	3135.6±249.5 (6)	62.3±17.0 (5)	530.8±115.6 (5)	1317.8±162.4 (5)	548.5±82.5 (6)
180	1683.5±163.1 (6)	58.3±9.3 (4)	267.4±40.1 (6)	754.9±194.2 (6)	771.1±67.6 (7)

B. Castrated.

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	799.1±78.1** (7)	-	666.1±86.1* (6)	451.6±51.9 (7)	722.1±65.4** (7)
60	1648.2±173.8*** (7)	35.8±3.1 (4)	676.8±28.8 (6)	292.6±51.1** (6)	519.0±66.9** (7)
75	1555.5±120.2*** (7)	-	469.2±51.9 (7)	156.6±21.3*** (6)	147.1±33.6*** (7)
90	3018.0±431.6* (9)	78.8±9.1 (4)	750.9±120.0 (6)	2247.3±461.7 (5)	1570.8±260.5 (6)
120	4173.4±327.2* (10)	78.1±9.2 (5)	731.8±80.9 (8)	2334.6±417.9* (7)	2346.7±451.5* (6)
180	4239.7±477.7*** (6)	40.3±7.3 (4)	1742.7±356.1** (6)	1874.2±275.3** (6)	2442.5±306.1*** (6)

Mean (± SEM),* $p < 0.05$, **$p < 0.01$, ***$p < 0.001$, numbers examined, in parenthesis.

Table 3

Concentrations of biogenic amines in the hypothalamus of female rats at various post-natal ages.

A. Control

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	1006.6±196.1 (6)	236.5±23.9 (6)	468.5±78.0 (5)	1101.7±158.8 (6)	2162.9±291.0 (6)
60	2024.8±187.6 (8)	23.0±1.3 (5)	306.5±29.4 (7)	650.4±48.2 (7)	936.3±31.3 (8)
75	5920.5±389.5 (6)	12.9±2.7 (5)	544.6±37.1 (6)	2510.8±424.6 (6)	1800.4±126.5 (6)
90	4310.9±308.9 (8)	47.3±8.7 (6)	1020.6±89.6 (7)	1974.5±211.6 (8)	2278.6±188.8 (8)
120	3796.5±198.8 (6)	16.9±1.2 (5)	619.2±90.7 (6)	1658.1±118.3 (6)	2031.9±113.0 (6)
180	4969.0±468.7 (7)	44.2±8.3 (4)	516.0±65.8 (5)	1778.5±176.6 (6)	1178.0±152.9 (6)

B. Androgenized

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	1094.8±87.2 (7)	231.0±46.8 (4)	425.8±84.6 (6)	734.8±148.3 (6)	1579.5±185.1 (6)
60	4944.1±205.6*** (8)	67.6±18.3* (4)	530.4±42.8*** (7)	1913.0±122.1*** (8)	1912.9±122.1*** (8)
75	4059.9±301.7** (7)	21.9±3.6 (5)	982.2±67.3*** (7)	1999.5±100.6 (7)	1344.9±144.9* (6)
90	3620.0±256.4 (9)	15.7±3.6* (5)	954.0±80.4 (9)	1390.3±112.7* (8)	1833.9±42.1* (8)
120	2006.4±117.0*** (11)	8.9±3.1 (8)	405.8±38.3** (11)	1389.9±112.7 (10)	763.1±63.7*** (10)
180	1709.5±48.1*** (10)	13.8±1.7* (4)	305.6±23.7** (8)	729.1±48.0*** (8)	770.5±83.9* (8)

Mean (± SEM), *p <0.05, **p <0.01, ***p <0.001, numbers examined in parenthesis.

fallen significantly and this fall was sustained in the other age groups examined. Androgenization during the period of brain sexual differentiation resulted in a more than two-fold increase in the NA levels at 60 days of age compared to the controls. This effect was soon reversed and NA levels began a steep decline which was maintained through to 180 days.

2. Amygdala (Fig. 24, Tables 4 and 5)

a. Males:

NA levels in the sham-castrated animals were observed to increase until 75 days of age and did not fall until the rats reached 180 days. Neonatal castration did not noticeably affect NA concentrations at 12 days. However at 60 and 75 days NA levels were much lower than in the controls ($p < 0.001$). At 90 and 120 days NA concentrations were seen to be similar to those in the sham-castrated animals but at 180 days they had risen very significantly. This pattern of change is exactly the same as was observed in the hypothalamus.

b. Females:

In the amygdala of control animals concentrations of NA showed no time-dependent changes through to 60 days. By 75 days however, they exhibited a three-fold increase to levels which were sustained throughout the remainder of the study. Neonatal androgenization did not alter NA levels at 12 days, but an abrupt and short lived rise was seen post-pubertally at 60 days of age. The NA content of the amygdala then dropped significantly ($p < 0.001$) to levels

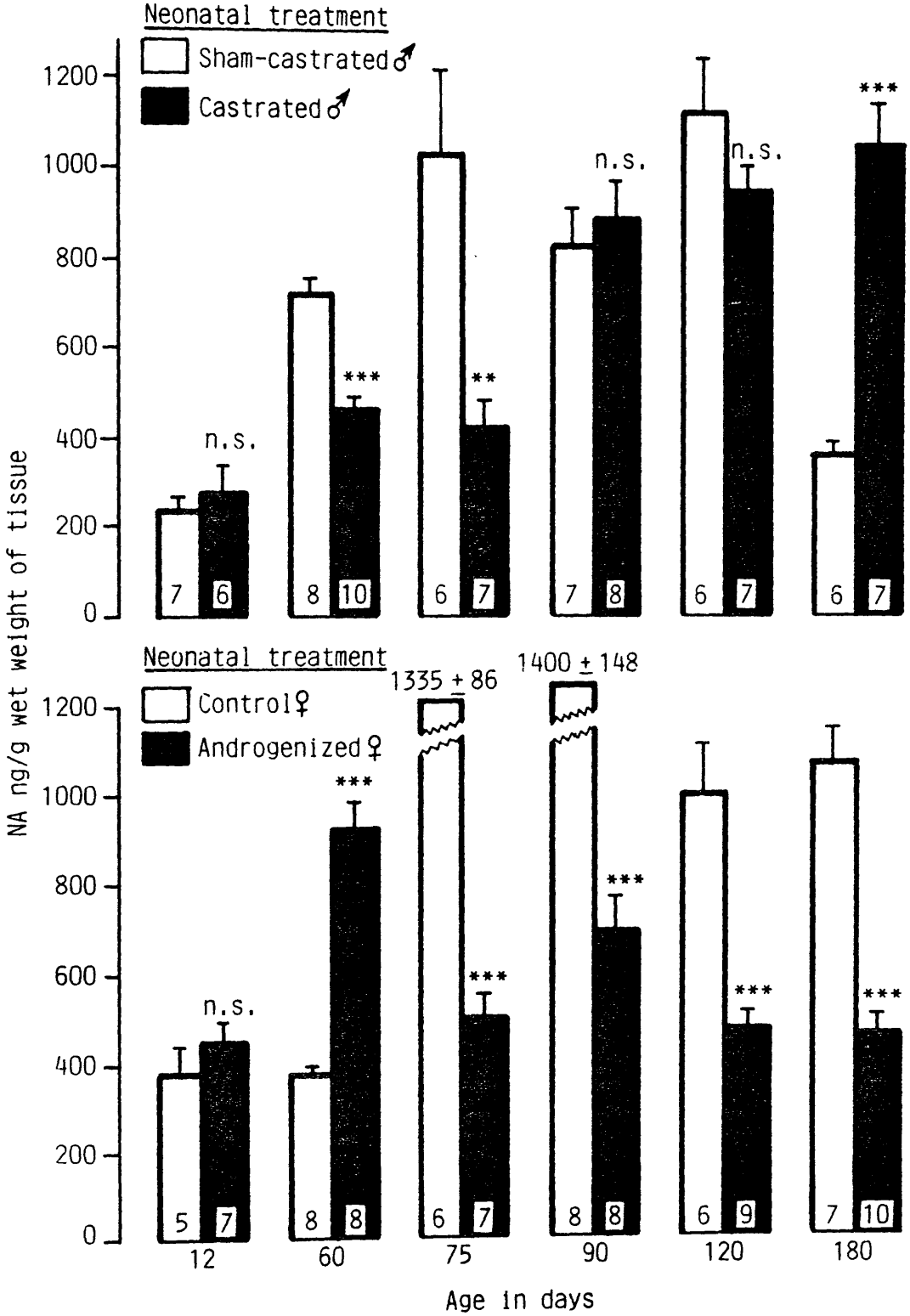


Fig. 24 Mean (± SEM) NA concentration in the amygdala at various postnatal ages in the control and experimental rats *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

Table 4

Concentrations of biogenic amines in the amygdala of male rats at various post-natal ages.

A. Sham-Castrated

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	236.9±34.2 (7)	63.7±7.1 (5)	273.0±39.1 (7)	255.9±33.7 (7)	231.4±32.8 (7)
60	714.3±41.1 (8)	34.0±7.6 (5)	621.7±121.3 (6)	596.2±108.1 (6)	1782.6±261.4 (6)
75	1028.7±195.9 (6)	8.6±3.1 (5)	374.2±68.2 (6)	824.6±92.4 (6)	940.6±152.0 (5)
90	821.9±88.9 (7)	-	591.8±70.6 (8)	1356.3±172.1 (7)	1269.4±108.9 (8)
120	1124.7±122.2 (6)	-	409.7±107.0 (6)	306.0±60.2 (6)	1516.8±241.4 (5)
180	349.3±31.3 (6)	18.3±1.4 (6)	420.2±56.6 (6)	296.0±36.1 (5)	667.6±55.5 (6)

B. Castrated

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	281.9±61.8 (6)	-	294.4±51.2*** (6)	441.9±63.9** (6)	343.9±70.9 (6)
60	464.8±29.8*** (10)	35.6±5.8 (5)	241.2±46.2** (8)	79.6±13.0*** (6)	457.6±37.0*** (10)
75	421.6±65.0*** (7)	70.1±15.6** (5)	261.3±37.8 (6)	96.4±25.5*** (5)	179.4±26.8*** (7)
90	877.5±86.5 (8)	53.2±9.6 (6)	403.3±52.7* (6)	605.6±47.9** (6)	2023.3±188.2** (6)
120	942.8±64.8 (7)	-	699.7±90.3* (6)	792.7±90.1** (7)	1957.3±131.7 (7)
180	1042.1±104.3*** (7)	-	983.7±162.4* (7)	1064.5±83.4*** (6)	2369.0±186.1*** (7)

Mean (± SEM), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, numbers examined in parenthesis.

Table 5

Concentrations of biogenic amines in the amygdala of female rats at various post-natal ages.

A. Control

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	375.6±63.3 (5)	-	242.1±39.3 (6)	391.6±45.1 (7)	1031.0± 131.1 (7)
60	371.3±24.0 (8)	20.8±3.8 (6)	468.1±53.3 (8)	864.8±78.4 (8)	914.1±45.6 (8)
75	1335.6±86.5 (6)	37.7±9.1 (4)	739.7±125.2 (5)	923.6±96.4 (5)	1802.7±122.0 (5)
90	1400.3±148.9 (8)	62.8±10.3 (4)	1342.0±154.5 (7)	1263.8±110.0 (7)	1750.0±130.1 (8)
120	1001.6±108.6 (6)	37.8±10.9 (4)	836.9±52.6 (6)	1131.2±79.3 (6)	1416.2±137.4 (6)
180	1073.5±84.0 (7)	6.7±2.5 (8)	932.6±89.8 (7)	806.2±109.6 (6)	1298.5±177.3 (7)

B. Androgenized

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	451.1±46.5 (7)	67.9±10.7 (8)	284.5±26.0 (7)	445.7±73.6 (6)	1415.6±187.7 (6)
60	924.8±63.8*** (8)	6.6±2.8** (6)	492.6±35.3 (9)	800.0±128.8 (9)	941.5±77.2 (7)
75	505.4±55.7*** (7)	4.3±1.0** (5)	695.3±61.4 (5)	692.8±102.7 (6)	1070.2±75.3*** (7)
90	686.6±78.4*** (8)	15.6±2.3*** (7)	682.4±66.4*** (9)	1375.8±158.3 (9)	1272.7±75.6** (9)
120	469.4±39.3*** (9)	6.9±1.6** (7)	281.7±35.2*** (8)	624.5±64.5*** (6)	744.8±48.7*** (10)
180	460.5±36.7*** (10)	17.6±3.8* (5)	243.8±34.2*** (7)	493.7±29.6** (9)	759.8±78.4** (10)

Mean (± SEM), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, numbers examined in parenthesis.

that were maintained at all other ages studied.

3. Cerebellum (Fig. 25, Tables 6 and 7)

a. Males:

In sham-castrated rats the NA content rose through to 60 days of age. This rise was sustained for not less than another two months after which a drop was observed at 180 days. However, following neonatal castration NA concentrations were observed to be significantly higher than in the controls at 12 days, significantly lower at 90 days and again significantly higher at 180 days.

b. Females:

NA concentrations in the cerebellum of control animals differed little throughout the period of study (except at 60 days). In testosterone-treated rats NA was elevated at 12 days of age compared to the controls. A steady decline in cerebellar NA levels was then observed in the experimental group throughout the remaining period of the study, which reached a statistically high significance ($p < 0.001$) compared to the controls at both 120 and 180 days.

4. Cerebral Cortex (Fig. 26, Tables 8 and 9)

a. Males:

Levels of NA were low in control animals at 12 days of age but by two months they exhibited a sharp rise. Levels then fluctuated markedly, but showed an overall decrease through to 180 days. Neonatal castration brought about an even greater increase in NA levels by 60 days of age than

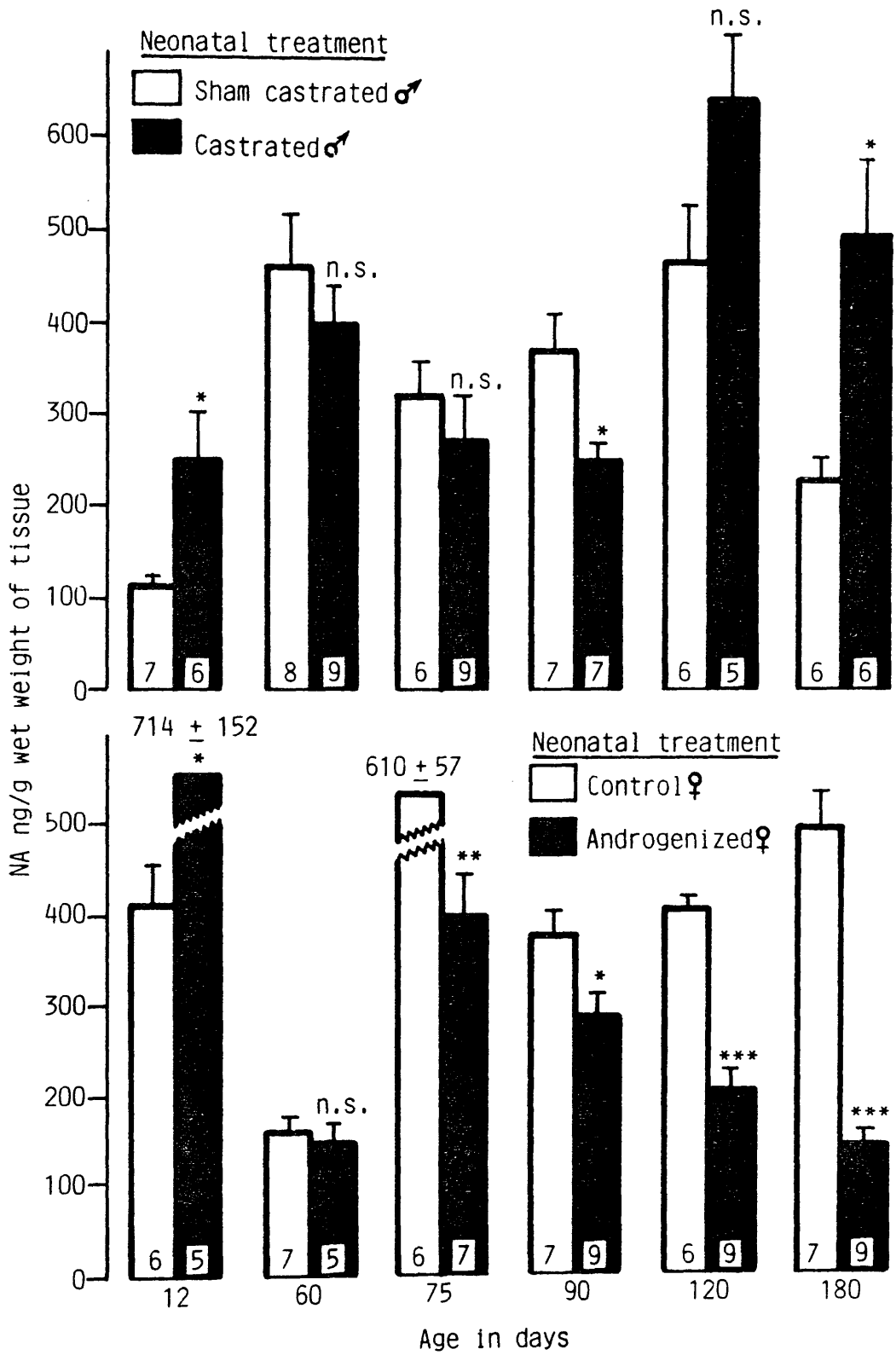


Fig. 25 Mean (± SEM) NA concentration in the cerebellum of the control and experimental rats at various post-natal ages. *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

Table 6

Concentrations of biogenic amines in the cerebellum of male rats at various post-natal ages.

A. Sham Castrated.

Ages (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	115.2±10.0 (7)	-	107.2±17.6 (6)	142.7±18.7 (7)	108.2±28.3 (5)
60	462.1±61.9 (8)	73.8±13.2 (7)	83.0±21.5 (6)	325.5±58.3 (8)	1119.8±89.6 (6)
75	324.3±38.3 (6)	33.8±6.4 (4)	165.2±20.8 (7)	69.9±2.2 (5)	431.2±67.1 (5)
90	371.8±39.4 (7)	155.6±15.6 (4)	255.0±24.5 (6)	180.0±40.8 (6)	494.2±97.3 (6)
120	474.1±60.9 (6)	146.7±26.9 (5)	89.0±19.9 (5)	426.9±99.5 (5)	147.2±25.8 (5)
180	228.8±25.5 (6)	31.9±17.6 (4)	80.99±22.9 (5)	267.8±55.2 (5)	245.2±43.8 (6)

B. Castrated

Ages (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	248.4±45.7* (6)	-	20.3±8.3* (5)	221.1±36.2 (6)	212.2±34.2* (5)
60	394.2±46.9 (9)	146.7±34.7* (5)	105.3±21.5 (7)	257.4±23.5 (7)	222.1±28.1*** (10)
75	274.4±43.8 (9)	33.7±7.6 (4)	208.1±36.1 (9)	239.2±23.9*** (7)	2710.7±522.2** (9)
90	247.8±22.3* (7)	42.7±15.0*** (7)	78.1±26.0*** (7)	209.3±43.5 (8)	1886.5±280.9** (7)
120	667.6±83.6 (5)	17.6±5.5** (4)	42.8±12.4* (6)	277.7±31.1 (7)	505.4±52.7*** (5)
180	497.3±86.5* (6)	38.7±14.6 (5)	205.0±25.0** (5)	314.9±53.9 (5)	725.0±138.3** (5)

Mean (± SEM), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, numbers examined in parenthesis.

Table 7

Concentrations of biogenic amines in the cerebellum of female rats at various post-natal ages.

A. Control

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	409.5±47.2 (6)	-	11.4±2.4 (4)	266.3±48.6 (6)	1018.0±175.5 (6)
60	162.3±17.4 (7)	53.3±13.7 (6)	51.6±9.4 (6)	159.4±10.0 (7)	396.8±53.1 (7)
75	610.3±57.5 (6)	2.9±1.0 (6)	70.2±11.7 (5)	90.1±14.0 (5)	417.7±43.8 (6)
90	379.1±24.2 (7)	123.6±17.9 (7)	31.5±4.3 (5)	195.2±21.3 (6)	428.7±67.9 (8)
120	413.1±16.8 (6)	35.8±5.4 (4)	20.5±2.0 (5)	233.9±35.5 (5)	394.6±41.5 (6)
180	493.8±40.2 (7)	30.0±5.4 (4)	57.3±12.8 (5)	118.8±19.2 (6)	453.1±99.2 (6)

B. Androgenized

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	710.8±151.8* (5)	40.9±7.1 (4)	13.6±3.3 (5)	351.7±64.1 (6)	1379.8±340.7 (6)
60	147.2±23.8 (5)	39.6±3.2 (4)	15.2±1.9** (7)	139.1±24.1 (6)	349.4±59.2 (7)
75	399.9±44.3** (7)	10.0±1.2*** (4)	16.5±2.7*** (5)	138.2±29.5 (7)	506.3±66.4 (7)
90	288.0±23.0* (9)	27.6±3.8* (4)	34.1±6.6 (8)	95.2±11.1** (7)	434.5±23.4 (7)
120	215.5±22.3*** (9)	71.2±10.3* (4)	38.8±6.5* (5)	31.9±6.4*** (5)	275.4±26.1* (8)
180	152.7±15.8*** (9)	80.8±11.4** (4)	129.5±19.6* (8)	31.0±6.7*** (7)	279.4±24.4* (9)

Mean (± SEM), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, numbers examined in parenthesis.

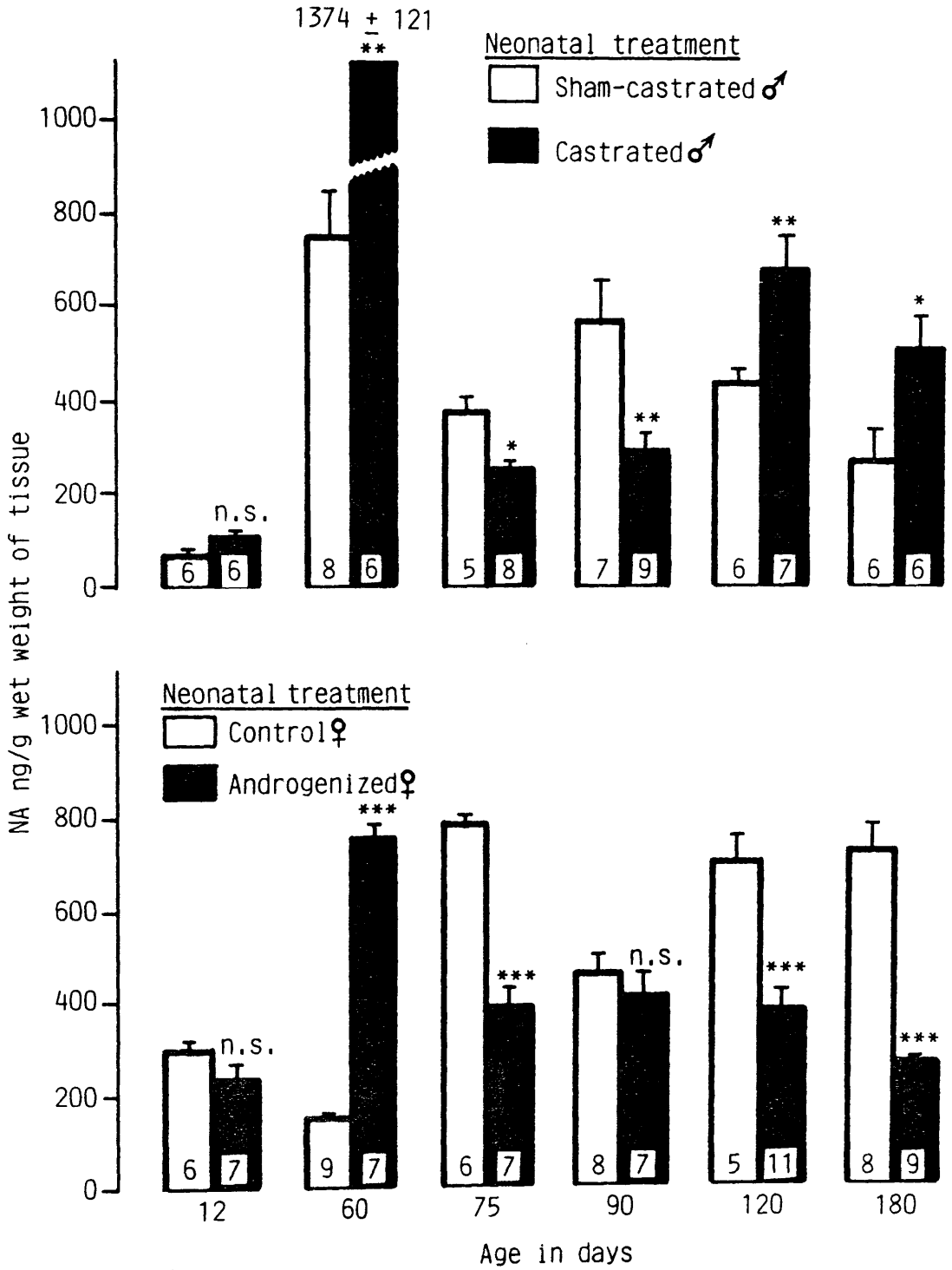


Fig. 26 Mean (± SEM) NA concentration in the cerebral cortex of control and experimental rats at various post-natal ages. *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

Table 8

Concentrations of biogenic amines in the cerebral cortex of male rats at various post-natal ages.

A. Sham-Castrated

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	67.8±18.0 (6)	-	101.0±11.1 (7)	103.9±22.4 (7)	162.3±20.0 (7)
60	759.3±100.3 (8)	43.8±6.7 (5)	286.1±37.6 (7)	679.2±116.6 (6)	1100.7±188.0 (8)
75	381.3±42.7 (5)	25.4±4.1 (4)	171.9±24.0 (5)	592.5±79.3 (5)	1440.9±209.1 (5)
90	577.3±88.7 (7)	-	144.8±24.7 (8)	520.0±99.6 (6)	846.3±128.7 (8)
120	451.2±33.5 (6)	47.1±4.1 (4)	37.8±6.1 (5)	297.5±63.6 (5)	286.2±39.2 (5)
180	292.9±69.5 (6)	41.8±11.3 (4)	25.2±4.2 (6)	48.5±6.8 (5)	411.9±96.2 (6)

B. Castrated

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	109.0±17.1 (6)	-	35.8±7.1** (5)	173.2±20.1* (7)	200.0±20.3 (7)
60	1374.2±121.3** (6)	-	119.5±15.1** (5)	869.6±96.2 (6)	1138.5±131.7 (5)
75	264.5±20.7* (8)	-	406.5±64.4* (7)	457.7±59.2 (6)	1953.7±283.1 (6)
90	303.6±36.6** (9)	23.4±4.1 (6)	158.9±19.9*** (7)	650.1±101.2 (6)	1590.0±199.2** (7)
120	697.6±72.8** (7)	126.0±34.8 (4)	39.1±7.8 (5)	397.6±50.5 (8)	2106.8±284.2*** (5)
180	529.2±73.8* (6)	96.6±14.4* (4)	205.8±38.1*** (6)	464.8±58.4*** (6)	2013.9±241.8*** (6)

Mean (± SEM), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, numbers examined in parenthesis.

Table 9

Concentrations of biogenic amines in the cerebral cortex of female rats at various post-natal ages.

A. Control

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	299.6±23.4 (6)	-	44.5±0.83 (6)	142.9±9.7 (7)	855.5±124.5 (6)
60	155.9±11.2 (9)	34.6±2.6 (4)	113.6±21.8 (7)	205.2±17.1 (6)	874.0±56.5 (7)
75	793.9±20.5 (6)	38.3±8.6 (5)	90.7±10.5 (6)	287.5±59.0 (6)	1366.8±115.0 (6)
90	468.3±40.9 (8)	78.5±17.2 (6)	136.6±14.4 (5)	891.4±53.1 (7)	2159.8±125.6 (7)
120	713.4±63.1 (5)	-	165.1±19.4 (6)	578.8±80.4 (5)	1148.5±154.9 (5)
180	740.7±56.0 (8)	18.1±3.3 (5)	150.1±21.2 (5)	205.4±12.4 (6)	969.7±77.6 (6)

B. Androgenized

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	236.2±31.6 (7)	32.9±5.9 (5)	70.6±0.84 (5)	334.9±68.2** (5)	960.4±175.4 (6)
60	760.0±31.4*** (7)	19.5±3.6* (6)	191.5±7.8** (6)	839.7±100.0*** (6)	1046.3±79.5 (8)
75	401.4±44.6*** (7)	15.3±3.6*** (6)	33.1±6.6* (5)	123.8±16.1* (6)	982.6±113.1* (6)
90	428.9±49.3 (7)	19.0±2.4* (5)	112.4±14.6 (7)	425.7±72.2** (7)	1273.3±130.2*** (9)
120	400.4±41.1*** (11)	21.2±2.1 (7)	68.7±9.9*** (7)	251.2±33.2*** (9)	641.6±30.6*** (9)
180	265.4±14.8*** (9)	24.7±3.7 (7)	52.2±7.0*** (8)	137.9±19.8* (8)	862.6±74.4 (9)

Mean (± SEM), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, numbers examined in parenthesis.

occurred in the controls. A large drop was then seen at both 75 and 90 days which was followed by a rise at 120 and 180 days which caused levels to be significantly higher than in the untreated group.

b. Females:

Low levels of NA observed at 12 and 60 days of age were followed by a two-three fold elevation by 75 days. Levels had fallen back by 90 days but then remained comparatively steady during the remainder of the study. Testosterone treatment of the neonates did not bring about any observable change from the controls at 12 days of age. However, the abrupt increase noticed in the hypothalamus and amygdala was also seen in the cerebral cortex at 60 days. This was followed by a decline at 75 days to bring levels down to a significantly lower level ($p < 0.001$) than in the controls. Although this reduction remained stable during the rest of the study, NA levels were still very significantly depleted in the androgenized groups as compared to untreated animals.

5. Corpus callosum (Tables 10 and 11)

NA concentrations both in the male and female showed no change either in the control or in the experimental groups through to 120 days of age. The only change was seen at 180 days when the situation with regard to NA levels was exactly reversed to each other. Neonatal castration caused the NA levels to be very significantly elevated as compared to controls. In the females neonatal androgen treatment resulted in a marked depletion at 180 days of age.

Table 10

Concentrations of biogenic amines in the corpus callosum of male rats at various post-natal ages.

A. Sham-Castrated

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
60	822.2±220.5 (8)	189.1±4.7 (3)	778.4±167.1 (7)	360.6±34.1 (6)	1150.3±178.0 (8)
75	225.2±56.6 (4)	20.4±3.7 (3)	229.4±50.4 (4)	241.2±15.9 (5)	365.6±60.2 (4)
90	330.1±133.7 (6)	202.8±59.1 (5)	1628.6±402.3 (7)	365.1±60.4 (6)	624.5±94.9 (7)
120	403.9±68.4 (5)	-	882.2±181.2 (5)	217.5±87.5 (5)	898.6±249.5 (5)
180	228.6±85.1 (4)	-	438.8±96.4 (4)	224.7±43.3 (4)	247.6±78.0 (4)

B. Castrated

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
60	521.1±13.8 (6)	-			
75	216.3±81.4 (8)	41.7±7.0* (4)	535.0±79.0* (6)	255.7±63.2 (7)	250.1±55.9 (7)
90	235.7±44.6 (6)	-	1071.9±109.3* (7)	145.2±20.1* (5)	1009.6±115.5* (5)
120	283.2±50.1 (11)	148.3±28.3 (7)	469.7±106.6* (8)	152.1±22.7 (11)	834.1±96.9 (7)
180	1248.3±343.2*** (6)	116.4±42.8 (4)	1198.5±321.7** (6)	481.9±191.3 (5)	1222.4±221.1*** (6)

Mean (±SEM), *p < 0.05, **p < 0.01, ***p < 0.001, numbers examined in parenthesis.

Table 11

Concentrations of biogenic amines in the corpus callosum of male rats at various post-natal ages.

A. Control

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
60	213.7±44.3 (6)	26.7±9.6 (4)	236.6±30.7 (5)	158.7±24.4 (5)	225.4±30.1 (5)
75	296.4±43.5 (6)	14.2±1.8 (5)	517.9±57.9 (5)	647.2±79.3 (5)	1009.8±72.9 (5)
90	328.2±96.3 (6)	499.6±76.8 (4)	1005.9±148.4 (6)	236.3±20.6 (6)	914.1±108.0 (6)
120	254.7±30.9 (5)	16.8±2.0 (4)	848.1±77.2 (5)	618.7±40.8 (5)	603.8±65.3 (5)
180	549.1±73.9 (7)	37.0±7.3 (4)	1809.0±175.7 (7)	204.9±42.7 (6)	957.7±135.9 (6)

B. Androgenized

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
60	270.8±34.0 (8)	32.5±6.6 (4)	318.9±39.3* (7)	232.7±32.7 (8)	321.7±42.9 (5)
75	200.8±33.5 (6)	19.9±7.4 (5)	464.5±102.0 (4)	303.8±39.4** (5)	549.7±75.5** (5)
90	131.8±14.9* (5)	61.4±6.5*** (4)	1501.5±189.8 (6)	235.1±22.8 (6)	1021.0±195.9 (6)
120	244.8±18.3 (6)	27.8±2.1 (4)	462.7±60.1** (6)	360.8±27.7** (5)	288.5±25.1*** (6)
180	175.4±15.5*** (6)	6.8±1.6*** (5)	663.7±100.3*** (6)	48.5±10.8* (5)	237.6±32.4*** (5)

Mean (± SEM), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, numbers examined in parenthesis.

6. Hippocampus (Tables 12 and 13)

Due to some storage problem measurement of neurotransmitters was not possible in the neonatally castrated animals of 60, 75 and 90 days of age.

a. Males:

The effect of neonatal castration which could only be observed at 120 and 180 days resulted in a very significant difference between the experimental groups and their controls ($p < 0.001$) with levels being 2-3 times higher in the former.

b. Females:

NA levels rose through to 75 days, but then remained almost unchanged during the remainder of the investigation. Neonatal androgenization caused a depletion in NA concentration by 75 days and this was sustained throughout the period of study.

7. Corpus striatum (Fig. 27, Tables 14 and 15)

a. Males:

Low concentrations of NA observed at 12 days of age in the control group showed a very sharp rise soon after the onset of puberty, i.e. 60 days. This increase was short-lived. NA levels had fallen by 75 days although they rose again at 90 days. A gradual decline then continued throughout the remainder of the study. Neonatal castration resulted in NA levels being significantly lower than in the controls at 12 and 60 days. Levels in the castrated group were significantly higher than in the sham-operated animals at 75 days and again at 180 days.

Table 12

Concentrations of biogenic amines in the hippocampus of male rats at various post-natal ages.

A. Sham-Castrated.

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
60	1058.3±166.4 (6)		1188.0±242.6 (6)	615.7±123.3 (5)	1877.0±409.0 (6)
75	349.0±100.6 (6)		979.0±294.0 (6)	900.1±191.7 (6)	1050.7±409.8 (6)
90	498.9±84.0 (6)		683.6±214.6 (6)	245.1±75.7 (5)	842.3±191.8 (5)
120	718.7±214.4 (6)		138.0±41.7 (5)	262.7±59.3 (6)	1716.0±203.1 (6)
180	421.6±147.0 (6)	38.4±19.0 (5)	69.6±10.9 (5)	147.8±15.5 (5)	437.4±173.7 (5)

B. Castrated.

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
60					
75					
90					
120	1411.4±212.7*** (5)		181.0±62.7 (5)	565.5±178.7 (5)	1289.0±297.3** (5)
180	1980.9±525.5*** (5)	491.0±150.0*** (5)	1200.3±203.3*** (5)	1992.2±254.5*** (4)	

Mean (± SEM), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, numbers examined in parenthesis.

Table 13

Concentrations of biogenic amines in the hippocampus of control rats at various post-natal ages.

A. Control

Age (days)	ng/g wet weight				
	NA	ADR	DA	5HT	5HIAA
60	391.3±70.2 (8)	17.6±2.4 (5)	128.5±17.9 (8)	318.9±70.2 (6)	893.8±86.0 (6)
75	910.3±148.1 (5)	-	553.7±266.3 (5)	1015.3±113.3 (4)	1722.1±83.1 (4)
90	1032.1±131.1 (8)	185.6±12.1 (7)	339.7±62.6 (5)	839.3±90.2 (7)	1129.3±104.8 (6)
120	928.2±92.8 (6)	14.9±2.0 (4)	115.0±17.7 (5)	618.4±75.2 (5)	690.0±88.4 (5)
180	1263.7±198.3 (7)	75.7±6.2 (5)	260.3±55.9 (6)	1157.8±68.8 (6)	637.3±51.0 (5)

B. Androgenized

Age (days)	ng/g wet weight				
	NA	ADR	DA	5HT	5HIAA
60	617.4±90.7** (7)	34.9±6.2* (5)	146.5±31.1 (6)	888.6±36.0*** (6)	1571.4±183.4** (6)
75	352.9±68.5** (6)	10.1±2.3 (5)	650.0±63.6 (5)	865.3±106.1 (5)	524.4±65.8*** (5)
90	529.6±83.1* (6)	149.0±27.2* (5)	1335.7±189.4** (5)	526.3±79.8* (6)	1138.2±165.5* (6)
120	305.7±22.7*** (9)	21.1±6.2 (4)	31.3±1.7*** (6)	204.6±22.2*** (8)	601.1±41.6 (8)
180	283.7±25.3*** (11)	19.7±3.3*** (6)	80.3±6.7** (9)	183.2±24.8*** (9)	731.8±75.3 (10)

Mean (± SEM), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, numbers examined in parenthesis.

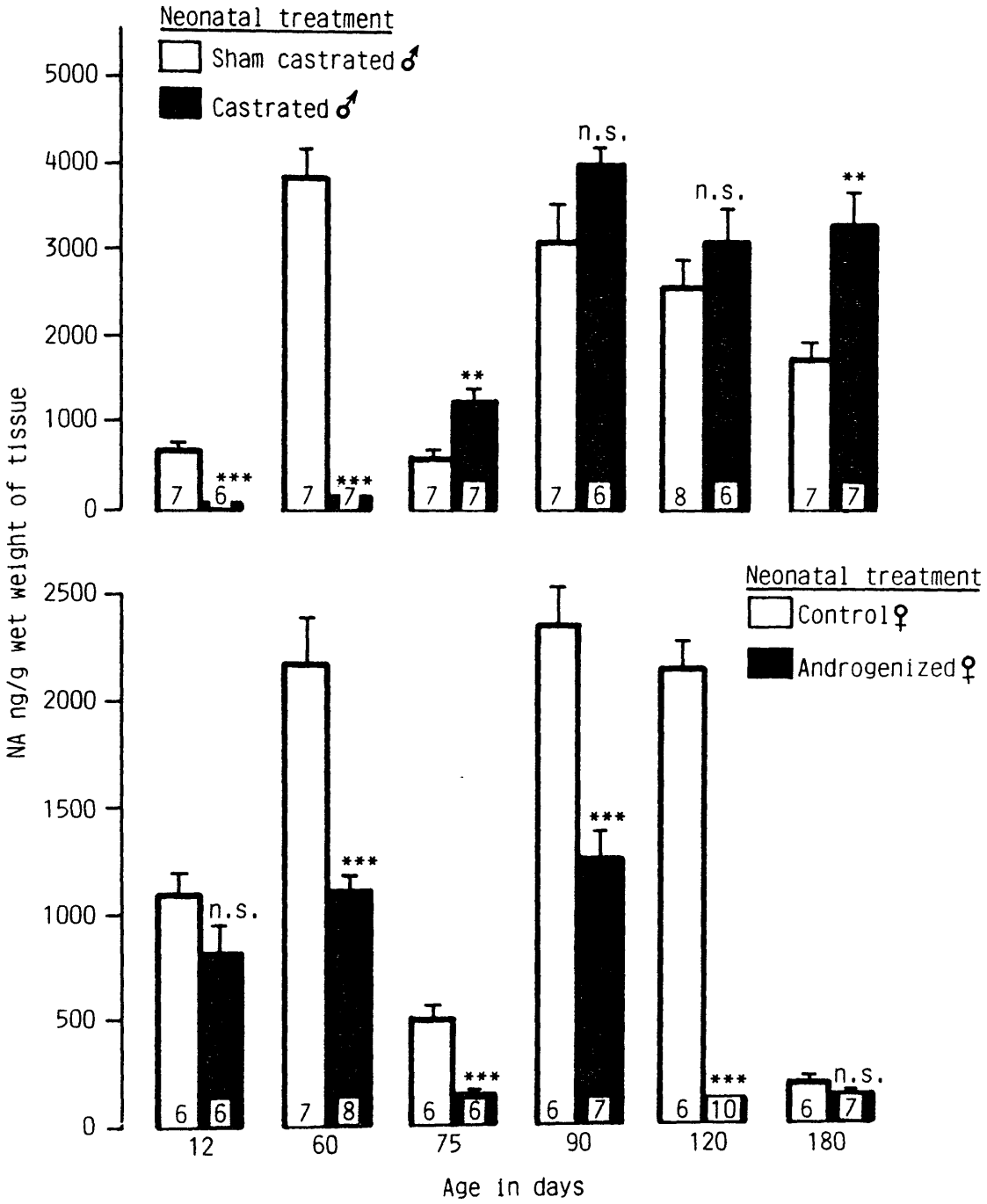


Fig. 27 Mean (± SEM) NA concentration in the Corpus Striatum in the control and experimental rats at various post-natal ages. *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

Table 14

Concentrations of biogenic amines in the corpus striatum of male rats at various post-natal ages.

A. Sham-Castrated

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	692.3±121.1 (7)	41.1±11.0 (3)	3003.3±311.9 (6)	247.9±48.2 (6)	1018.7±206.2 (6)
60	3847.4±345.5 (7)	12.5±2.3 (3)	7083.0±467.0 (12)	531.0±71.1 (7)	3267.2±498.4 (8)
75	594.7±93.8 (7)	21.5±4.7 (6)	4885.7±630.9 (8)	772.7±119.8 (7)	627.4±145.7 (6)
90	3128.0±453.2 (7)	-	9882.0±852.0 (9)	515.7±40.9 (8)	1982.7±209.5 (8)
120	2656.6±307.1 (8)	22.2±8.0 (5)	10453.5±732.9 (8)	207.2±19.7 (6)	1608.6±370.4 (6)
180	1824.0±196.4 (7)	-	3094.3±270.0 (7)	89.2±14.7 (6)	1049.1±117.7 (8)

B. Castrated

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	107.9±12.6*** (6)	-	5237.2±803.1* (6)	732.9±108.8** (7)	1283.6±195.0 (6)
60	166.7±10.2*** (7)	-	8955.3±518.2* (8)	212.9±33.5** (6)	362.1±58.2*** (6)
75	1271.5±156.8** (7)	3.4±2.0* (4)	9367.6±1139.8** (7)	276.9±41.3** (6)	118.3±32.6** (5)
90	4067.6±188.4 (6)	-	15591.3±763.1*** (6)	1016.7±179.0** (6)	6630.1±314.5*** (6)
120	3158.7±420.2 (6)	10.7±1.4 (4)	11849.2±609.2 (7)	600.6±78.9*** (7)	3154.2±565.7* (7)
180	3396.6±382.1** (7)	-	8375.7±1114.1*** (7)	459.4±56.1*** (7)	2646.8±323.3 (7)

Mean (± SEM), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, numbers examined in parenthesis.

Table 15

Concentrations of biogenic amines in the corpus striatum of female rats at various post-natal ages.

A. Control

Age (days)	ng/g wet weight				
	NA	ADR	DA	5HT	5HIAA
12	1094.4±104.5 (6)	-	3316.9±212.4 (6)	536.0±70.3 (6)	2583.0±394.4 (6)
60	2169.9±203.5 (7)	15.5±4.1 (5)	6083.8±501.4 (9)	357.7±52.7 (6)	3530.2±289.4 (9)
75	495.9±55.3 (6)	34.7±9.4 (3)	10863.4±1185.9 (6)	466.2±55.8 (6)	3476.0±356.6 (6)
90	2357.3±184.3 (6)	8.6±1.5 (3)	11126.3±507.7 (9)	788.3±45.0 (9)	2179.1±237.2 (8)
120	1876.1±206.5 (6)	19.6±2.3 (4)	10806.8±666.6 (6)	770.2±76.4 (6)	2014.3±160.0 (6)
180	198.5±28.2 (5)	23.6±4.7 (5)	15766.6±1039.7 (7)	1027.9±76.9 (6)	2392.3±107.0 (5)

B. Androgenized

Age (days)	ng/g wet weight				
	NA	ADR	DA	5HT	5HIAA
12	826.9±137.1 (6)	-	3029.5±350.0 (5)	263.6±65.5* (5)	2008.4±275.6 (5)
60	1113.5±87.4*** (8)	37.6±6.0* (5)	2585.7±112.0*** (8)	190.6±16.6* (8)	1179.2±166.5*** (8)
75	138.1±18.8*** (6)	20.9±3.9 (4)	3792.6±293.0*** (6)	202.8±24.9** (7)	4439.2±460.8 (6)
90	1249.3±120.8*** (7)	51.3±11.6* (5)	7421.3±417.0*** (9)	261.8±48.2*** (8)	2707.3±367.7 (9)
120	119.3±13.3*** (10)	17.7±3.6 (6)	3438.6±234.7*** (9)	281.7±26.7*** (11)	2301.1±233.8 (10)
180	134.4±17.4 (7)	13.8±1.7 (5)	4904.3±275.8*** (8)	222.8±30.0*** (9)	2580.5±361.5 (8)

Mean (± SEM), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, numbers examined in parenthesis.

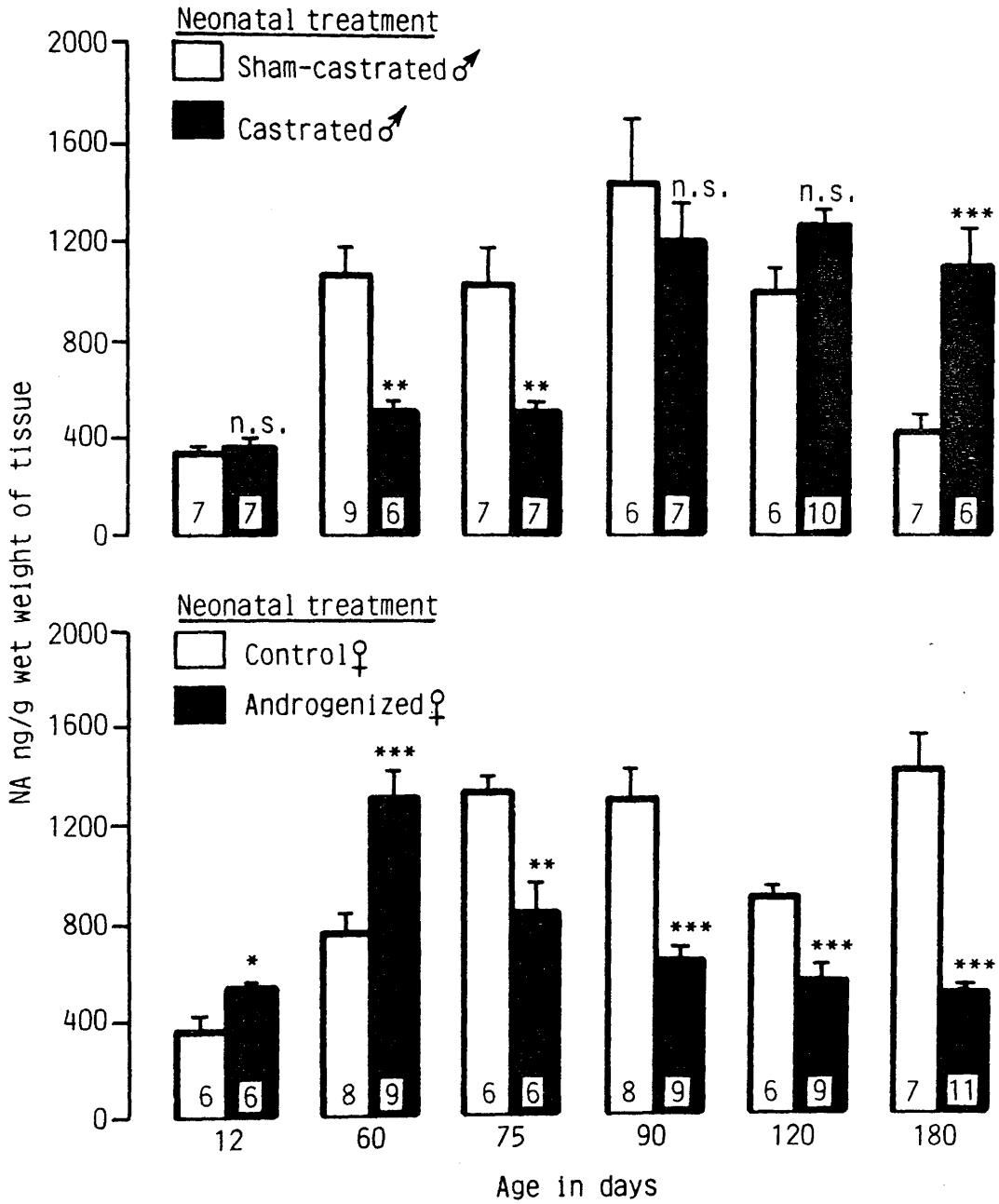


Fig. 28 Mean (± SEM) NA concentration in the corpora quadrigemina at various post-natal ages in the control and experimental rats *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

Table 16

Concentrations of biogenic amines in the corpora quadrigemina of male rats at various post-natal ages.

A. Sham-Castrated.

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	332.2±22.2 (7)	40.5±4.4 (5)	66.4±14.8 (6)	583.5±67.7 (7)	861.7±125.8 (6)
60	1062.1±129.3 (9)	108.9±11.3 (5)	199.2±29.7 (7)	628.9±165.5 (5)	2766.5±301.6 (8)
75	1042.2±152.9 (7)	21.2±6.0 (4)	294.8±22.1 (7)	129.7±16.1 (5)	2735.0±533.7 (5)
90	1456.5±251.0 (6)	86.1±20.9 (5)	431.1±22.5 (7)	1469.4±172.1 (8)	844.4±170.7 (8)
120	1013.0±97.1 (6)	-	266.9±41.5 (5)	275.3±67.6 (6)	3955.0±786.1 (6)
180	464.5±65.2 (7)	66.3±21.3 (5)	191.2±37.4 (7)	301.5±70.0 (5)	1320.1±150.4 (6)

B. Castrated.

Age (days)	ng/g wet weight of tissue				
	NA	ADR	DA	5HT	5HIAA
12	345.8±36.2 (7)	171.5±38.4** (3)	35.1±6.8 (5)	431.1±59.3 (6)	812.2±122.8 (6)
60	524.8±39.7** (6)	21.1±3.8*** (5)	36.1±6.9*** (6)	102.5±11.1** (6)	179.0±23.4*** (6)
75	518.3±33.7** (7)	75.8±22.3 (5)	272.9±28.1 (7)	232.9±33.1* (6)	328.3±64.8*** (7)
90	1232.0±154.8 (7)	24.9±4.3* (4)	160.0±17.1*** (6)	834.4±192.0* (6)	5308.0±916.3*** (8)
120	1254.3±70.8 (10)	22.8±2.7 (4)	218.0±22.3 (7)	915.0±73.2*** (8)	2171.1±231.5* (9)
180	1149.0±133.7*** (6)	25.7±4.6 (4)	551.1±77.2** (6)	845.9±140.7* (6)	3837.7±324.8*** (6)

Mean (± SEM), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, numbers examined in parenthesis.

Table 17

Concentrations of biogenic amines in the corpora quadrigemina of female rats at various post-natal ages.

A. Control

Age (days)	ng/g wet weight				
	NA	ADR	DA	5HT	5HIAA
12	364.8±64.8 (6)	92.7±10.2 (3)	90.5±15.6 (4)	1202.1±182.3 (6)	3870.6±366.9 (6)
60	751.5±85.6 (8)	31.8±3.6 (6)	158.7±20.2 (8)	885.7±69.5 (8)	2169.0±217.8 (7)
75	1344.6±68.1 (6)	5.1±2.7 (5)	401.6±55.1 (5)	1702.5±296.9 (5)	2615.4±47.8 (5)
90	1311.2±118.7 (8)	61.0±6.2 (6)	257.9±40.6 (6)	1592.0±240.3 (7)	2301.3±91.9 (6)
120	910.6±43.5 (6)	26.3±6.8 (4)	190.8±37.6 (5)	1026.1±66.8 (6)	1813.7±106.3 (6)
180	1427.7±147.9 (7)	23.0±3.5 (5)	305.1±52.3 (7)	1227.7±149.6 (7)	1995.9±275.7 (6)

B. Androgenized

Age (days)	ng/g wet weight				
	NA	ADR	DA	5HT	5HIAA
12	538.3±24.5* (6)	-	38.1±9.3* (5)	594.1±74.7* (5)	3592.1±430.0 (5)
60	1318.3±94.8*** (9)	27.5±3.5 (4)	202.2±24.6 (8)	1347.5±130.2** (7)	1855.5±195.0 (9)
75	835.9±125.0** (6)	3.4±1.0 (3)	171.9±23.8** (5)	404.1±68.5** (5)	1500.2±106.7*** (6)
90	647.7±65.5*** (9)	19.8±4.5*** (5)	235.0±25.6 (7)	379.6±55.5*** (7)	2593.1±209.8 (8)
120	558.7±52.4*** (9)	23.9±2.0 (5)	151.4±29.3 (6)	432.4±54.5*** (8)	1350.1±73.8** (10)
180	490.6±33.5*** (11)	11.9±2.8* (6)	70.2±10.9*** (7)	309.4±22.1*** (9)	1742.8±113.2 (11)

Mean (± SEM), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, numbers examined in parenthesis.

b. Females:

Androgenization caused a very significant reduction in NA levels at all ages studied with the exception of 12 and 180 days of age.

8. Corpora quadrigemina (Fig. 28, Tables 16 and 17)

a. Males:

In sham-castrated rats NA levels rose through to 90 days after which they showed a steep decline. Neonatal castration significantly reduced NA concentrations at 60 and 75 days compared to the control groups. At 90 and 120 days differences between control and experimental animals did not reach significance, but at 180 days, due to the depleted concentrations of NA in the control animals, levels in the castrated rats were observed to be significantly higher ($p < 0.001$).

b. Females:

In control animals NA concentrations showed a three-fold increase by the age of 75 days and levels then remained comparatively stable until the end of the study. Neonatal androgen administration, as in the hypothalamus, amygdala and cerebral cortex caused a very significant elevation in NA levels at 60 days of age. This was followed by a fall which was maintained through to 180 days.

Distribution of Dopamine in the Rat Brain

Hypothalamus, amygdala, cerebral cortex, cerebellum, corpus striatum and corpora quadrigemina (Figs. 29-34).

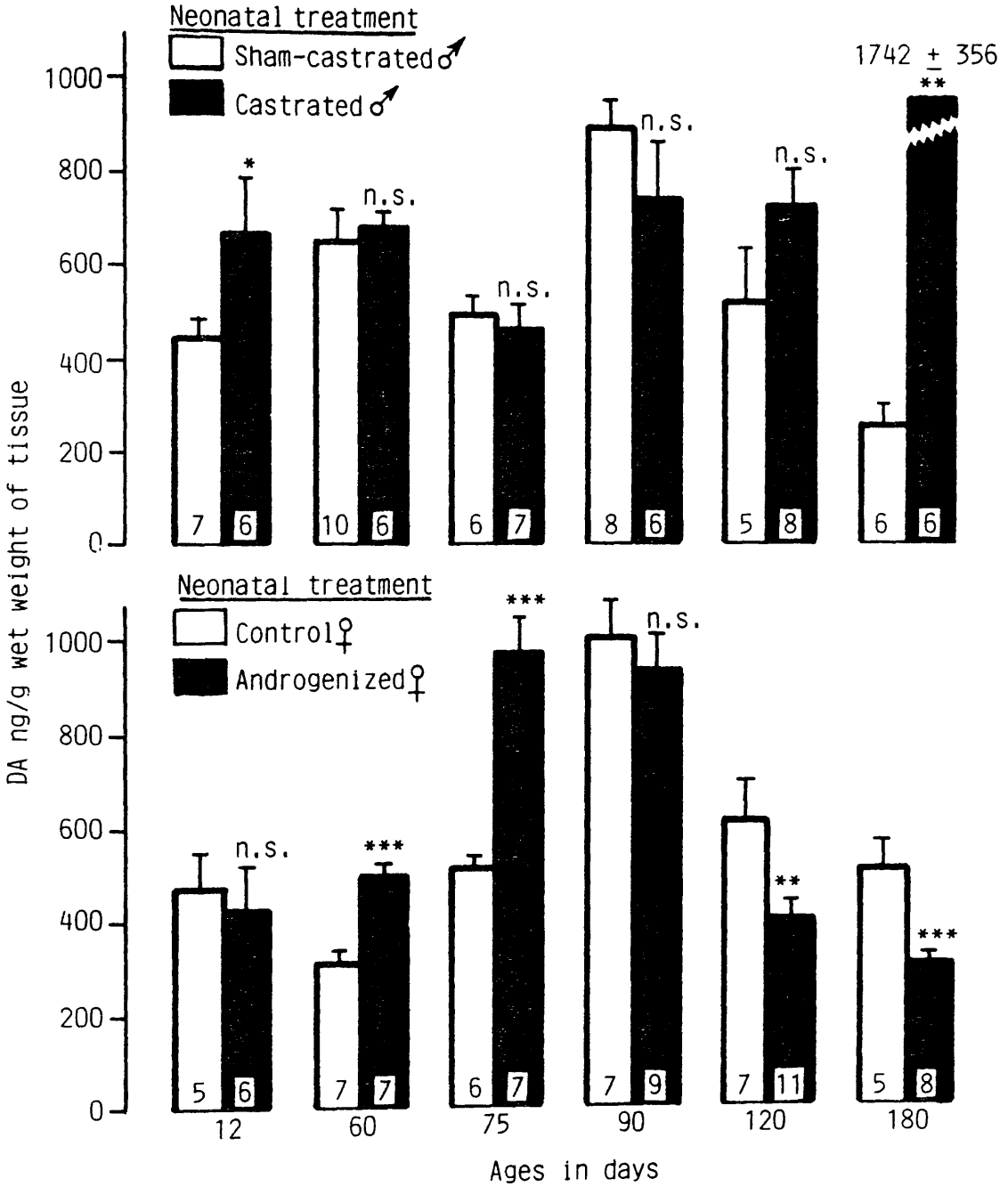


Fig. 29 Mean (± SEM) DA concentration in the hypothalamus at various postnatal ages in the control and experimental rats *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

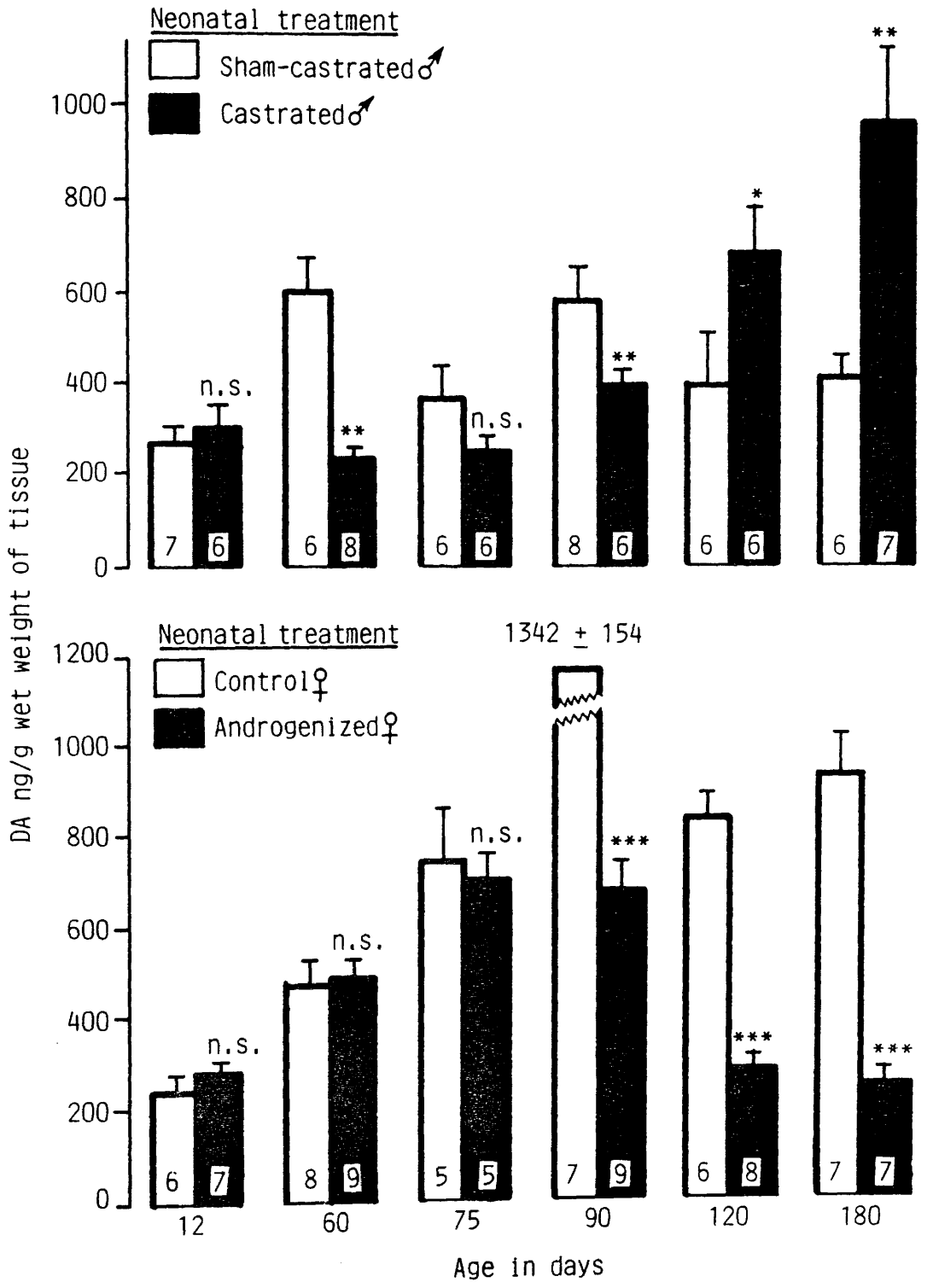


Fig. 30 Mean (± SEM) DA concentration in the amygdala at various postnatal ages in the control and experimental rats *p<0.05, **p<0.05, ***p<0.001, n.s. not significant.

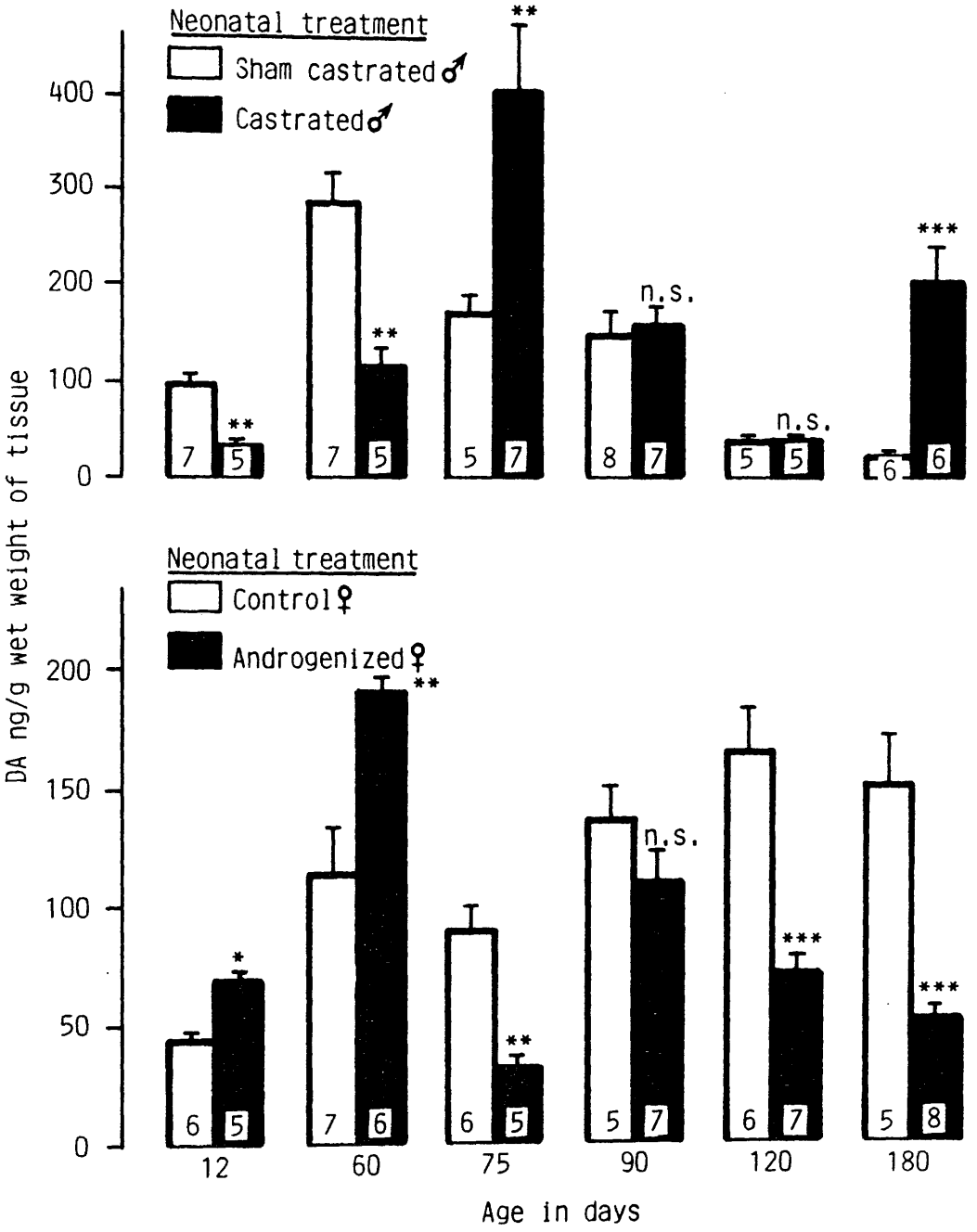


Fig. 31 Mean (± SEM) DA concentrations in the cerebral cortex of the control and experimental rats at various post-natal ages. *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

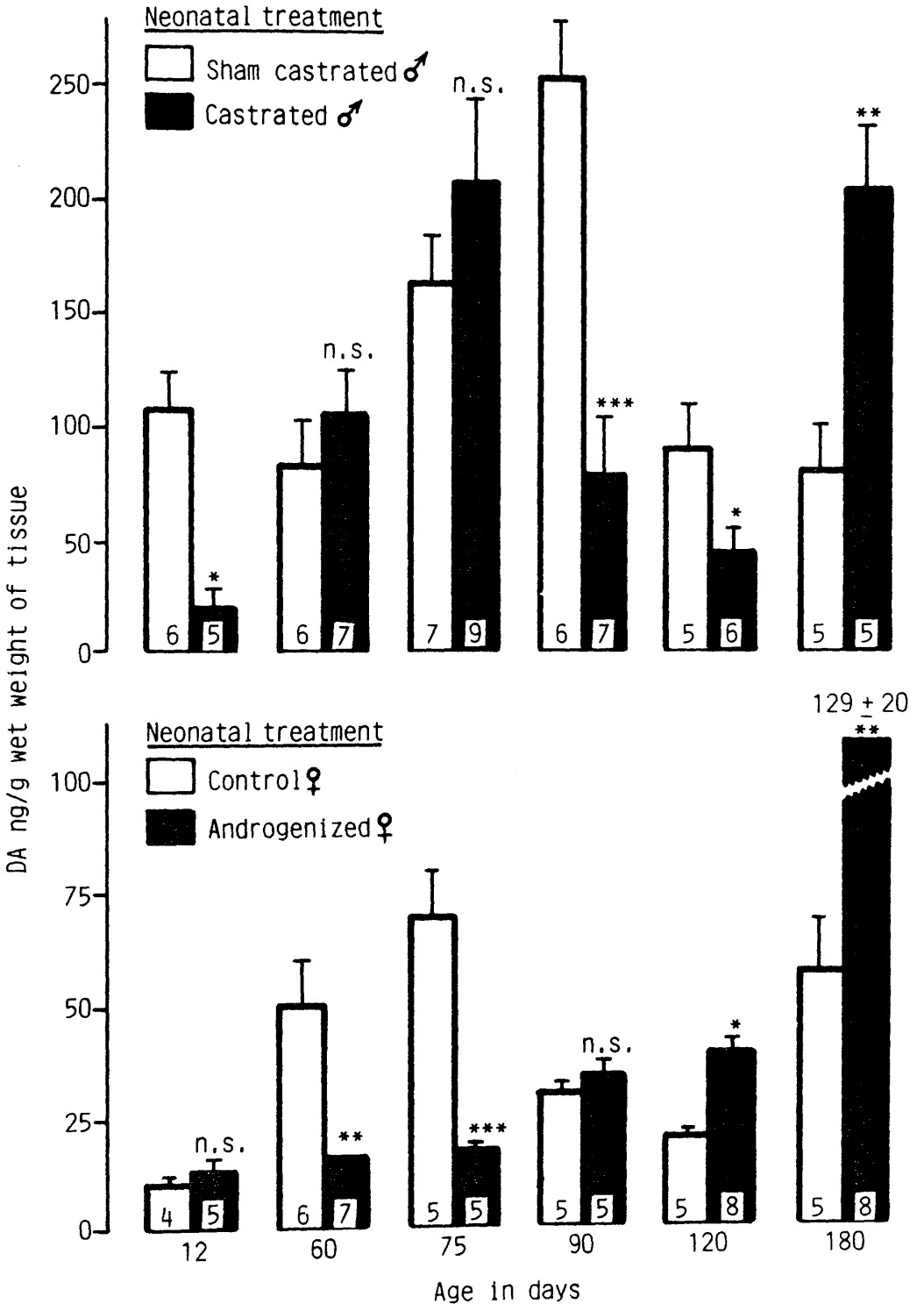


Fig. 32 Mean (± SEM) DA concentration in the cerebellum of the control and experimental rats at various post-natal ages. *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

a. Males:

In both the control and experimental groups DA levels showed no clear pattern of change in most areas of the brain studied with the exception of the corpus striatum. At 180 days however, DA levels in all brain regions were significantly higher in the experimental group than in the controls. DA concentrations rose consistently through to 120 days after which they fell dramatically. In those animals neonatally castrated DA levels in the corpus striatum were significantly elevated in comparison to the controls at all ages examined.

b. Females:

DA concentrations in the untreated animals showed a gradual increase until 90 days in the hypothalamus and amygdala. After this time they declined very substantially. Androgenization caused DA levels to be elevated at 60 and 75 days, in the hypothalamus (although not in the amygdala), but by 120 days levels had fallen dramatically.

Concentrations of DA in the cerebral cortex of control females exhibited little change during the whole period of the study. Neonatal androgenization however, resulted in a marked increase in DA concentrations in the infant and young rats while in older animals (120 and 180 days of age) there was a significant reduction in DA levels compared to the controls. This reduction in the older experimental groups was observed in other regions as well i.e. hypothalamus, amygdala, corpus callosum, hippocampus, corpus striatum and corpora quadrigemina.

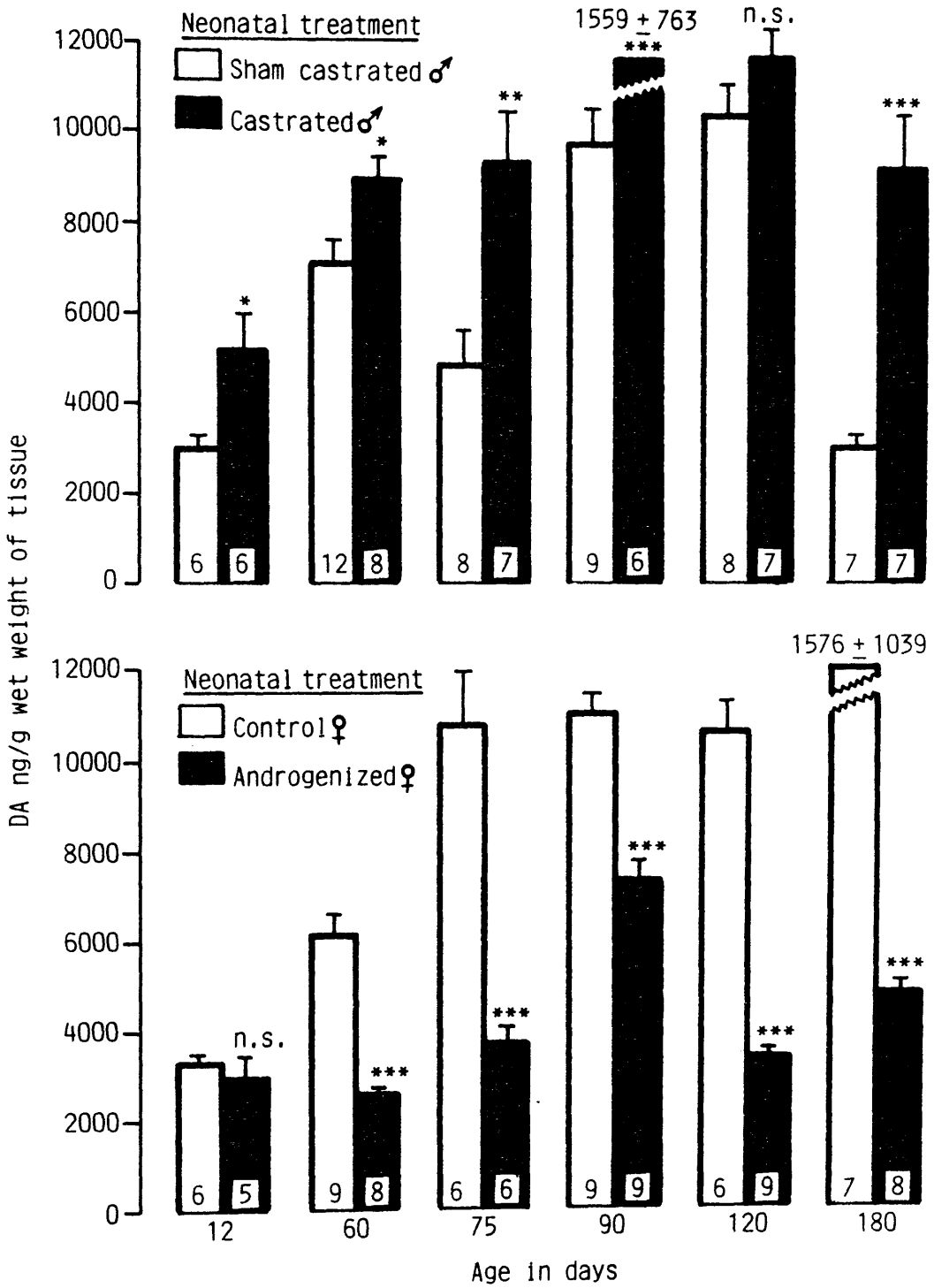


Fig. 33 Mean (± SEM) DA concentration in the Corpus Striatum in the control and experimental rats at various post-natal ages. *p<0.05, **<p0.01, ***p<0.001, n.s. not significant.

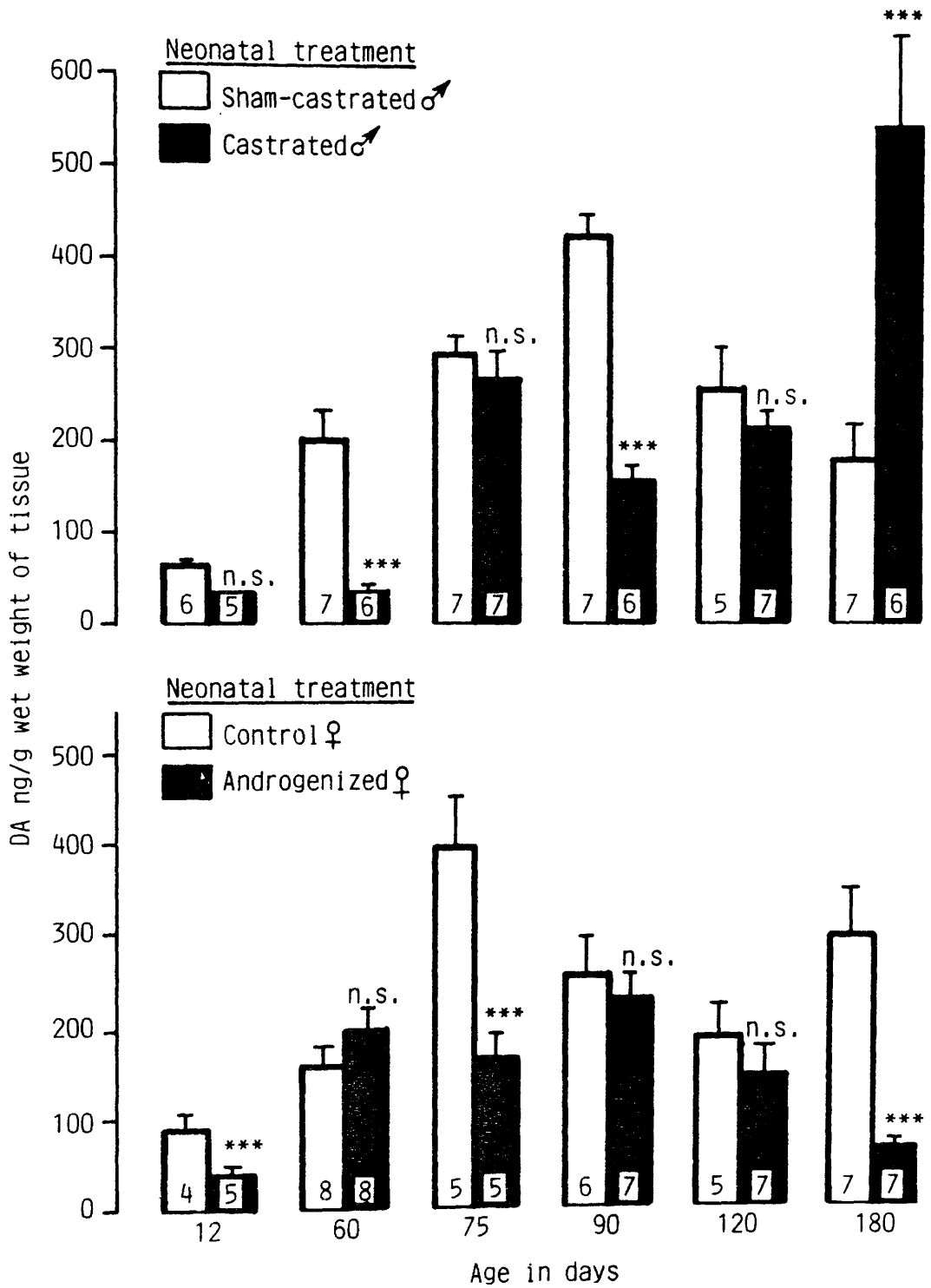


Fig. 34 Mean (± SEM) DA concentration in the corpora quadrigemina at various post-natal ages in the control and experimental rats *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

Cerebellar levels of DA were low in both control and experimental groups at 12 days of age. They remained low in the androgenized animals, but increased substantially in the controls to be significantly higher at 60 and 75 days. No significant difference between the two groups was observed at 90 days, but DA levels then rose in the androgenized rats to become significantly higher than in the untreated groups at 120 and 180 days.

Corpus Callosum and Hippocampus (Tables 10-13)

In the corpus callosum levels of DA in both control and experimental groups did not show any recognizable pattern of change throughout the study. In the sham-castrated animals DA concentrations in the hippocampus were observed to be very high at 60 days, but they then declined sharply. In those rats castrated neonatally, DA levels were very significantly higher at 180 days than in their controls.

In oil-treated animals DA concentrations both in the corpus callosum and hippocampus varied considerably in the different age groups. Neonatal testosterone treatment did not produce a significant effect until 90 days, when DA levels were greatly elevated in comparison to the controls. In the older animals (120 and 180 days) however, DA levels had fallen substantially to become significantly lower than in the oil-treated groups.

DISCUSSION

Discussion

Time-related noradrenergic changes in the hypothalamus, amygdala and cerebral cortex of the male rat

Neonatal manipulation of the androgen environment in the rat has been shown to bring about sexually dimorphic anatomical and functional changes in the brain, especially in terms of the neural control of sexual function and behaviour, by causing permanent changes in neuronal organization (Gorski, 1971; Naumenko, Serova and Pack, 1985).

A major finding of the present study was that the high concentrations of NA normally present in the above brain regions of male rats up to 90 days of age were very significantly lowered following neonatal testosterone deprivation. In the hypothalamus this reduction in the NA concentrations was evident at 12 days and was maintained until 90 days, while in the amygdala and cerebral cortex it only became apparent at a later stage (i.e. at 60 and 75 days respectively). It is possible that the removal of testosterone suppresses the growth of axonal processes and reduces interneuronal connections. The delayed effect observed in the amygdala and cerebral cortex might be due to a generalized influence of androgens on the noradrenergic cell bodies originating from the lateral tegmental area and in the locus coeruleus. Jones and Moore (1977) have described how noradrenergic innervation originating from the lateral tegmental area ascends in the medial forebrain bundle to the level of the hypothalamus. The paraventricular and periventricular nuclei of the hypothalamus receive as much

as 40% of their noradrenergic innervation from cells in the locus coeruleus. It is not however known whether noradrenergic neurones in the lateral tegmental and dorsal medullary systems (groups A1, A2, A5 and A7) project specifically to the median eminence or whether the terminals present in the median eminence are collaterals of neurones passing through the hypothalamus on the way to the forebrain regions (Moore and Johnson, 1982). Nevertheless, it is generally agreed that the neocortical noradrenergic innervation originates in the locus coeruleus (Lindvall and Björklund, 1978). Thus it has been demonstrated that after lesions in the locus coeruleus or the dorsal tegmental bundle (which originates exclusively in the locus coeruleus) there is in the neocortex a strong decrease of histochemically demonstrable noradrenergic terminals (Lindvall, Björklund and Divac, 1978). Furthermore biochemically measurable NA (Kobayashi, Palkovits, Kopin and Jacobowitz, 1974) and the major metabolite 4-hydroxy-3-methoxyphenylglycol (Korf, Aghajanian and Roth, 1973) are also reduced after such lesions.

The finding of high concentrations of NA in the hypothalamus of intact males during the present study cannot be attributed only to the presence there of the SDN-POA; high terminal densities are also seen in the supraoptic, paraventricular, dorsomedial, periventricular nuclei; the internal and sub-ependymal layers of the median eminence and the suprachiasmatic section of the hypothalamus (Ajika, 1979). Lower terminal densities occur in the arcuate nucleus, the medial and lateral

preoptic nuclei and part of the posterior hypothalamic area. Levels of NA in the hypothalamus, amygdala and cortex which initially declined following neonatal castration showed a very significant elevation in the older age groups (i.e. 120 and 180 days). This pattern of change observed in the older experimental animals was similar to that seen in the younger controls (up to 90 days), where the NA concentrations were high. These higher concentrations of NA seen in control animals up to 90 days indicate that the presence of testicular hormones during the period of sexual differentiation has probably been accelerating noradrenergic synaptogenesis. A similar pattern of change in NA concentrations has also been noted in the arcuate nucleus of adult rats (Crowley et al., 1978). The arcuate nucleus has been implicated in the control of anterior pituitary gonadotrophin secretion (McCann et al., 1977) and is a target tissue for both androgens and oestrogens (Sar and Stumpf, 1975). In the younger experimental animals it seems possible that the absence of gonadal steroids may have brought about a reduction in the central NA concentrations by limiting the availability of cofactors involved in the biosynthetic pathways of this amine (Coyle, 1973). The restoration of NA levels to control values seen in the older age groups may be attributed to a combined effect of hyperactive adrenals (following castration) and the increased adrenal corticosteroid receptor binding activity distributed all over the brain (Stumpf and Sar, 1978; Turner and Weaver, 1985). This increase in the NA concentrations does not however

necessarily mean that normal neuroendocrine function of the brain in the neonatally castrated rat is restored after a certain period of time. The high plasma LH levels seen in neonatally castrated rats throughout the period of study confirms that this is not so, and evidence is now accumulating to indicate that gonadal steroids permanently alter postnatal neuronal maturation and neural circuit formation (MacLusky and Naftolin, 1981; Arai, 1984). Nevertheless the suggestion can be made that organizational influences also occur outside of the critical period for differentiation of the neuroendocrine system that regulates cyclic gonadotrophin release.

The pattern of gradual increase in NA concentrations seen in both the hypothalamus and amygdala of neonatally castrated animals suggests that deprivation of testosterone during the critical period has delayed the maturation of the noradrenergic system. Concentrations of NA which were detected at 90 days in the control animals were not attained until 180 days in the experimental group. A probable explanation for this difference has already been given.

In the cerebral cortex the pattern of change seen following neonatal steroid manipulation was nearly identical to that which was observed in the other two regions. However, the condition observed in the control animals was quite different. It is suggested that the situation existing in the control groups could be due to the spatial temporal changes occurring during brain maturation, when there is a constantly shifting pattern of neuronal relationships (Coyle, 1973; Goldman-Rakic and Brown, 1982). The changes seen in the cerebral cortex

of the experimental groups might be related to other non-reproductive functional activities of this region which also require gonadal steroid exposure during a critical period of neuronal development.

The hypothalamus and the amygdala are regions which have a well documented neuroendocrine role and there is very similar patterns of time-related change in NA concentrations in both the control and the experimental animals. This finding is in agreement with other observed testosterone-dependent structural dimorphic changes in the CNS such as neuronal numbers, synaptic organization and nuclear volume (Raisman and Field, 1973; Staudt and Dörner, 1976; Gorski et al., 1978; Ayoub et al., 1982; Güldner, 1982). On the other hand although the cerebral cortex showed a similar pattern of change to the hypothalamus and amygdala in the experimental animals, the control levels of NA were quite different from those observed in the other two regions. Other workers (Pappas et al., 1978; MacLusky, 1979; Diamond, Young, Sukhwinder and Johnson, 1981; MacLusky et al., 1986) examining the cortex have found sex differences in the number of oestrogen receptors. Changes in NA concentrations in the cerebral cortex following neonatal castration may be explained in terms of a generalized effect of the treatment.

The cortex may receive catecholaminergic inputs from sources other than just the lateral tegmental area and locus coeruleus. However neonatal hormonal manipulation has noticably affected the NA concentrations in almost the same way as in the other two regions. The maturation of the noradrenergic

system in the cerebral cortex continues well into postpubertal life and exhibits considerable variation depending upon its topographic and perhaps functional localization within the cortex. In assessing the biological significance of these findings, it should be emphasized that the changes in NA content and synthesis observed in the rat probably occur well after most brainstem afferents have entered the cerebral cortex. However with the extensive collaterization of noradrenergic neurones throughout the brain it is conceptually difficult to relate to them a specific endocrine function.

Time-related dopaminergic changes in the hypothalamus, amygdala and cerebral cortex of the male rat

In the hypothalamus, amygdala and cerebral cortex no clear pattern of change in DA concentrations was observed until around 120 days of age in both the control and experimental animals. This result does not coincide with the data published by Crowley et al. (1978), who reported a reduction in DA levels in the hypothalamus of adult male rats following castration on day 1 of neonatal life. Our finding with regard to time-dependent changes in concentrations in the hypothalamus, amygdala and cerebral cortex also disagree with those of Vaccari (1980), who observed that DA levels are consistently higher in females than in their male littermates. In general, our work suggests an inconsistent pattern of DA distribution in neonatally testosterone-deprived rats until 120 days of age. Whereas an increase in the DA content of the hypothalamus, amygdala and cortex of androgen-deprived animals was a very

consistent feature at 180 days, this was the only time that the findings agreed with those of Gladue, Humphrys, Debold and Clemens (1977).

Dopamine-containing cell bodies have been identified in various regions of the CNS and the three regions under consideration receive dopaminergic pathways from many sources. A major group of hypothalamic dopaminergic neurones is located in the arcuate nucleus (A12 cell group) which gives rise to tuberoinfundibular dopaminergic projections. Few dopaminergic cells are present along the third ventricle in the preoptic periventricular nuclei (A14 cell group) (Palkovits, 1984). DA-containing cell bodies have also recently been identified in the paraventricular nucleus by immunohistochemistry (Swanson et al., 1981). A small number of DA-containing neurones also exist in the zona incerta and dorsal to the dorsomedial nucleus (A13 cell group) and these innervate the dorsal hypothalamic nuclei and the substantia nigra.

The largest dopaminergic cell group in the CNS occupies the territory of the ventral tegmental area (A10 cell group), the zona compacta (A9 cell group) and the pars lateralis (A8 cell group) of the substantia nigra. The rich dopaminergic innervation of the median eminence is derived from fibres that project from dopaminergic cell bodies lying in the tuberoinfundibular area. Mesocortical dopaminergic pathways arising from the ventral tegmental area ascend in the lateral part of the medial forebrain bundle to the cingulum. These pathways are also joined by mesolimbic dopaminergic projections arising from the A8, A9 and A10 cell groups (Palkovits, 1984).

The presence of neonatal androgen has only resulted in an elevation of DA concentrations in the 60 day-old age group. This may possibly be the time when the enzymatic machinery for catecholamine synthesis is fully functional in the amygdala and cerebral cortex. The situation in the hypothalamus is however not so clear. Our work showed a consistently significant rise in DA concentrations in the three areas examined in the experimental animals compared to the control groups. This observation again lends support to the theory that removal of androgens during the neonatal period may lead to a lowering of DA concentrations throughout the CNS. The fact that DA concentrations only rose to control levels in the older groups of castrated animals points towards the possibility that as with the noradrenergic system other functions not related to reproduction may be involved in this long-term rise.

As yet no explanation for the DA-increase in the cortex can be presented.

Time-related catecholaminergic changes in the cerebellum, hippocampus, corpus striatum, corpora quadrigemina and corpus callosum of the male rat

In the rat it has been reported that all areas of the cerebellum receive a catecholaminergic innervation that is probably exclusively noradrenergic (Bloom, Hoffer and Siggins, 1971). Our study however indicates that this is not true as very low concentrations of DA were detected in the cerebellum.

NA-containing pathways which originate in the locus coeruleus and A4 cell group reach the cerebellum through the superior peduncle (Bloom, Krebs, Nicholson and Pickel, 1974; Palkovits, 1984). Recent studies by Dietrichs and Zheng (1984) and Dietrichs and Haines (1986) on both rats and cats have demonstrated the presence of a small population of hypothalamic neurones projecting to the cerebellum, spinal cord and amygdala. These projections may be suggested as a possible source of dopamine. In our study neonatal castration did not appear to alter the concentrations of NA and DA in the cerebellum, as it did in the hypothalamus and amygdala, during young age. This finding further substantiates the concept that the cerebellum may be a general modulator and coordinator of a wide variety of central nervous responses (Dietrichs and Haines, 1986).

The tremendous increase in NA concentrations observed at 120 and 180 days in the hippocampus of the castrated rats is an indicator of the effects of neonatal androgen deprivation. In the hippocampus sex differences in cytological parameters such as neuronal size, dendritic spine and axonal density have recently been reviewed (Toran-Allerand, 1984). Neonatal castration may have resulted in an increased uptake activity for NA in the above cytological parameters.

The detection of NA in the hippocampus is in accordance with the observation made by Jones and Moore (1977) that the hippocampal catecholaminergic innervation seems to be exclusively noradrenergic and like that in the neocortex is

derived from the NA cell bodies of the locus coeruleus. No other noradrenergic sources have so far been identified with certainty. The terminal distribution of NA fibres in the hippocampal formation of the rat has been described by Swanson and Hartman (1975). The ventral part of the entorhinal cortex receives, in addition, a dopaminergic innervation that originates in the mesencephalic dopaminergic cell group (Carter and Fibiger, 1977).

The dopaminergic innervation of the entorhinal cortex seems to be confined to its most ventral part (Lindvall, Björklund, Moore and Stenevi, 1974). The dopaminergic fibres occur - intermingled with NA-containing ones - in characteristic clusters localized in layers I - II (Lindvall and Björklund, 1978). The dopaminergic fibres reach the entorhinal cortex from the medial forebrain bundle via the ansa lenticularis system, running caudally through the amygdaloid area (Lindvall et al., 1974). Therefore, the changes observed in DA concentrations may not be a true reflection of dopaminergic activity in the hippocampus. Additional evidence of the catecholaminergic activity comes from the receptor studies of Stumpf and Sar (1978). They reported the presence of corticosteroid receptors in the hippocampus in addition to androgen and oestrogen receptors. An interaction between the gonadal steroids and the corticosteroids could influence the catecholaminergic systems of the hippocampus. The dendritic sexual dimorphism in this region may also be influenced by inputs from other brain areas that have gonadal hormone receptors.

Although no neuroanatomical information could be found on the noradrenergic innervation of the corpus striatum, significant concentrations of NA were observed in this region throughout the period of postnatal development investigated. Gordon and Shellenberger (1974) reported that the NA content of the corpus striatum was higher in males than in females. Later, in 1978, Crowley et al. reported that NA levels did not alter in adult animals following neonatal castration. Our findings which report NA concentrations at various developmental stages of the CNS do not agree with either of the aforementioned studies. Increased noradrenergic activity in the control animals may be a consequence of the onset of puberty although this region has not previously been implicated in the modulation of reproductive function. However, before this possibility can be accepted a more detailed study needs to be carried out to investigate the whole cascade of events that take place in all regions of the brain preceding and following puberty. A reduction in NA concentrations from control values observed in the experimental groups at 12 and 60 days of age was reversed at 75 days. No satisfactory explanation is available for this finding. The irregular pattern of changes in noradrenergic concentrations seen in the corpus striatum may not be directly related to sexual differentiation. Rather these differences could reflect sex differences in motor activity, food and water intake and other parameters of body weight regulation (Gordon and Shellenberger, 1974). The increased NA concentrations seen in the corpus striatum of

castrated males at 180 days was again a feature common to other brain areas investigated. Perhaps the same explanation for this finding would stand as that given earlier.

Dopaminergic inputs to the corpus striatum are mainly derived from the substantia nigra. Some axonal inputs are also received from the pyramidal neurones in the lamina V of the cerebral cortex and from the intralaminar thalamic nuclei.

In our work a significant increase in DA concentrations was observed in all age groups (with the exception of 120 days) following the neonatal deprivation of testosterone. The coordinated increase observed until 120 days in control animals might be attributed to the rise in activity of the enzymes concerned with DA synthesis (Coyle and Axelrod, 1972).

The rise in DA concentrations following neonatal castration observed throughout the period of investigation again conflicts with the observations made by Vaccari (1980). This author detected a higher concentration of DA in the adult male rat than in the female. This discrepancy could possibly be a strain specific one as Vaccari made his observations on Wistar rats whereas the Sprague-Dawley strain was used in our research.

In the present study a significant elevation in dopamine levels seen at 180 days was a feature common to all other areas of the CNS investigated in the castrated male rats. This observation also supports the argument that the absence of testosterone during the neonatal period has a generalized effect on both the noradrenergic and dopaminergic cell bodies

at this particular time of brain development. We suggest that this effect could be explained either by a restoration of synaptogenesis halted by the lack of testosterone during the critical period of CNS differentiation or availability of factors required for biosynthetic pathways.

The use of both fluorescence and dopamine-β hydroxylase immunohistochemical techniques indicate that the catecholaminergic innervation in this region is predominantly noradrenergic and originates from the locus coeruleus. This belief is also supported by the fact that unilateral destruction of the locus coeruleus by electrolytic lesioning will bring about a significant decrease in NA concentrations in the inferior colliculi (Kobayashi et al., 1974). The innervation of the superior colliculi is probably both noradrenergic and dopaminergic. The noradrenergic component should be identical with the projections from the locus coeruleus, whereas the superior collicular afferents of periventricular origin would represent the dopaminergic component.

It could be postulated that the absence of gonadal steroids at a critical time of brain development might affect the corpora quadrigemina by failing to preserve the catecholaminergic cell bodies. Some regeneration might occur at a later stage of brain development under the influence of hyperactive adrenals. This hypothesis is in agreement with a recent report by Björklund (1986) who indicated that at least certain types of grafted neurones can substitute both anatomically and functionally for damaged connections in the lesioned

adult brain.

In this study catecholamine concentrations in the corpus callosum did not show any change between control and experimental rats of both sexes. However, a recent report by De Lacoste-Utamsing and Holloway (1982) suggests that differences in the shape of the splenium of the corpus callosum in adults are sex-dependent. More recently Bell and Variend (1985) made a quantitative and morphological study in the corpus callosum and failed to confirm any gender specific differences during childhood.

Discussion

Time-related catecholaminergic changes in the various brain regions of the female rat

In the female rat, the hypothalamus can be influenced by testicular hormones rather longer than can that of the male - until about the tenth day of neonatal life. In this study the dose of testosterone administered caused a variable degree of androgenization. In most cases vaginal opening occurred very late (at about two months of age). After a short period of irregular cyclicity eventual permanent cornification of the vagina took place. In a small number of cases a few normal oestrous cycles occurred until the onset of anovulatory infertility and persistent vaginal cornification (delayed anovulatory syndrome). It is as if the normal process of ageing, during which older females cease to ovulate and show persistent vaginal cornification from about 200 days of age (Booth, 1979), has been speeded up by exposure to androgens during infancy. Experiments on DAS animals have been reviewed by Barraclough and Gorski (1961), who put forward a hypothesis for the hypothalamic control of gonadotrophin secretion in the rat. They proposed that the POA is normally responsible for the cyclic discharge of gonadotrophins and ovulation in the female. The basal hypothalamus, including the arcuate and ventro-medial nuclei would then control the 'tonic' discharge of gonadotrophins necessary to maintain the testes in the male, and follicular growth both in the normal and androgenized female.

To match the androgenizing effect the control females used in this study were always killed on the day of oestrus nearest to that of the assigned age group. The DAS may not result solely from the action of neonatal androgens upon the brain. Exposure of developing female rats to androgens has been reported to cause permanent changes in other tissues, e.g. the vagina (Ohta and Iguchi, 1976), the uterus (Hahn, Lai and Greenslade, 1974), the ovaries and the anterior pituitary (Mennin, Kubo and Gorski, 1974). In the case of the ovaries and pituitary gland, sensitivity to gonadotrophins and GnRH may be altered as a result of exposure to androgens during infancy.

One consequence of neonatal testosterone treatment was the altered catecholamine concentrations seen in various regions of the brain investigated. The observations made just after the process of brain sexual differentiation is presumed to have occurred (12 days), disagree with the findings of Hardin (1973) who reported that no differences in NA levels existed between the forebrain, hypothalamus and cerebral cortex at this age. Two other studies have also failed to find significant sex differences between NA levels in the hypothalamus, cerebral cortex, mesencephalon and hindbrain of rats aged from 8-14 and 12-20 days (Gladue et al., 1977; Vaccari, Brotman, Cimino and Timiras, 1977).

Concentrations of NA and DA in androgenized females were very significantly higher than in control animals at 60 days of age. This finding was the exact reverse of that

seen in the neonatally castrated male rats, where NA levels were very low, being similar to those observed in the control females. Alterations in catecholamine levels following androgenization has been reported to contribute to an acyclic pattern of gonadotrophin release and decreased sensitivity to ovarian hormones in adulthood (Barraclough, 1979; Booth, 1979).

The lower brain NA content of untreated females compared to intact males and androgenized females may be a reflection of a greater and faster rate of NA utilization in the midbrain of female rats. NA, has indeed been shown to be consistently more depleted (lower steady state levels) in the female than in the male brain, after inhibition of the rate-limiting enzyme tyrosine-hydroxylase with α-methyl-paratyrosine (Gordon and Shellenberger, 1974). An additional, indirect proof of a faster 'functioning' of the catecholamine system in adult female rats is provided by the observation that brain slices of pro-oestrous females take up more NA than do those of males (Wirz-Justice and Lichtensteiger, 1976).

The increase in the NA and DA content of the hypothalamus observed at 60 days of age in the experimental rats is in agreement with the findings of Crowley *et al.* (1978), who investigated the changes in the catecholamine content in hypothalamic nuclei of neonatally androgenized rats at 66 days of age. These authors observed an elevation of NA and DA in the arcuate nucleus, an area which has been implicated in the control of anterior pituitary secretion (McCann *et al.*,

1977).

The arcuate nucleus is a target tissue for both androgen and oestrogen (Sar and Stumpf, 1975). Furthermore it is interesting to note that the most effective locus for inducing acyclicity in female rats with neonatal testosterone implants has been shown to be the medial basal hypothalamus, including the arcuate nucleus (Crowley et al., 1978).

The rise in NA levels observed in the androgenized females at 60 days is similar to that seen in the males of the same age. However, the changes observed at this time do not appear to bear any relationship to the effects of puberty. Our results suggest that the effect of neonatal androgens on the brain content of NA and DA observed in the younger females, did not persist into later life. The short-lived rise seen at 60 days could perhaps be attributed to the use of testosterone itself and not testosterone propionate which is described as a more potent and effective androgen.

In the hypothalamus an increase in volume of the SDN-POA following the subcutaneous administration of exogenous testosterone propionate to newborn female rats has also been reported (Gorski et al., 1978; Jacobson and Gorski, 1981). In a more recent observation Arnold and Gorski (1984) reported that manipulation of the hormonal environment in the neonatal female does not however lead to a sex-reversal of the volume of SDN-POA. They postulated that the volume of the SDN-POA could be due to:

a) possible nonhormonal factors influencing its differentiation, or

b) a requirement for a greater, earlier or more prolonged exposure to androgen in the female.

However, prolonged treatment with testosterone propionate (2 mg daily to the pregnant rat from gestational day 16, and 100 µg daily to individual pups until day 10 of postnatal life) does not completely sex reverse the brain of the female in terms of SDN-POA volume (Döhler et al., 1982b). Although this observation does not exclude the possibility of a non-hormonal component, it does indicate that the steroid hormonal environment alone cannot fully determine final SDN-POA volume. No comments have been reported on the monoamine content of the SDN-POA.

The pattern of change with time in catecholamine content after neonatal hormonal manipulation observed in the hypothalamus during this study is typical of almost all other brain regions. The only exception was the change in DA concentrations seen in the cerebellum where, in the androgenized females, it was the reverse of what was observed in all other regions of the CNS studied. The situation with regard to the cerebellum is discussed later.

One effect of neonatal testosterone upon the developing CNS is to alter the cytoarchitecture in the neuroendocrine regions of the brain. As mentioned earlier, our results indicate that the characteristic change observed in most areas investigated might be due to a generalized suppressive

effect of androgens on both the noradrenergic and dopaminergic cell bodies in the lateral tegmental area, locus coeruleus and substantia nigra. Ascending pathways from the lateral tegmental area provide a noradrenergic input to the preoptic, ventromedial and arcuate nucleus of the hypothalamus. The presence of structural sexual dimorphism in terms of these region's functional properties has been widely reported (Gorski et al., 1978; Döhler, 1982a).

In control females concentrations of catecholamines showed a rise when the animals reacted 75 days of age and this was maintained throughout the period of study. This finding appears consistent with the hypothesis of Staudt and Dörner (1976) who postulated that the lower the androgen levels during the critical period of sex-specific brain differentiation, the higher appear to be the nuclear volumes of the medial and central parts of the amygdala throughout life. However Nishizuka and Arai (1981) dispute this theory. They report that synaptogenesis can be stimulated by androgens in the arcuate nucleus of the hypothalamus as well as in the medial amygdaloid nucleus.

The reduced hypothalamic content of NA seen in the androgenized females might prevent the appearance of the appropriate neural signal required for the discharge of LHRH from median eminence axon terminals, thus leading to reduced LH levels in this group. However, this does not explain the elevated LH values recorded at 120 and 180 days. Barraclough and Gorski (1961) originally suggested that the POA of the

brain is deletoriously affected by neonatal androgen exposure such that the cyclic regulation of gonadotrophin secretion is permanently disrupted. This early conclusion was based on the observation that neonatal androgenization (or ultimately oestrogenization) produced an ovarian polyfollicular, anovulatory syndrome which closely resembles that obtained after the destruction of the POA (Barraclough, Yrarrazavi and Haton, 1964). More recently Barraclough and Wise (1982) have established that in the adult androgen-sterilized female rat:

a) the numbers of GnRH perikarya and the distribution of their processes throughout the hypothalamus does not differ from normal female rats that have been ovariectomized and given steroid replacement (King et al., 1980);

b) sufficient GnRH is synthesized, transported and stored in median eminence axon terminals of the preoptico-suprachiasmatic tubero infundibular system of androgen sterilized rats to evoke preovulatory-like LH and FSH surges when depolarizing stimuli are delivered to the medial preoptic nucleus (Chappel and Barraclough, 1976);

c) pituitary responsiveness to LHRH is the same in androgen-sterilized and in cyclic pro-oestrous rats (Castro-vazquez and McCann, 1975);

d) in androgen-sterilized rats oestrogen nuclear receptor concentrations and binding affinities are normal in the preoptico-hypothalamic region and in at least one oestrogen-induced expression of neuronal genomic programming, namely induction of progestin cytosol receptors (Etgen, 1981).

In spite of the apparently normal physiological processes detailed above occurring in the preoptico-suprachiasmatic tuberoinfundibular system of androgen sterilized rats, nevertheless the positive feedback induction of LH and FSH surges by steroids (Harlan and Gorski, 1977), and the spontaneous release of these gonadotrophins do not occur. Recently Barraclough and Wise (1982) produced evidence to indicate that the neural trigger responsible for the preovulatory surges of GnRH and LH in pro-oestrous rats, or in oestrogen-treated ovariectomized animals is NA. This leads one to the conclusion that the early effects of androgens in the undifferentiated endocrine brain might involve the catecholaminergic system. It can therefore be suggested that the mid- and hindbrain noradrenergic systems are involved as initiators of the cyclic gonadotrophin surges.

The explanation given above for the reduced NA and DA concentrations seen in the brains of androgenized females seems to hold true at least for the hypothalamus and amygdala. However the fact that similar patterns exist in other regions of the brain draws attention to the possible deleterious effect of androgens upon the developing female CNS. The results of studies upon the SDN-POA and the ventromedial nucleus of the amygdala suggest that steroid hormones act at very fundamental levels of developmental neurobiology. Therefore the observations on the other brain regions points towards the involvement of these structures in sexually dimorphic functions that are independent of neuroendocrinology and

sexual behaviour.

Our measurements of NA and DA concentrations in the hypothalamus and amygdala show a similar pattern for sham-castrated males and androgenized females in adulthood. This finding is in agreement with the observations made by Adcock, Fenela, Fitzpatrick and Greenstein (1985). In their experiments on sexual dimorphism in the neonatal rat it appeared that administration of oestradiol benzoate converted a female pattern of mRNA-directed protein synthesis in the hypothalamus into a male pattern. Our findings confirm this report and this also seems to be true for the amygdala. Adcock et al. (1985) surprisingly found in one experiment that injection of oil alone had the same effect on the pattern on mRNA directed protein synthesis as did oestradiol benzoate. This raises the possibility that stress may also influence transcription in the neonatal brain. A reduction of dopamine concentrations in regions of brain not directly involved in reproductive function also points towards the pathogenesis of Parkinsonism, Alzheimer's disease and the Shy-Drager syndrome, conditions which involve the degeneration of catecholamine-containing neurones throughout the CNS (Black and Petito, 1976; Rinne, 1979; Eisler, Thorner, MacLeod, Kaiser and Calne, 1981; Hiramatsu, Kabuto and Mori, 1986). At postmortem, such patients typically demonstrate profound loss of dopaminergic neurones in the substantia nigra and of the noradrenergic neurones in the locus coeruleus (Forno, 1966; Black and Petito, 1976; Rinne, 1979). However, the fate of neurones in other catecholamine-containing cell groups has not been systematically investigated.

Outside the hypothalamo-amygdaloid complex there are regions which display oestrogen-binding activity: H^3-oestradiol visibly accumulates in the septal nuclei and hippocampus, in the midbrain, in the dorsal region of the pons (nucleus parabrachialis, locus coeruleus and central gray), in the cerebral cortex, in the corpus striatum, in the cerebellum and in the spinal cord of the rat (Stumpf and Sar, 1979).

The concentrations of DA observed in the corpus striatum of females androgenized neonatally were significantly reduced from control values by 60 days of age. This reduction was evident throughout the remainder of the study and paralleled the situation seen in the males. This finding disagreed with that of Vaccari (1980). The discrepancy could be due to oestrogen modulation of extra-pyramidal functions (Maggi and Perez, 1985) i.e. oestrogen both antagonizes and potentiates the activity of the dopaminergic system in the corpus striatum.

Biochemically oestrogen:

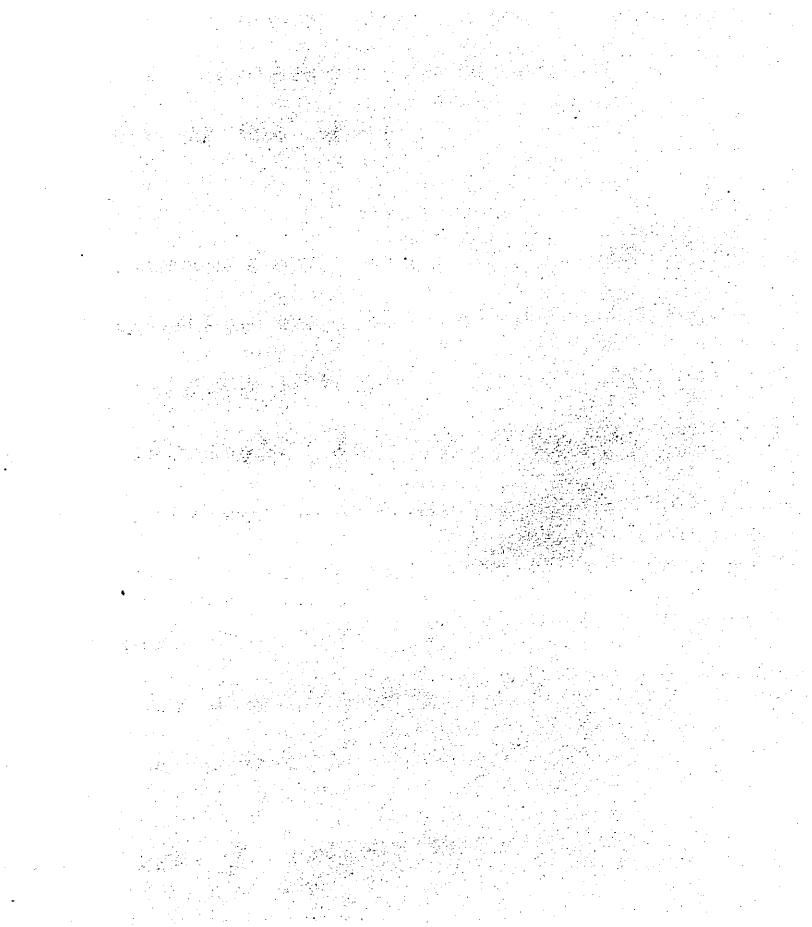
a) increases the number of DA receptors in the corpus striatum (Hruska and Silbergeld, 1980);
b) interferes with catechol-O-methyl transferase activity (Ball, Knupper, Haupt and Breur, 1972);
c) increases DA synthesis and turnover (Becker, Beer and Robison, 1984);
d) potentiates DA release stimulated by amphetamine or K^+ (Euvrard, Labrie and Boissie, 1979).

At the same time oestrogen receptors are also reported to: a) reduce striatal DA concentration (Foreman and Porter, 1980); b) reduce the response of striatal cholinergic neurones to DA (Euvrard et al., 1979).

Electrophysiologically it has been observed that neurones in the substantia nigra which respond to DA have a higher resting firing rate in animals treated with oestrogens (Chiodo and Caggiula, 1980). In similar animals a reduced sensitivity to ionotophoretically applied DA has been shown (Arnaud, Dufy, Pestre and Vincent, 1981).

As previously mentioned the presence of DA in the cerebellum is still a matter of dispute. Bloom et al. (1971) for example have reported that all areas of the cerebellum receive a catecholaminergic innervation that is probably exclusively noradrenergic. In the present study a small amount of DA was detected in this region in all age groups. Neonatal androgenization caused a significant reduction in cerebellar DA concentrations at 60 days of age compared to the controls. Later (at 120 days) this situation was reversed. Autoradiographic studies by Stumpf and Sar (1979) demonstrated that H^3-oestradiol visibly accumulates in the cerebellar cortex. Additional immunohistochemical evidence has recently been provided by Dietrichs and Haines (1986). They suggested the presence of a small population of hypothalamic catecholaminergic neurones projecting to both the cerebellum and spinal cord. These authors also reported the presence of divergent

axon collaterals extending from the hypothalamus to the amygdala and cerebellum which may be the source of DA in the cerebellum. The effects of androgenization seen in the cerebellum could thus be merely a reflection of what has taken place primarily in the hypothalamus and amygdala.



Results

Distribution of 5-hydroxytryptamine (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) in various regions of the rat brain

Hypothalamus (Figs. 35 and 36; Tables 2 and 3)

a. Males:

In sham-castrated rats the levels of both indoleamines rose through to 75 days. A further elevation was observed at 90 days, which was then followed by a very significant depletion. Neonatal castration however, resulted in a significant diminution in the concentrations of 5-HT and 5-HIAA compared to the controls through to 75 days. An abrupt rise then occurred which was only sustained in the experimental animals during the remainder of the study.

b. Females:

In the controls a relatively low concentration of 5-HT and its metabolite was observed until 60 days. Levels had doubled by 75 days and then remained comparatively stable through to 180 days. Androgen-treatment during the period of sexual differentiation caused an initial increase in 5-HT and 5-HIAA levels as compared to the untreated groups. However, these soon fell and eventually declined by 180 days to become very significantly lower in comparison to the controls ($p < 0.001$).

Amygdala (Figs. 37 and 38; Tables 4 and 5)

a. Males:

5-HT concentrations in the sham-castrated rats were seen to gradually increase until 90 days of age after which

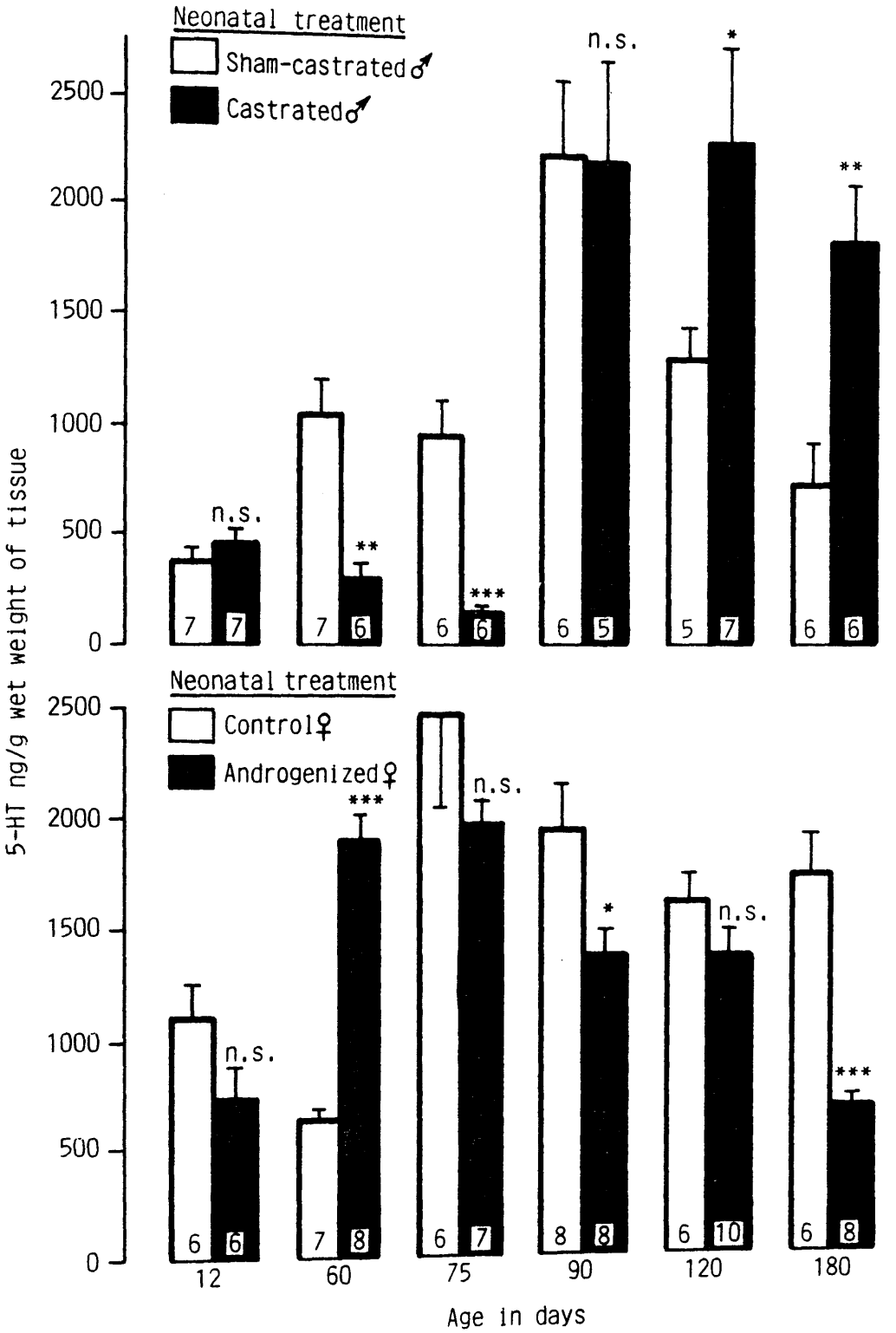


Fig. 35 Mean (± SEM) 5-HT concentration in the hypothalamus at various post-natal ages in the control and experimental rats *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

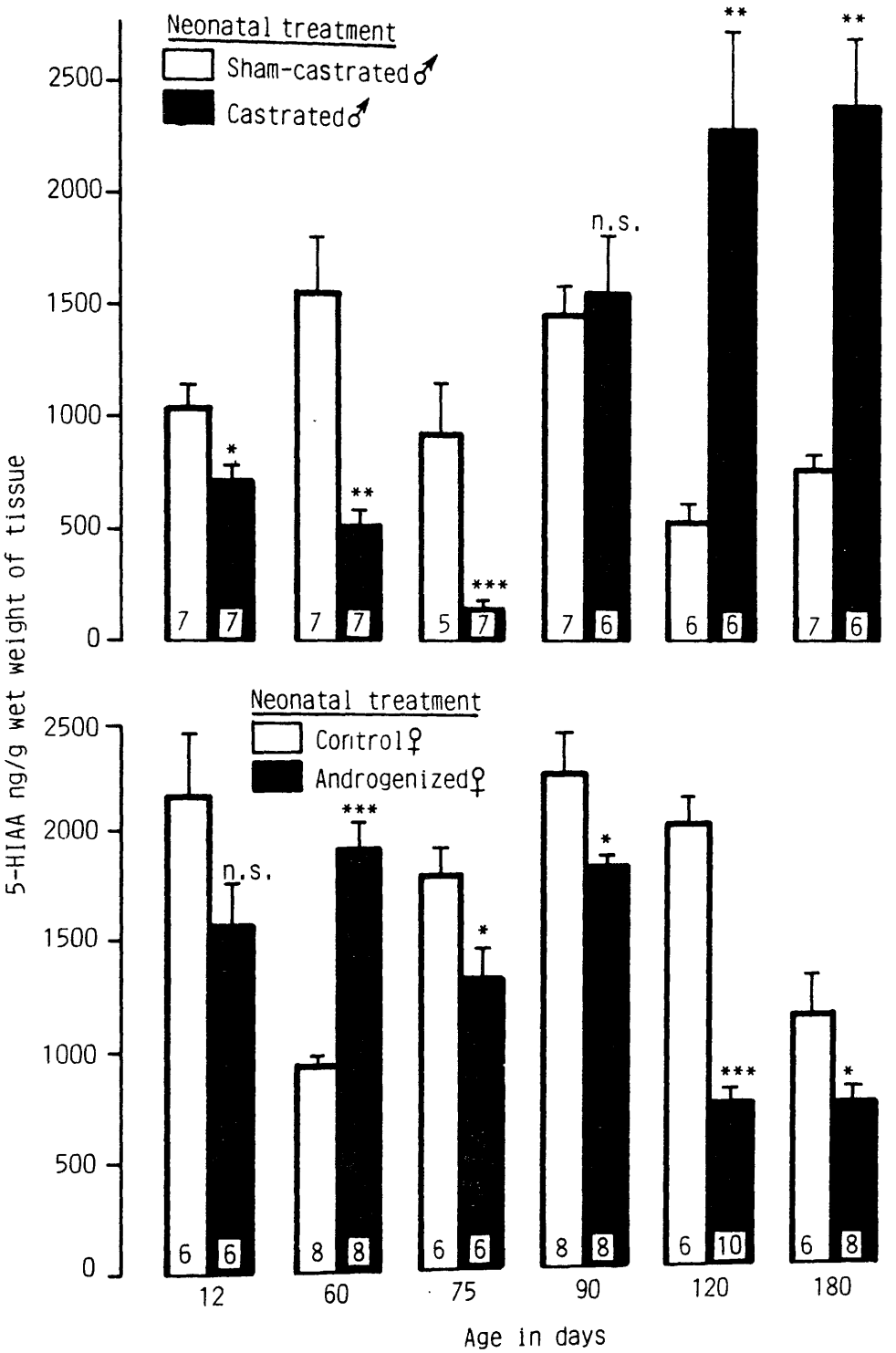


Fig. 36 Mean (± SEM) 5-HIAA concentration in the hypothalamus at various post-natal ages in the control and experimental rats *<0.05, **p<0.01, ***p<0.001, n.s. not significant.

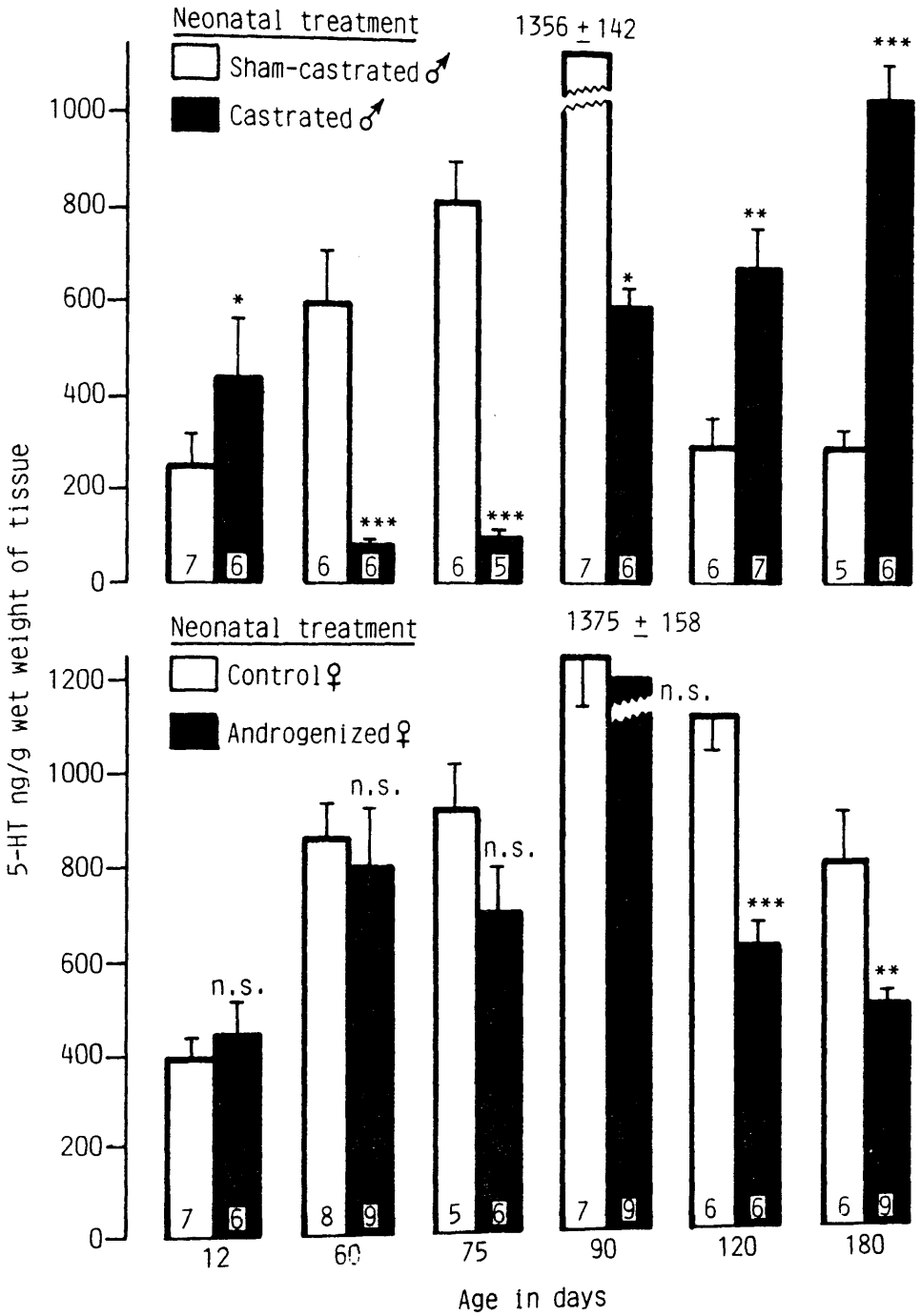


Fig. 37 Mean (± SEM) 5-HT concentration in the amygdala at various postnatal ages in the control and experimental rats *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

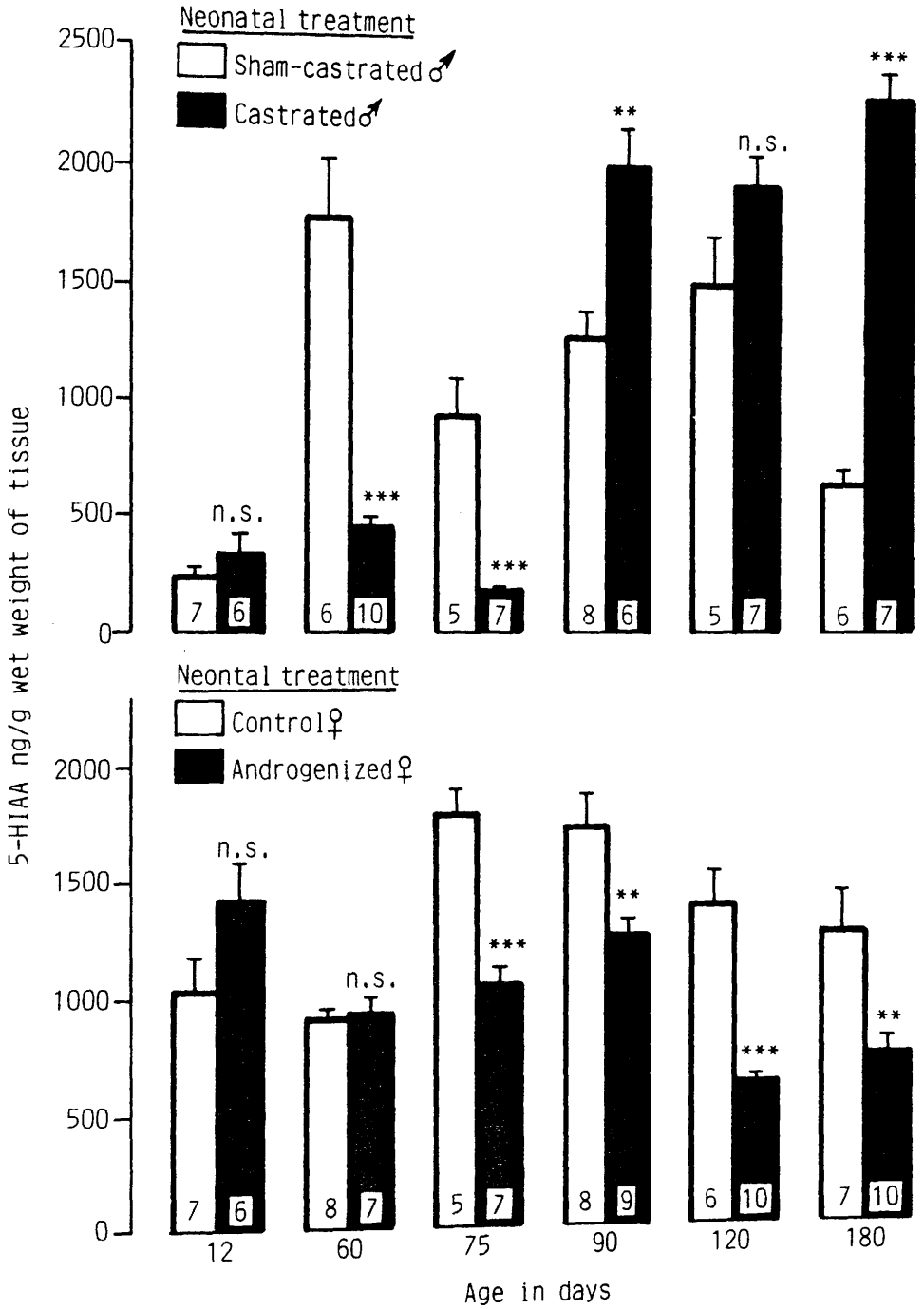


Fig. 38 Mean (± SEM) 5-HIAA concentration in the amygdala at various postnatal ages in the control and experimental rats *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

they fell back to values similar to those recorded in the 12 day-old group. 5-HIAA levels were significantly higher in the 60 and 75 day old controls, than in those castrated as neonates (Fig. 38). At 90 and 180 days however, 5-HIAA values were found to be significantly lower in the controls than in the experimental groups.

Neonatal castration caused an immediate rise in 5-HT levels (observed at 12 days of age), but this was followed by a very sharp drop. However by 120 days the situation had become reversed with the 5-HT content of the amygdala being very much higher in the experimental group than in the controls. A similar situation was observed at 180 days.

b. Females:

Interestingly, the pattern of age-dependent 5-HT-changes observed in untreated animals were very similar to those seen in both the androgenized females and sham-castrated male rats. However at 120 and 180 days of age 5-HT levels were significantly lower in the females androgenized as neonates than in their controls. This picture was similar to that observed in the sham-castrated males.

In the androgenized females 5-HIAA levels were significantly lower than in the controls in all but the youngest two age groups.

Cerebral cortex and cerebellum (Figs. 39.-42; Tables 6-9)

a. Males:

5-HT concentrations in the above regions showed a very similar pattern of change in both the neonatally castrated

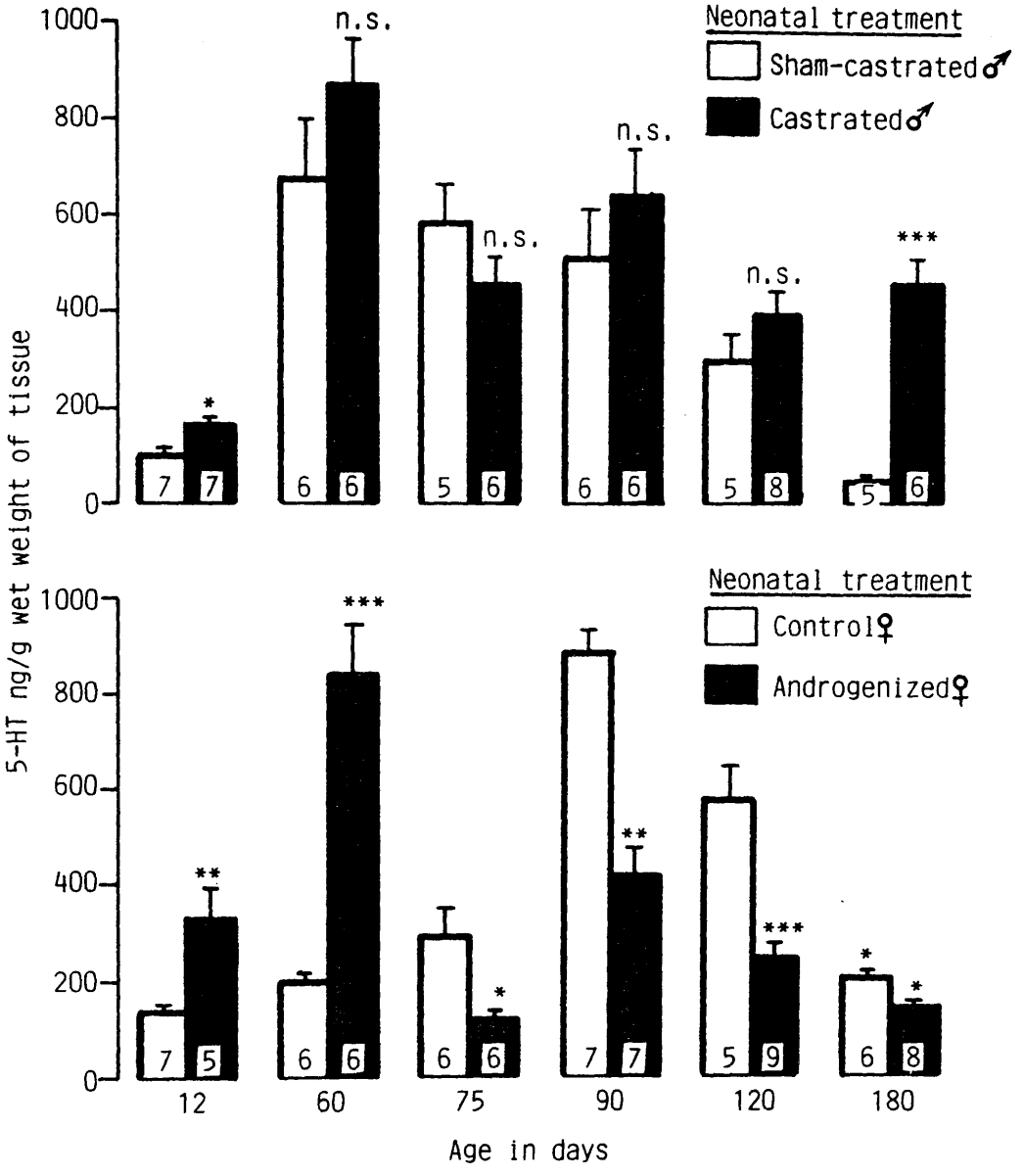


Fig 39 Mean (± SEM) 5-HT concentration in the cerebral cortex of control and experimental rats at various post-natal ages. *p<0.05, **p<0.01, **p<0.001, n.s. not significant.

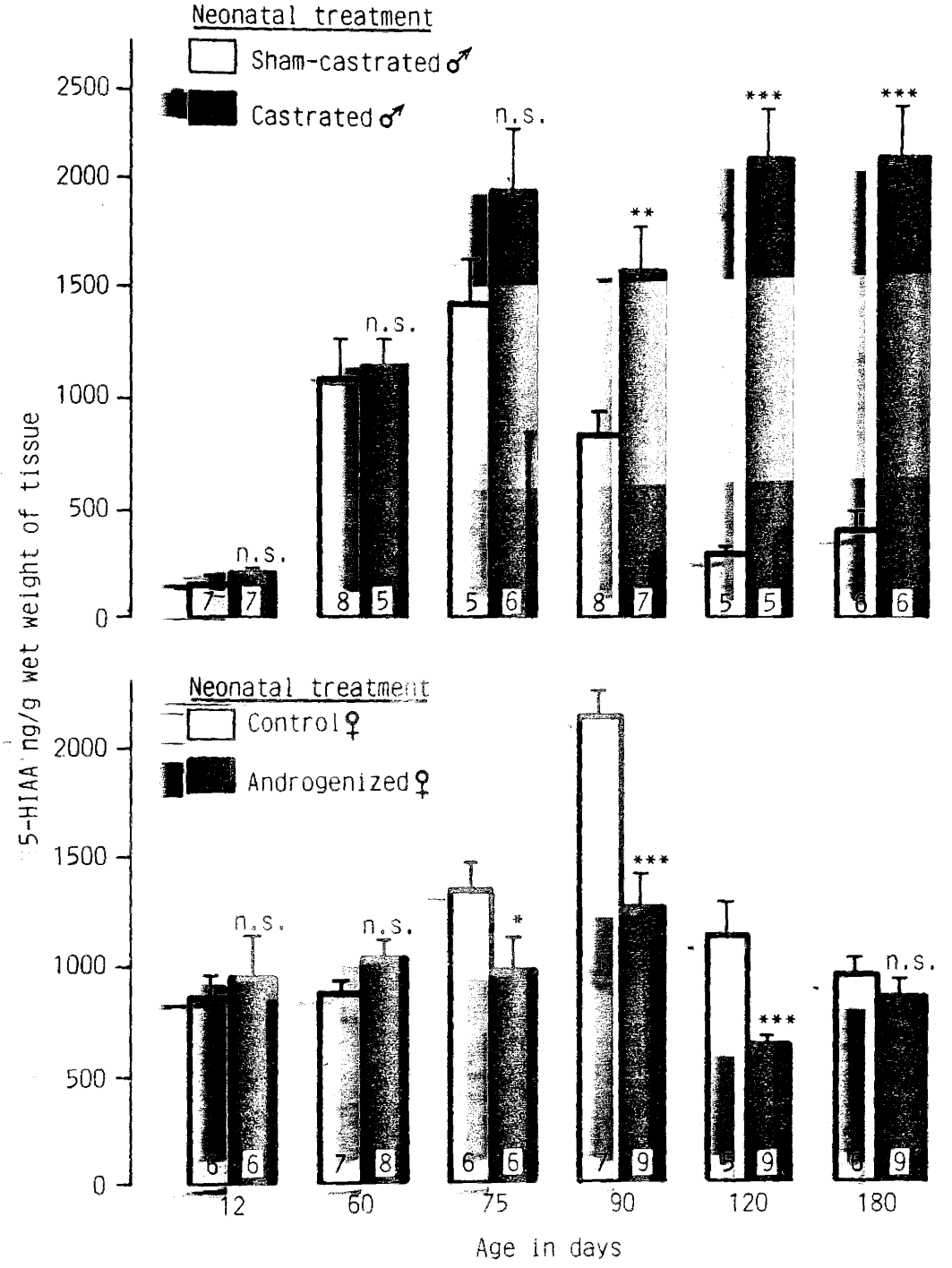


Fig. 40. Mean (± SEM) 5-HIAA concentration in the cerebral cortex of control and experimental rats at various post-natal ages. *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

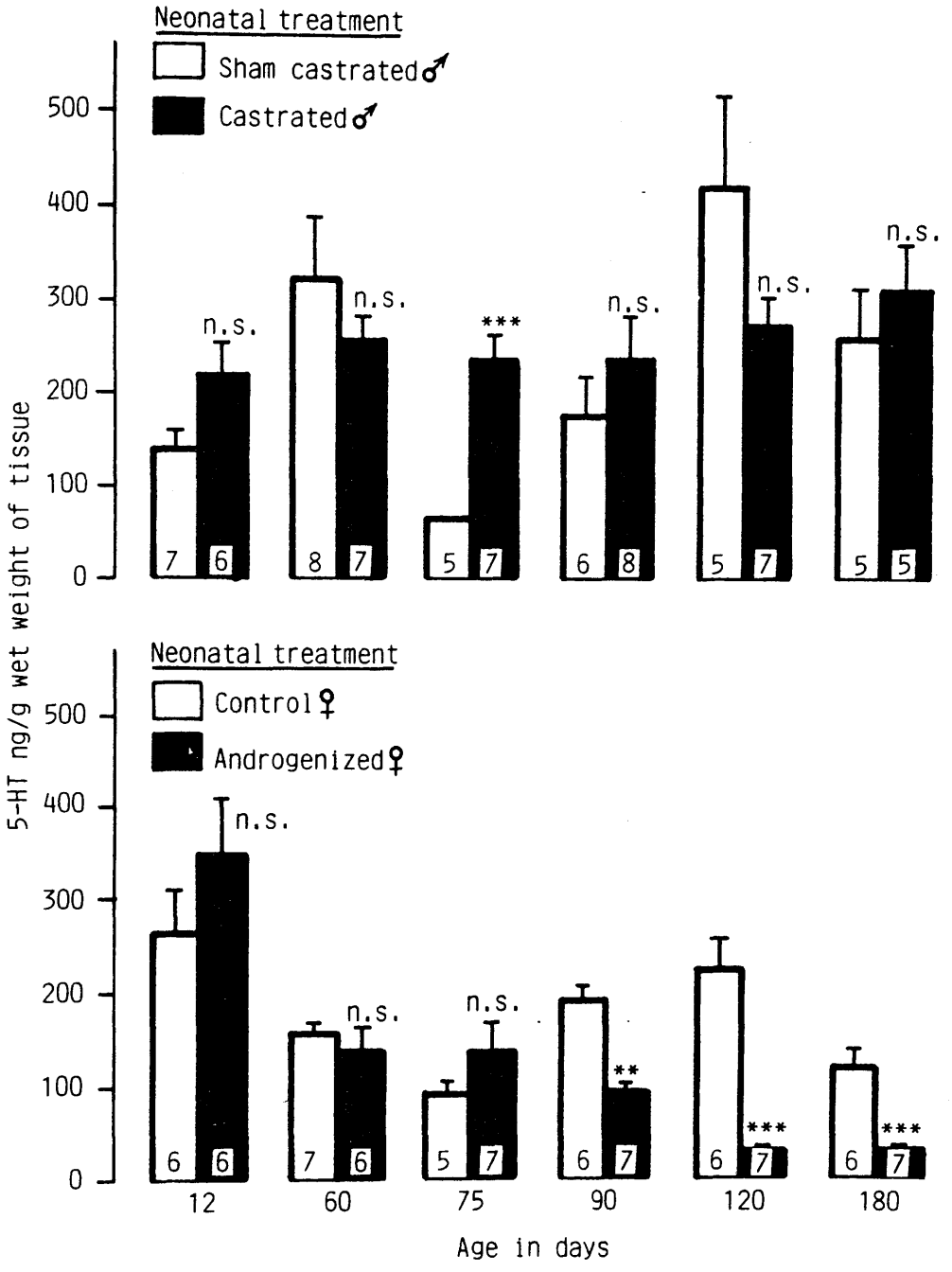


Fig. 41 Mean (± SEM) 5-HT concentration in the cerebellum of the control and experimental rats at various post-natal ages. *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

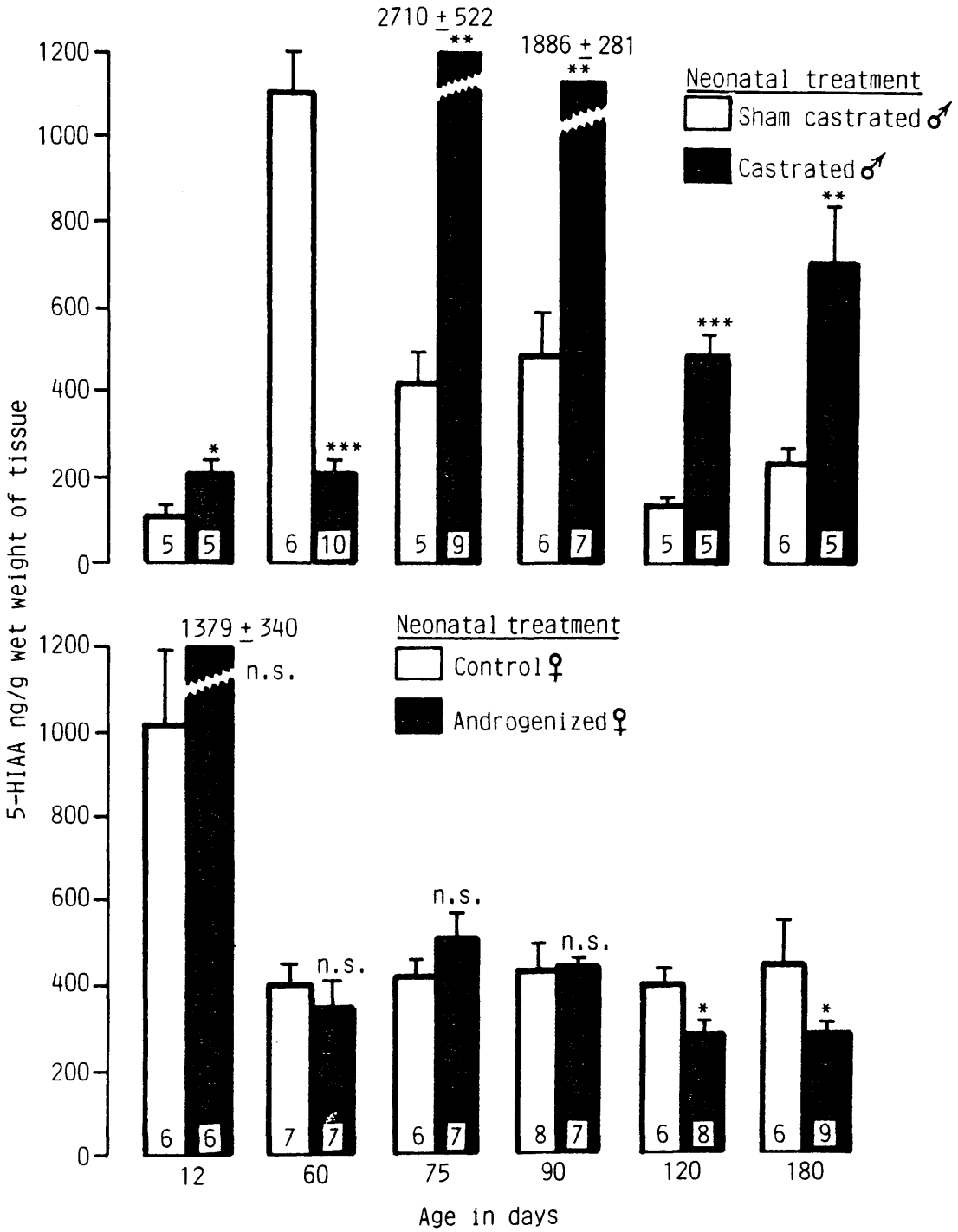


Fig. 42 Mean (± SEM) 5-HIAA concentration in the cerebellum in the control and experimental rats at various post-natal ages. *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

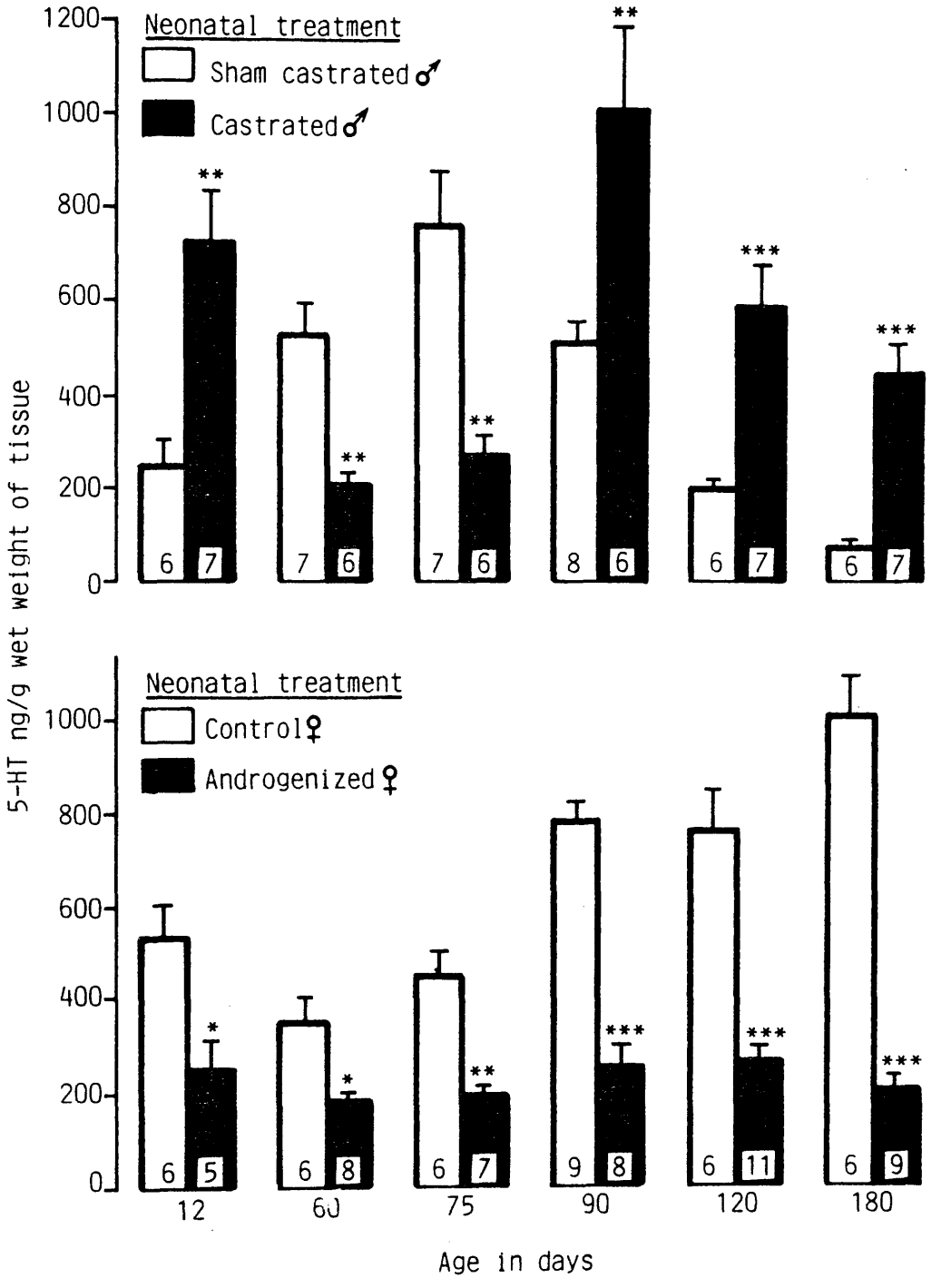


Fig. 43 Mean (± SEM) 5-HT concentration in the Corpus Striatum in the control and experimental rats at various post-natal ages. *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

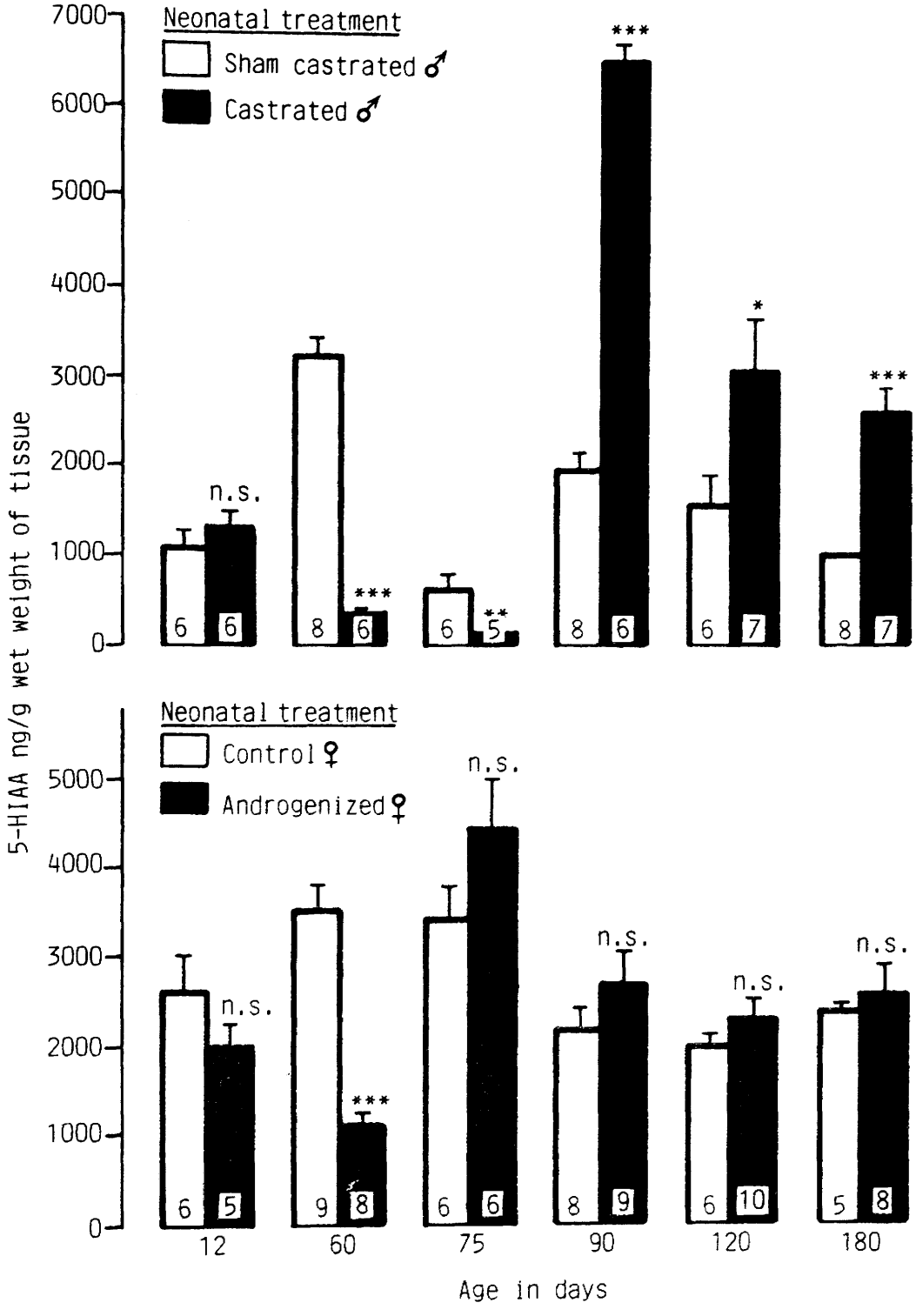


Fig. 44 Mean (± SEM) 5-HIAA concentration in the Corpus Striatum in the control and experimental rats at various post-natal ages. *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

content of this brain region was significantly lower in the castrated animals until 90 days when the situation was reversed.

b. Females:

5-HT concentrations in the untreated animals showed a steady increase through to 180 days, but this was prevented by neonatal androgenization. As a result 5-HT levels were significantly lower in the androgenized animals throughout the period of study. 5-HIAA levels in the corpus striatum did not exhibit any recognizable pattern of alteration between control and experimental animals during almost the whole period of the study. The exception was at 60 days when levels were seen to be significantly lower in the androgenized group.

Corpus callosum and hippocampus (Tables 10-13)

a. Males:

5-HT and 5-HIAA levels in sham-castrated rats were seen to fluctuate throughout the period of study and no recognizable pattern of change could be identified in the experimental animals, whenever a measurement was possible.

b. Females:

In both regions 5-HT and 5-HIAA concentrations in the untreated animals showed an increase until 75 days. Neonatal androgenization brought about an elevation in the levels of both indoleamines in the younger age groups but from 90 days levels declined throughout the remainder of the study. They then showed some variation (e.g. 5-HT levels in the hippocampus of the control were very high at 180 days as were 5-HIAA

levels in the corpus callosum.

Corpora quadrigemina (Figs. 45 and 46; Tables 16 and 17)

a. Males:

Levels of both 5-HT and 5-HIAA fluctuated markedly throughout the period of study, at certain ages being significantly higher in the controls and at other ages, the situation being reversed. Furthermore, although levels of 5-HT and 5-HIAA in the sham-castrated rats did not bear any relationship to each other, in the neonatally castrated group they did exhibit a similar pattern of change at certain times (12, 60, 180 days).

b. Females:

Levels of both 5-HT and 5-HIAA in the untreated animals did not show any consistent pattern of change throughout the whole period of study. Neonatal androgen administration caused a significant reduction in 5-HT levels at 12 days of age, but this was reversed at 60 days. Levels of 5-HT were seen to be consistently lower in the androgenized females than in the controls from 75 through to 180 days of age. The situation in the androgenized females with regard to 5-HIAA was similar to that seen in the untreated group.

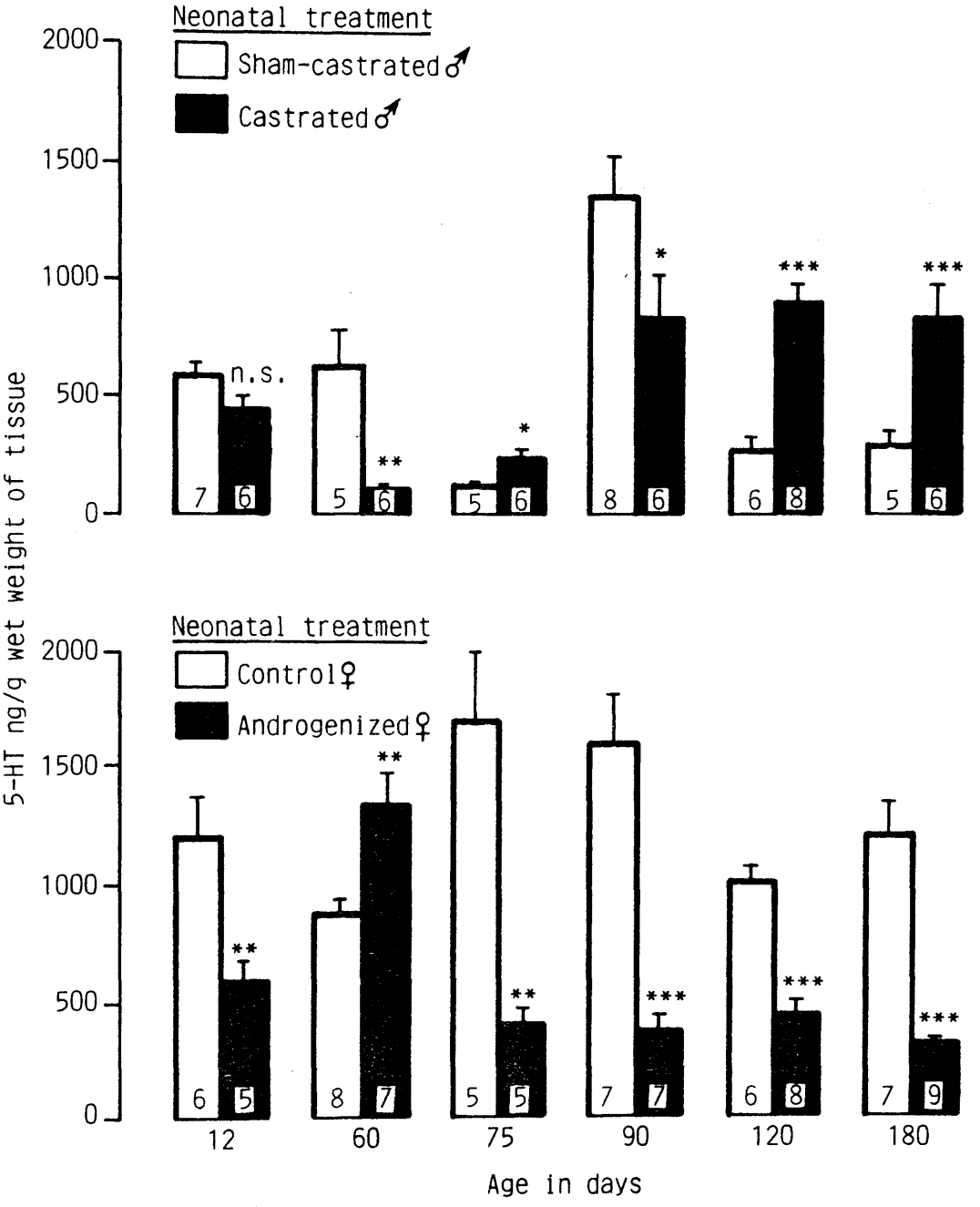


Fig. 45 Mean (± SEM) 5-HT concentration in the corpora quadrigemina at various post-natal ages in the control and experimental rats *p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

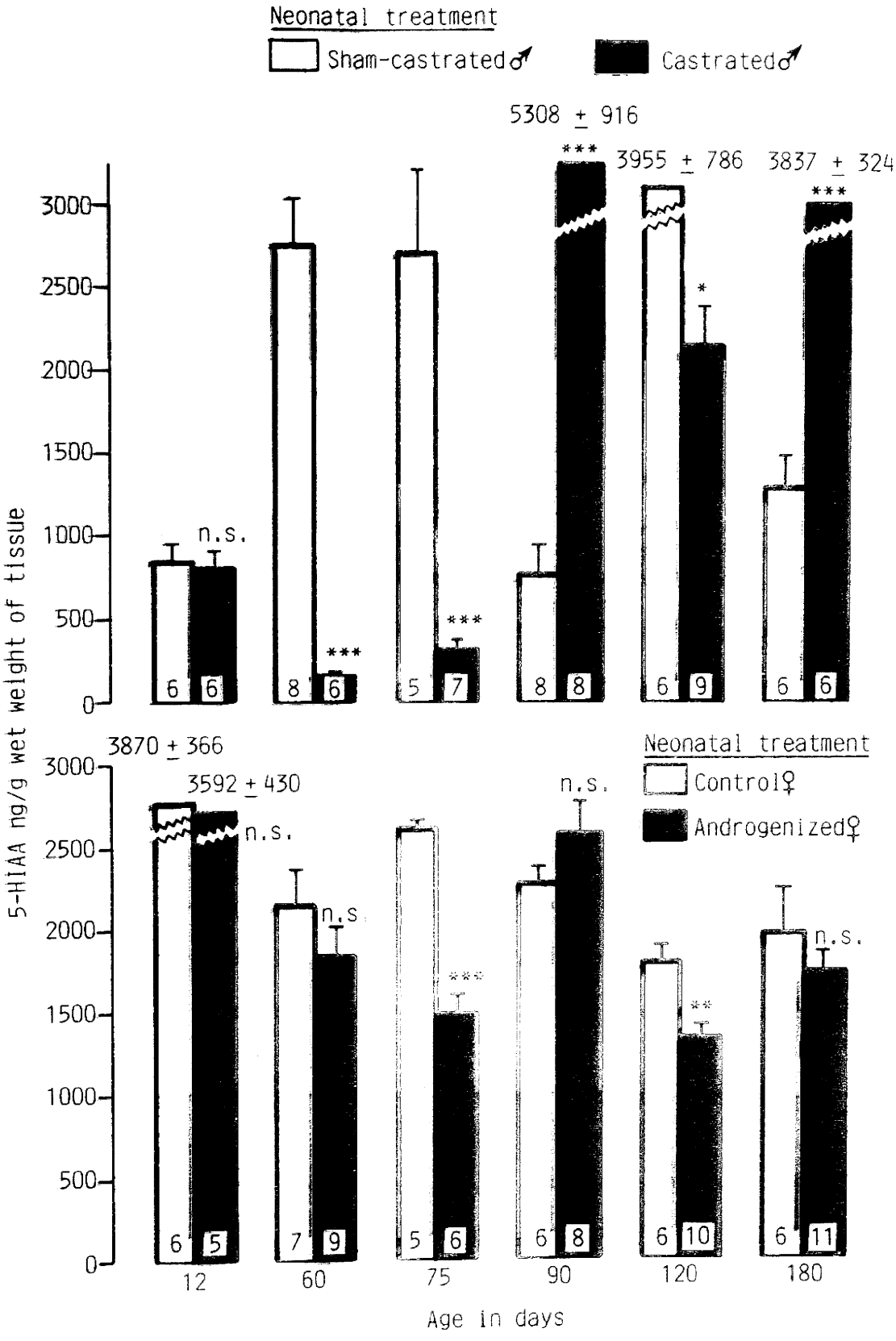


Fig. 46 Mean (± SEM) 5-HIAA concentration in the corpora quadrigemina at various post-natal ages in the control and experimental rats
*p<0.05, **p<0.01, ***p<0.001, n.s. not significant.

Time-dependent changes in plasma LH levels

Plasma LH levels measured by RIA in the sham-castrated rats were not detectable until they reached 90 days of age (see Table 18). From this time low concentrations were seen which were maintained through to 180 days. Neonatal castration, by interrupting the negative feedback mechanism, caused the LH levels to become greatly elevated in comparison to the sham-operated males.

Oil-treated female rats (killed on the day of oestrus) had low concentrations of LH throughout the period of study. However, in the neonatally androgenized animals plasma LH levels were not detectable until 90 days. At 120 and 180 days levels had risen very significantly when compared to the untreated groups.

Effect of Prostaglandin E_2 on the brain neurotransmitter levels of fetal rabbits

This part of the study was carried out in collaboration with Josephine Odber (1986). Concentrations of brain monoamine measured in the fetal rabbits are presented in ng/mg protein.

Distribution of biogenic amines in various brain regions of near-term rabbits

Concentrations of biogenic amines in various brain regions of newborn rabbit fetuses obtained from pregnancies terminated by hysterotomy exhibited only a small number of recognizable sex differences (Table 19). These are outlined below:

Table 18

Plasma LH levels in control and experimental rats measured at various post-pubertal ages.

Age (days)	LH concentrations ng/ml				
	60	75	90	120	180
Sham-castrated	N.D. (14)	N.D. (14)	7.63±2.12 (10)	6.13±1.32 (9)	4.9±1.08 (7)
Castrated	5.95±15.04 (10)	30.28±3.96 (13)	46.0 ±4.72*** (8)	37.4±5.18*** (8)	36.7±5.65*** (8)
Oil-treated	1.9±0.34 (8)	2.54±0.37 (9)	1.42±0.19 (11)	1.69±0.21 (9)	1.87±0.2 (9)
Androgenized	N.D.	N.D.	N.D.	22.2±2.45***	12.26±1.41***

Mean (±SEM), ***p < 0.001, N.D. not detectable, numbers examined in parenthesis.

Table 19

Concentrations of biogenic amines in various brain regions of fetal rabbits obtained by hysterotomy.

Brain Region	ng/mg protein					
	Sex	NA	ADR	DA	5HT	5HIAA
Hypothal-amus	(6)	6.8±0.5	1.5±0.4	1.9±0.6	8.4±2.2	6.9±1.5
	(5)	8.4±2.2	0.8±0.5	5.0±0.7*	6.7±1.2	17.8±1.6***
Amygdala	(5)	1.5±0.1	0.1±0.1	1.2±0.6	2.6±0.8	8.8±4.7
	(6)	3.7±0.9	0.4±0.2	2.5±0.9	3.1±0.8	4.7±1.3
Cerebellum	(5)	3.1±0.8	0.1±0.1	1.9±0.5	6.9±3.9	4.3±2.2
	(6)	3.9±0.7	0.5±0.3	3.9±1.4	2.9±0.7	4.6±2.3
Cerebral Cortex	(4)	0.9±0.2	-	0.5±0.2	1.2±0.3	1.4±0.8
	(5)	1.6±0.4	-	0.8±0.4	0.6±0.2	5.8±3.1

Mean (± SEM) *p < 0.05, ***p < 0.001, numbers examined in parenthesis.

1. The hypothalamic content of both DA and 5-HIAA was significantly higher in the female fetuses than in their male counterparts.
2. In the amygdala NA concentrations were also higher in females as compared to males.

The three does that were given intravenous injection of PGE_2 (0.5 - 1 mg) failed to deliver spontaneously. Oxytocin (ranging from 300 mIU to 2 IU) was then administered intravenously to all three, but only one was induced to deliver. The other two were sacrificed and the pups surgically removed.

The biogenic amine content in the brain regions of the pups from all three does that had been treated with prostaglandins could not be determined. This was due to the monoamine peaks being completely obscured by the prostaglandin, or a metabolite of it (Fig. 47). This observation clearly demonstrates that the prostaglandins do enter the fetal brain and interfere with the ability to measure the biogenic amines present by HPLC-ECD.

The results of the investigations carried out on mid-term human fetuses from prostaglandin terminated pregnancies are reported in Appendix I. Those results from the study undertaken to examine the post-mortem stability of rat brain monoamines are described in Appendix II.

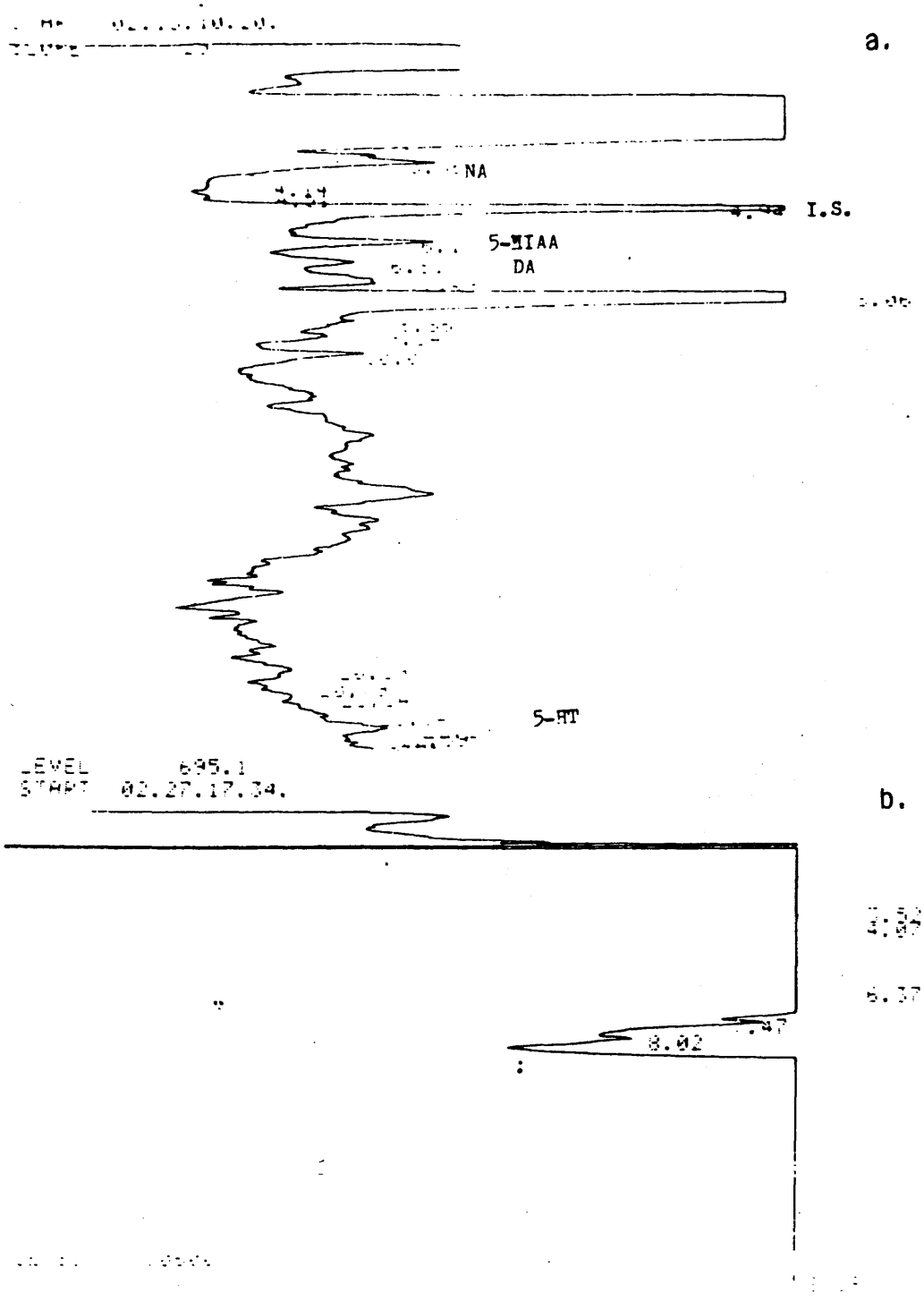


Fig. 47 Chromatograms of rabbit fetal brain samples (a) obtained by hysterotomy (b) obtained from PGE_2-terminated pregnancy.

DISCUSSION

Discussion

Time-related serotoninergic changes in the various brain regions of the male and female rat

In the present study androgen-dependent sex differences in 5-HT concentrations in certain regions of the rat brain were apparent at 12 days of age. Neonatal castration resulted in a significant elevation of the 5-HT content of the amygdala, cerebral cortex and corpus striatum. In contrast neonatal androgenization brought about a significant reduction in 5-HT concentrations of the corpus striatum and corpora quadrigemina but a significant rise in the concentrations of this neurotransmitter in the cerebral cortex.

The results of the present study are only in partial agreement with earlier findings. Hardin (1973) and Gladue *et al.* (1977) reported that there were no sex differences in the 5-HT content of the rat CNS within the first 10 days of life. However Ladosky and Gaziri (1970) and Giulian *et al.* (1973) stated that whole brain 5-HT levels were lower in the male at 12 days. Similarly Gladue *et al.* (1977) found that at this age 5-HT levels were significantly lower in the male hypothalamus and Brotman *et al.* (1976) claimed the same to true for the mesodiencephalon and pons-medulla. The latter authors also observed that the 5-HT content of the corpus striatum was higher in the female than in the male. Hardin (1973) revealed that the sex difference seen in the brain 5-HT content of two day old rats was due to a difference in the activity of the enzyme systems synthesizing this neuro-

transmitter, and not to the monoamine oxidase system responsible for its breakdown. Bourgoin, Faivre-Baumann, Héry, Ternaux and Hamon (1977) later indicated that high free tryptophan levels are present in the newborn female rat brain, but that a dramatic decrease occurs during the latter part of the first postnatal week. This finding is also supported by the work of Daszuta, Gandin-Chazal, Faudon, Barrit and Ternaux (1979) who recorded high 5-HIAA levels in the two week old rat brainstem, most probably due to high 5-HT-specific MAO A activity. They also suggested that during later postnatal life the increase in 5-HT levels seen in the forebrain was more marked than that observed in the brainstem. Gaziri and Ladosky (1973) have demonstrated that there is no sex difference in the activity of the MAO enzymes on day two, but that on day 12 their activity is increased in the anterior hypothalamus of the male. This sex difference in enzyme activity may be reflected in our findings that the hypothalamic 5-HT content of the intact male at 12 days appears to be considerably less than that recorded in the normal female. Interestingly, the situation with regard to all other brain regions (excluding the cortex) seems to be identical to that seen in the hypothalamus.

The latter observation may reflect an increased activity in all these brain areas of the intact male pups. On the other hand, it could also be due to an increased 5-HT brain content in the 12 day old oil-treated females as a result of a demasculinization of the CNS serotoninergic system. The results of Vaccari *et al.* (1977) and Dazuta, Barrit and Faudon

(1982) have indicated that the development of the brain MAO system responsible for the deaminating of 5-HT in female rats and mice reaches a maximum during the second week of age; this is particularly evident in the brainstem. The concentrations of 5-HIAA (the metabolite of 5-HT) formed by the action of MAO was also present in high levels in 12 day old female rats, most particularly in the corpora quadrigemina. The marked elevation seen in this region could perhaps result from our method of dissection, in which a deep incision was always made to separate the corpora quadrigemina from adjacent areas of the brain. There was thus the possibility that along with the corpora quadrigemina an area of the midbrain raphe was also taken. It has been shown that MAO-deaminating activity is very high in the midbrain raphe leading to elevated concentrations of 5-HIAA in this region (Bourgoin-Hamon, 1976).

Neonatal castration of the male pups did not lead to any significant alteration in the 5-HT content of the hypothalamus, cerebellum and corpora quadrigemina at 12 days of age. Similarly neonatal testosterone administration to 12 day old female rats did not bring about any significant change in 5-HT concentrations of the hypothalamus, amygdala and cerebellum. These observations are supported by the finding of a recent study by Jarzab and Döhler (1984). These workers observed that postnatal inhibition of 5-HT synthesis does not interfere with the effects of postnatal testosterone propionate administration on either the future pattern of gonadotrophin release or the expression of male and female

sexual behaviour. No appropriate explanation has been found in the literature for the unaltered 5-HT levels seen in the cerebellum, amygdala and corpora quadrigemina after neonatal androgen manipulation. It could therefore be postulated that MAO activity, which is responsible for 5-HT metabolism in the CNS, may be unaffected by the presence of testosterone, at least in certain areas of the brain.

Our results lend support to the idea that reduced 5-HT concentrations in the brain are involved in its masculinization, as the overall concentrations of both 5-HT and 5-HIAA measured in all the regions (excluding the cortex) are significantly higher in the intact females as compared to the males. It would therefore seem that the sex differences in brain 5-HT levels observed in 12 day-old rats are a consequence rather than a cause of masculinization. On the other hand there are a variety of experimental data which suggest the active participation of the serotoninergic system in sexual differentiation of the brain immediately after birth. Shirama and his associates (1976) observed that postnatal treatment of female rats with the 5-HT precursor 5-hydroxytryptophan (5-HTP) delayed the permanent anovulatory sterility-inducing effect of postnatal testosterone propionate. An even stronger protective effect on androgen-induced permanent anovulatory sterility was observed when rats were treated postnatally with the 5-HT synthesis inhibitor para-chlorophenylalanine (p-CPA) (Reznikov et al., 1979).

In the neonatally castrated rats levels of 5-HT and 5-HIAA were very significantly reduced from control values by 60 days of age in the hypothalamus, amygdala, corpus striatum and corpora quadrigemina. However by 120 days the situation was reversed. It has been pointed out earlier (Gaziri and Ladosky, 1973) that neonatal castration significantly reduces the synthesis of 5-HT in the anterior hypothalamus of the youngest age group by increasing the activity of MAO A. This phenomenon has also been reported in the forebrain and brainstem (Bourgoin, Artaud, Adrien, Héry, Glowinski and Hamon, 1977). In addition Bourgoin et al. (1977) have shown that a reduced clearance rate of 5-HIAA and formation of 5-HIAA from peripheral 5-hydroxytryptophan do not contribute to the steady state levels of 5-HT.

The present results also indicate that neonatal androgenization has resulted in a very significant elevation in 5-HT levels at 60 days of age in the hypothalamus, corpora quadrigemina and cerebral cortex. This finding is in general agreement with the earlier studies of Ladosky and Gaziri (1970); Hyyppä and Rinne (1971); Guilian et al. (1973) and Gladue et al. (1977). These workers all observed that in pre-pubertal animals (30 days) 5-HT levels in the cortex and hypothalamus are greater in males than in females; a situation which is the opposite to that found around the time of puberty.

Neuroanatomical mapping of the serotoninergic system within the CNS indicates that this neurotransmitter is fairly widely distributed. Ascending pathways, originating from the

midbrain and pontine raphe cells project to the forebrain and cerebellum (Palkovits, 1980a, 1984). Immunoreactive 5-HT cells have been described in the hypothalamic dorsomedial and ventrolateral nuclei (Frankfurt, Lauder and Azmitia, 1981; Sakumoto, Sakai, Jouvet, Kimura and Maeda, 1982) whereas in the cerebral cortex, 5-HT innervation is relatively dense and uniform across all layers (Lidov et al., 1980). The present study indicates that manipulation of the androgen environment in the neonatal male leads to an increase in 5-HT levels in almost all regions of the brain in the adult animals. Neonatal androgenization in females leads to a gradual decline in the 5-HT content of almost all regions of the brain during adulthood.

In the oil-treated females 5-HT and 5-HIAA concentrations in the hypothalamus, amygdala and corpora quadrigemina were seen to be generally higher at 75 days than in the intact males. Later at 120 days of age this sex difference also became apparent (mainly due to reduced levels of 5-HT in the intact males) in the cerebral cortex, hippocampus and corpus striatum and was maintained throughout the remainder of the study in all the regions mentioned.

The above observations are in agreement with the reports of a higher activity of tryptophan hydroxylase and hydroxy-tryptophan decarboxylase (Rosecrans and Schechter, 1972; Vaccari et al., 1977), in the female brain Watts and Stanley (1984) have also suggested that there is a higher rate of 5-HT synthesis in the hypothalamic-preoptic brain area of adult female rats as compared to that in adult males. It

has been postulated that the 5-HT neurones of the female rat brain have a greater storage capacity and a higher enzymatic activity with a higher rate of 5-HT synthesis, and are thus generally more developed than are those in the male. This sex difference in capacity of the 5-HT system may also be of fundamental importance for such functions as sexual behaviour and aggression, which show the same sex-linked pattern in mammals. The inhibitory role of 5-HT upon these functions is well established in animal experiments (Booth, 1979; Ahlenius, Larsson and Svensson, 1980; Meyerson, 1984). In this study 5-HIAA (the metabolite of 5-HT) displayed an almost identical pattern of change to that of 5-HT in both sexes (exceptions may have been due to a reduced clearance rate or formation of 5-HIAA from peripheral 5-HTP). Interesting in this context are reports of higher CSF 5-HIAA levels in women than in men (Asberg, Bertilsson, Tuck, Cronholm and Sjøquisk, 1973), as well as various studies indicating a correlation between aggressive behaviour and low CSF 5-HIAA levels (Asberg, Bertilsson and Martensson, 1984).

In the older animals neonatal castration appears to have abolished sex differences in the 5-HT content of the brain. This would imply that testosterone is responsible for the weaker behavioural response to pargyline and tryptophan seen in males (Carlsson, Svensson, Erikson and Carlsson, 1985). Another interesting finding in this context are the results from the study by Engel, Ahlenius, Almgran, Carlsson, Larsson and Söderstom (1978) who found that neonatal castration caused

an increase in 5-HT synthesis in the limbic forebrain and the diencephalon; this effect was counteracted by testosterone. Fishette, Biegon and McEwen (1984) have suggested that androgens exert their effect postsynaptically by reducing the sensitivity of the 5-HT receptors.

The marked changes in the concentrations of 5-HT and 5-HIAA seen in the hypothalamus - and to a lesser extent in the corpora quadrigemina of the younger animals probably result from changes in plasma tryptophan metabolism (Schmidt and Sanders-Buch, 1971; Tricklebank, Kantamaneni, Hunt, Patel and Curzon, 1982) and tryptophan hydroxylase activity (Deguchi and Burchas, 1972; Hamon and Bourgoin, 1982). However, a comparison of the data obtained throughout the period of study shows that the concentrations of each amine did not change to the same degree in each brain region which would suggest that changes in plasma tryptophan metabolism and enzyme activities affect each area to a different extent (Watts and Stanley, 1984).

The results of the present study are in general agreement with the published data on sex differences in the 5-HT content of the hypothalamus, amygdala and mesencephalon. In almost all the previous studies sex differences were investigated in adult rats, generally without specifying their age. However, this study investigating changes in 5-HT content in various stages of postnatal development is interestingly comparable with a similar investigation by Watts and Stanley (1984). These authors did not find any sex differences until 80 days of age both in the hypothalamic POA and midbrain raphe. Our

findings though generally disagree with the results of the aforementioned study however, the content of 5-HT seen at 90 days in the hypothalamus and corpora quadrigemina may be comparable with the observation made by Watts and Stanley (1984) at 80 days. The present study also could not produce evidence of any sex difference in 5-HT content of the amygdala and cerebellum. The discrepancy with the above study could be partly explained by circadian variations in brain 5-HT levels. Studies on serotoninergic circadian rhythms (Héry, Faudon, Dusticier and Héry, 1982; Semba, Toru and Mataga, 1984) have revealed that the brain 5-HT content is approximately 20% higher during the afternoon than in the morning. All animals utilized in this research were killed during the afternoon whereas Watts and Stanley (1984) sacrificed their animals at 10.00 hr. However, it is interesting to note that the levels of 5-HT during our study observed at 90 days were similar to those reported by Watts and Stanley (1984) at 80 days. This finding probably confirms the explanation put forward by Watts and Stanley (1984) that the sex differences in 5-HT concentrations reported by Ladosky and Gaziri (1970) were due to changes in brain areas outside the hypothalamic POA and midbrain raphe.

GENERAL DISCUSSION

General Discussion

Among the many recent discoveries of sexual dimorphism in the CNS of various species (Arnold and Gorski, 1984) are examples of sex differences in neuronal numbers (Gorski et al., 1978) size (Pfaff, 1966) and pattern of connectivity (Raisman and Field, 1973). Steroid hormone exposure during early development has been proven responsible for the male neural structures differing from those of the female. The precise role of the neurotransmitters as neurotransducers within the mammalian CNS has been very difficult to characterize, in as much as the presynaptic inputs to these cells cannot be isolated. However, considerable evidence has accumulated to suggest that during a critical period of brain sexual differentiation, the neuroendocrine system is vulnerable to internal and external environmental influences (Dörner, 1977). These alterations are manifested in steady state concentrations of neurotransmitters, which, however, gives little information about the ongoing activity of the system being studied. The state of activity can be inferred from information about

i) the concentrations of the neurotransmitter and

ii) of its precursors and metabolites,

iii) the activities of synthesizing and degrading enzymes, and

iv) the receptor content.

The presumed involvement of, for example, monoaminergic and cholinergic systems in sexually dimorphic functions such as gonadotrophin release and sexual behaviour (Evritt et al., 1975; Héry et al., 1976; Clemens, Dohanich and Witcher, 1981;

Meyerson, 1984) suggests that in any case sex differences in the activity of these systems can be expected.

The technique of HPLC-ECD used in this study enabled us to investigate the effects of manipulating the gonadal steroid environment on the developing male and female CNS. The biogenic amines NA, ADR, DA, 5-HT and 5-HIAA present in various regions of the brain were measured with a high degree of sensitivity. Much recent work has focussed upon the concentrations of biogenic amines in specific areas of the CNS (Héry et al., 1976; Saavedra, 1979). In cats, sheep and primates concentrations of these neurotransmitters in the hypothalamus and other parts of the brain have been reported by Brown, Crane and Goldman (1979), while more recently Carlsson and his associates (1980) have investigated the levels of these substances in the adult human brain collected during post-mortem studies.

In the present research, alterations of the androgenic equilibrium during the period of brain sexual differentiation was followed by a long-lasting modification of the central biogenic amine systems. Data from several laboratories indicate that mean serum androgen levels are higher in male than in female rat embryos (Slob, Ooms and Vreeburg, 1980; Weisz and Ward, 1980; Tobet et al., 1982). This sex difference is most apparent 3-4 days before birth (when serum androgen levels appear to peak in males) and is also observed during early postnatal life (Döhler and Wuttke, 1974; Weisz and Ward, 1980).

Morphological studies of the developing CNS have led to the hypothesis that maximal sensitivity to gonadal hormones may be associated with the appearance of receptors for both androgens and oestrogens and serve as a signal for the onset of the 'critical period' of sexual differentiation (Vito and Fox, 1982). The appearance of receptors in the hypothalamic POA of rats follows very closely the appearance of postmitotic neurones. The neuroblasts which subsequently populate the POA and limbic system undergo their final mitotic divisions during mid- to late gestation. Also the presence of receptors in the embryonic rat brain is coincident with the appearance of postmitotic neurones in the SDN of the rat (Jacobshon and Gorski, 1981). Thus, neurones in these brain regions may be responsive to sex steroids as soon as they become postmitotic and begin to differentiate, thereby defining the beginning of the 'critical period' of sexual differentiation. The presence of androgen receptors is consistent with the hypothesis that some androgens in testicular secretions might act directly and indirectly via conversion to oestrogen (Vito and Fox, 1982).

Another parameter which provides insight into the initiation of the critical period for the sexual differentiation of the brain is the development of aromatizing activity required for the conversion of testosterone into oestradiol. Aromatase activity, following its appearance in the diencephalon after day 14 of gestation, reaches a peak in the hypothalamus at least 2 days prior to birth. Thereafter, the activity rapidly falls to postnatal day 4 after which it declines more

gradually. Naftolin and Ryan (1975) and George and Ojeda (1982) have found aromatase activity in both the mediobasal hypothalamus and POA but not in the cortex and cerebellum. The specific activity of aromatization in the fetal rat hypothalamus is several fold higher than that previously measured for other regions of the brain (George and Wilson, 1978).

The fact that oestradiol can stimulate the growth and branching of hypothalamic neurones (Toran-Allerand, 1976; Arai and Matsumoto, 1978) indicates that locally formed oestrogen may be involved not only in sexual differentiation, but also in more general aspects of brain development. In addition, aromatase activity has been demonstrated in the CNS of a variety of species, including fish, amphibians, reptiles and birds as well as in mammals (Callard, Petro and Ryan, 1978). The wide phylogenetic conservation of this enzymatic process in the brain reinforces the possibility of a fundamental relationship between aromatization and brain development.

In the rat the period of organization of the gonadal axis coincides with a phase of rapid development of transmitter systems in the forebrain, as illustrated by the time-course of receptor development described above. Similar temporal relationships appear to exist in the human fetus which exhibits high serum testosterone levels between 11 and 17 weeks of pregnancy (Dörner, Stahl, Rohde, Görtzlehner, Wittkowski and Saffert, 1977; Reyes, Boroditsky, Winter and Faiman, 1979).

In 14-16 week old male fetuses, Gilmore and Wilson (1983) found high concentrations of NA and DA in the hypothalamus; levels declined after the 17th week. No such changes were observed in the frontal cortex.

The work presented in this thesis supports the theory that the sexual differentiation of CNS is androgen-dependent and alterations of the androgenic equilibrium during the critical period are followed by a long-lasting modification of the biogenic amine systems in the brain. Our study suggests that a general, and to a certain extent, selective modification of the central neurotransmitter system is produced by neonatal androgen manipulation. In this connection, the effect of gonadal steroids would appear to reinforce the prenatal influence of adrenocortical steroids (Naumenko and Dygalo, 1980; 1984) although a certain distinction seems to exist in the character of certain neurotransmitter systems in the CNS.

In our experiments the steroidal milieu of both male and female neonatal rats was manipulated during the period when the rat brain monoaminergic neurones were acquiring a particular pattern for adult life. Olso and Seiger (1972) and Schlumpf *et al.* (1980) using immunofluorescent methods first detected the presence of monoamine cells in the rat hypothalamus by embryonic days 13-14. In the mesencephalon and lower brainstem they first appear on days 14-15. Fibres originating from catecholaminergic cells in the mesencephalon and lower brainstem penetrate telencephalic

*

Control males and androgenised females showed a similar pattern of change between 12 and 60 days in the biogenic amine contents of most regions examined: they increased more than in control females and castrated males. Between 60 and 120 days the changes were variable between groups and there was no clear pattern; at 180 days the control females and castrated males in general showed similar and relative high values; the control males and androgenised females showed similar and relatively low values.

structures and reach the developing neocortex on day 16 (Schlumpf et al., 1980).

Biochemical studies indicate that the fetal rat brain possesses all the enzymes involved in the biosynthetic pathway for the catecholamines by days 15-16 of gestation. Thus according to Coyle and Axelrod (1972) tyrosine hydroxylase activity is present in the rat as early as day 15 of the prenatal life. Parvez and Parvez (1980) have detected catechol-O-methyl transferase and monoamine oxidase in the whole brain of the rat from days 16 and 18, respectively. An active uptake mechanism for NA develops in the whole brain of the rat by day 18 of the prenatal period (Coyle and Axelrod, 1972).

The actions of androgens on the developing CNS have not yet been fully elucidated. Testosterone however, by affecting the process of transcription and translation etc. has been shown to modify the developing neurotransmitter system of the brain as a genetic inducer (Adcock et al., 1985).

*

However, the fact that certain similarities exist in the brain amine content of different groups may not necessarily mean that these are related to reproductive function.

It might be expected that the situation with regard to brain amine content seen at 60 days (a time after which puberty has occurred) would be maintained thereafter. However, this was not so; as is evident from Figures 48-53. After 60 days a long period of time followed during which no particular pattern was evident. It was not until the rats had reached four-six months of age that the situation seen at 60 days reappeared. The pattern of brain amine content attained by 180 days in both experimental groups confirms the notion that alteration of androgen balance during the perinatal period of life results in a long-lasting, general, and to a certain extent, selective modification of brain neurotransmitter systems (Beatty, 1979; De Vries, Buijis and van Leeuwan, 1984; Maggi and Perez, 1985).

Until recently there was little evidence to document the existence of adrenaline in the CNS. This was because of the chemical methods for measuring ADR levels, or for detecting activity attributable to the synthesizing enzyme (phenylethanolamine -N-methyl transferase), were unable to provide unequivocal data. However, with the development of highly sensitive methods such as HPLC-ECD the existence of ADR-containing neurones in the CNS has been confirmed. These cells are found largely in two groups, intermingled with noradrenergic cells of the lateral tegmental system and dorsal medulla.

In the present study concentrations of ADR were seen to be present in only extremely low quantities. Nevertheless,

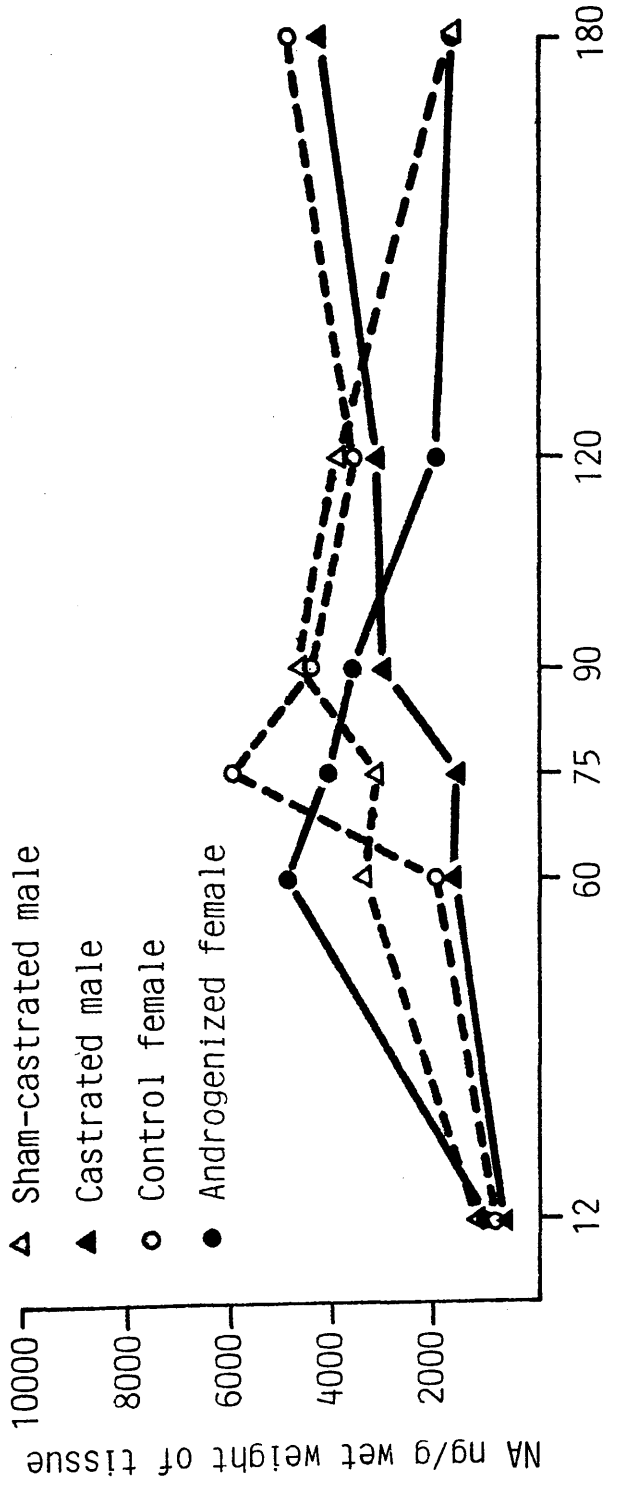


Fig. 48 Age-related changes in NA concentration in the rat hypothalamus.

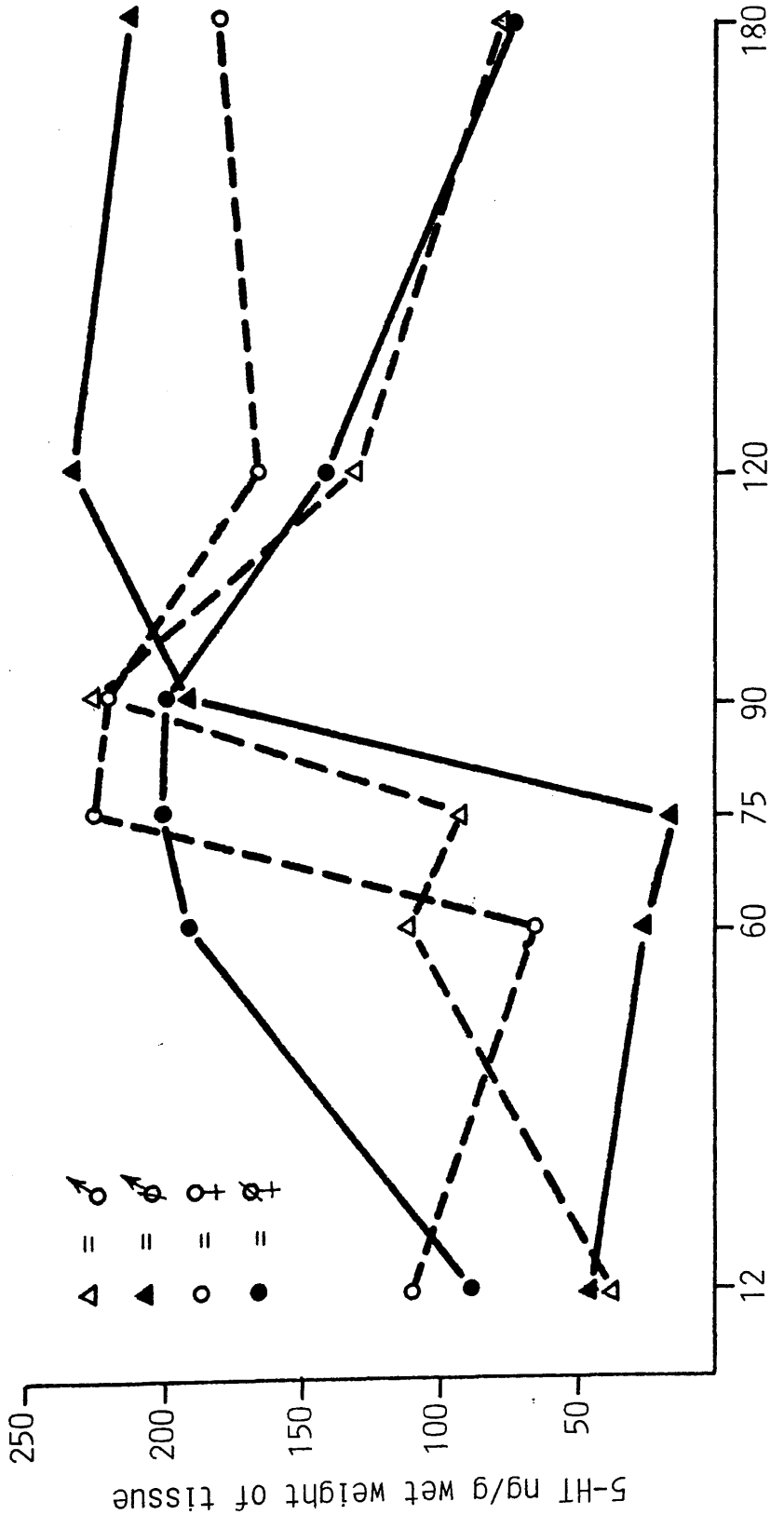


Fig.49 Age-related changes in 5-HT concentration in the rat hypothalamus.

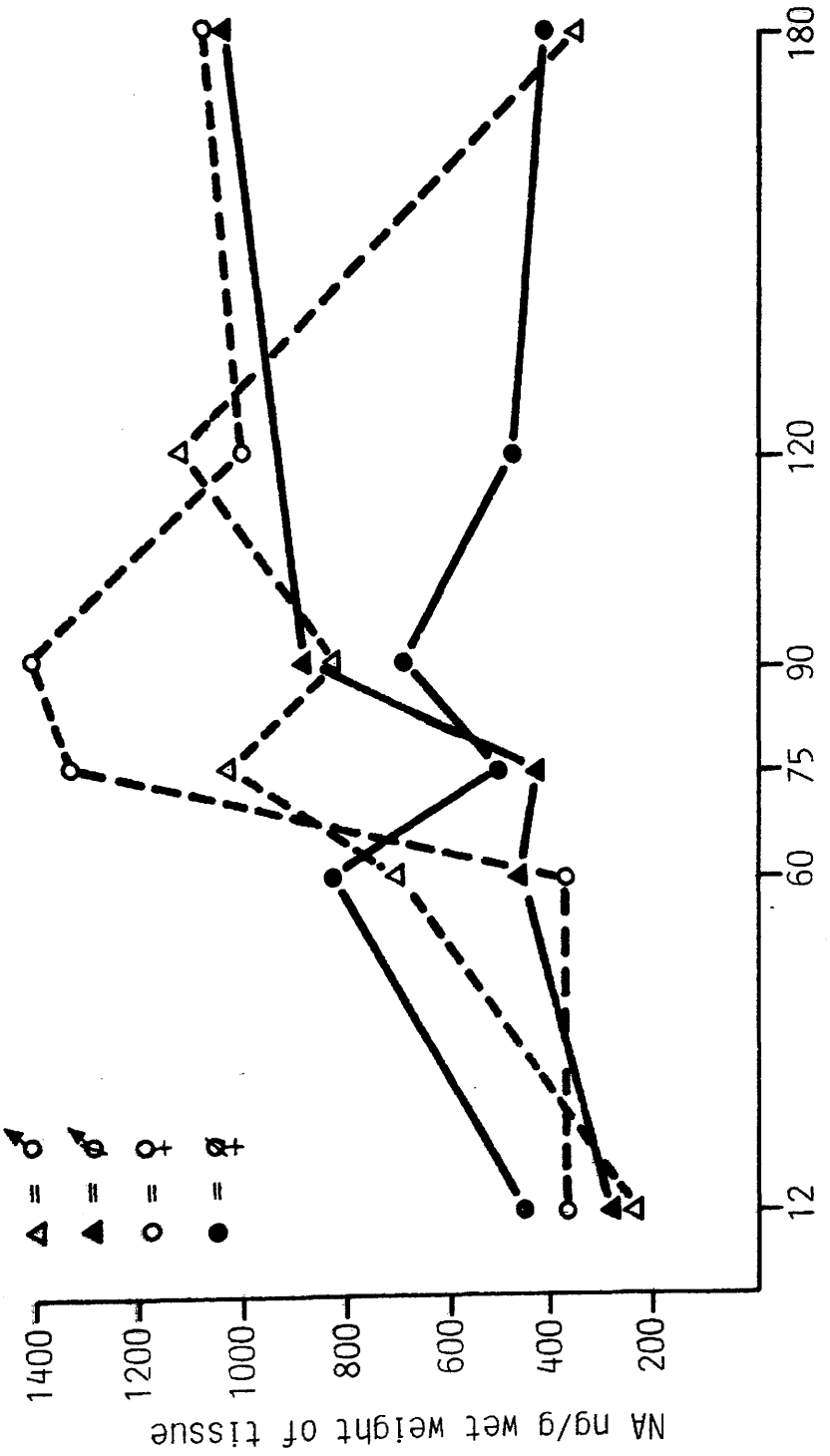


Fig.50 Age-related changes in NA concentration in the rat amygdala

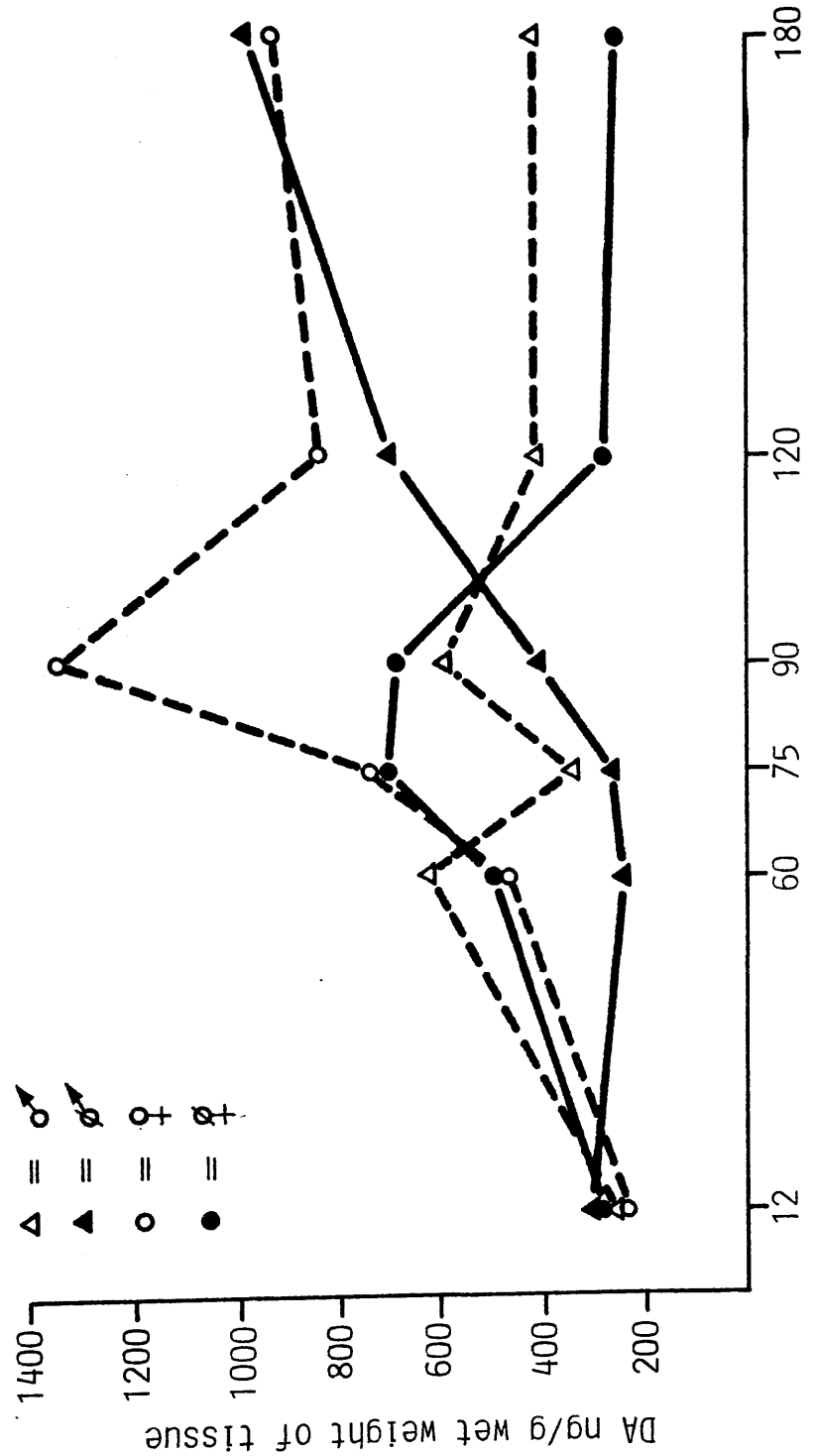


Fig.51 Age-related changes in DA concentration in the rat amygdala.

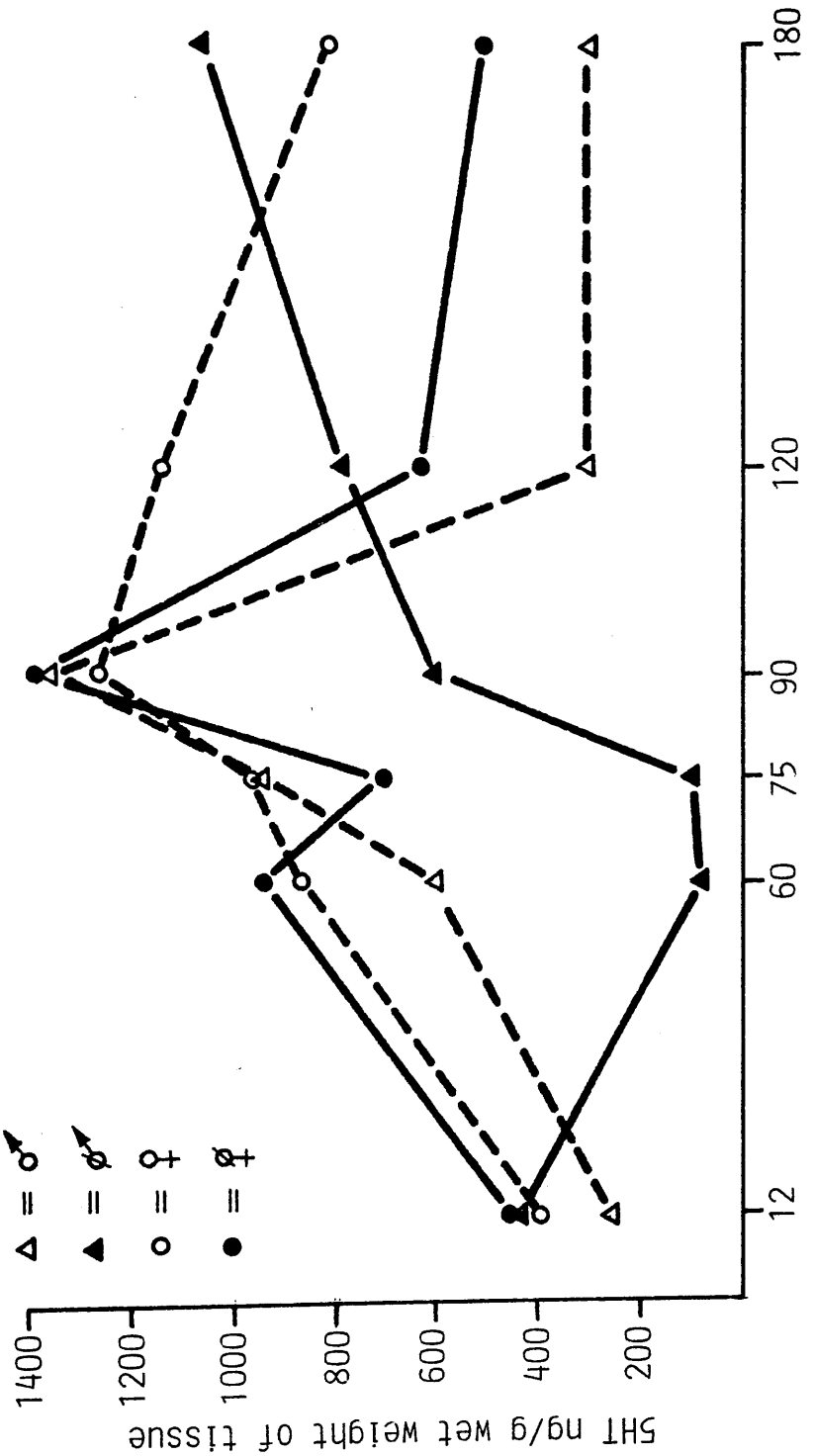


Fig.52 Age-related changes in 5HT concentration in rat amygdala.

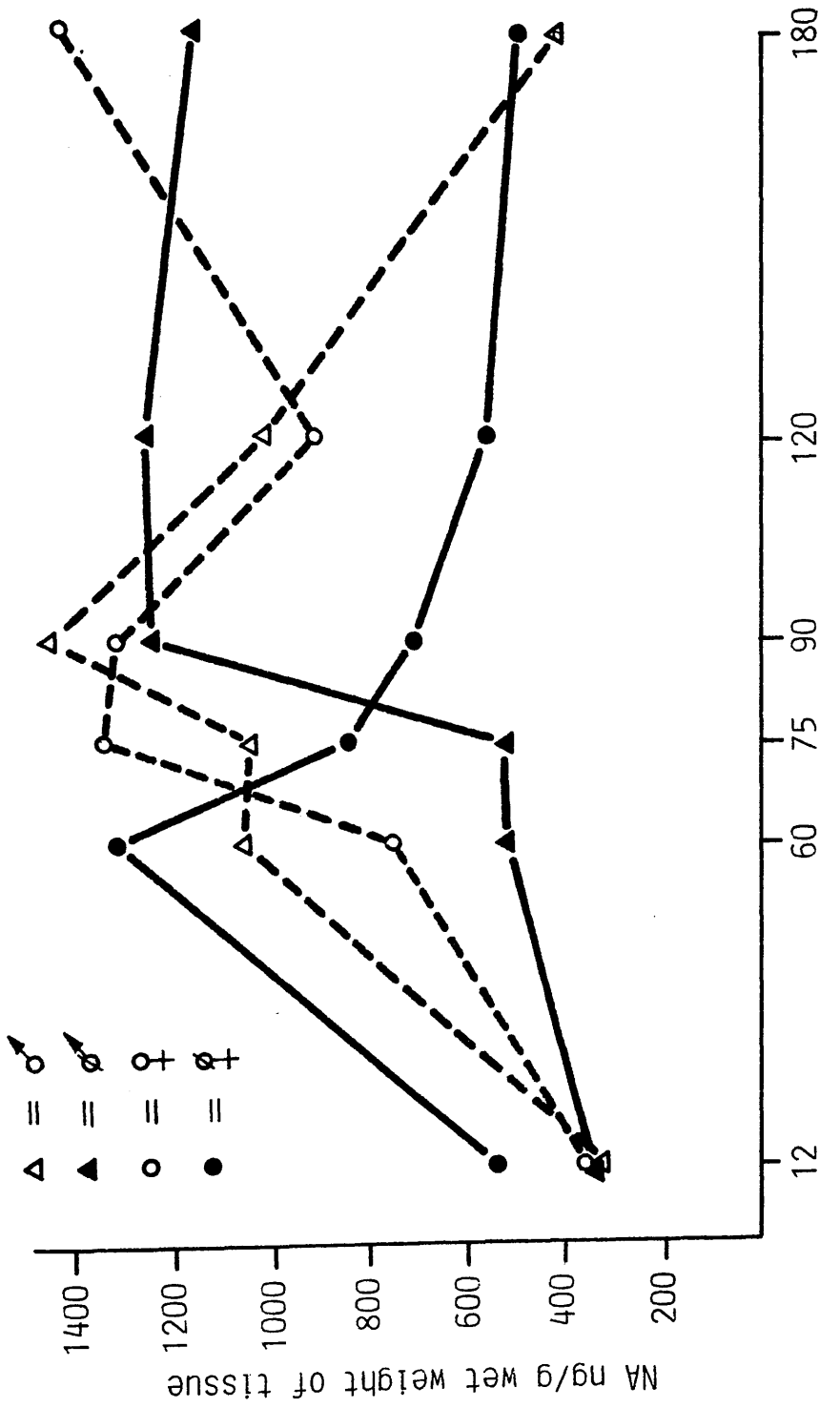


Fig.53 Age-related changes in NA concentration in rat corpora quadrigemina

as mentioned earlier ADR levels were found to be significantly reduced from control values in androgenized females of 60 and 90 days of age. It is difficult however, to interpret the meaning of this reduction because of the scarcity of literature relating to the involvement of the adrenergic system in the process of brain sexual differentiation.

In earlier research carried out in this laboratory, Gilmore and Wilson (1983) have shown that in the human too, the biogenic amines are involved in the androgen-dependent sexual differentiation of the brain. Using spectrofluorometric methods to measure the content of biogenic amines in mid-term human fetuses obtained by hysterotomy, Gilmore and Wilson (1983) reported sex differences to exist in the NA, DA and 5-HT content of the hypothalamus. However, during the present study upon human fetuses obtained from PGE_2-terminated pregnancies using HPLC-ECD, little additional information could be acquired (Siddiqui, Clark and Gilmore, 1985). This was because there was a considerable variation in the biogenic amine content of all three regions studied. The source of this variation may have been partly due to the time delay in obtaining the specimens. Another cause could have been the method of pregnancy termination. Fetuses obtained from PGE_2-terminated pregnancies are subjected to a considerable degree of stress because of the intense uterine contractions brought about by the prostaglandin. Another handicap encountered during the present investigation was that during the amine analysis a consistent shoulder appeared along with the NA peak (See Figure 54). This made it very difficult to

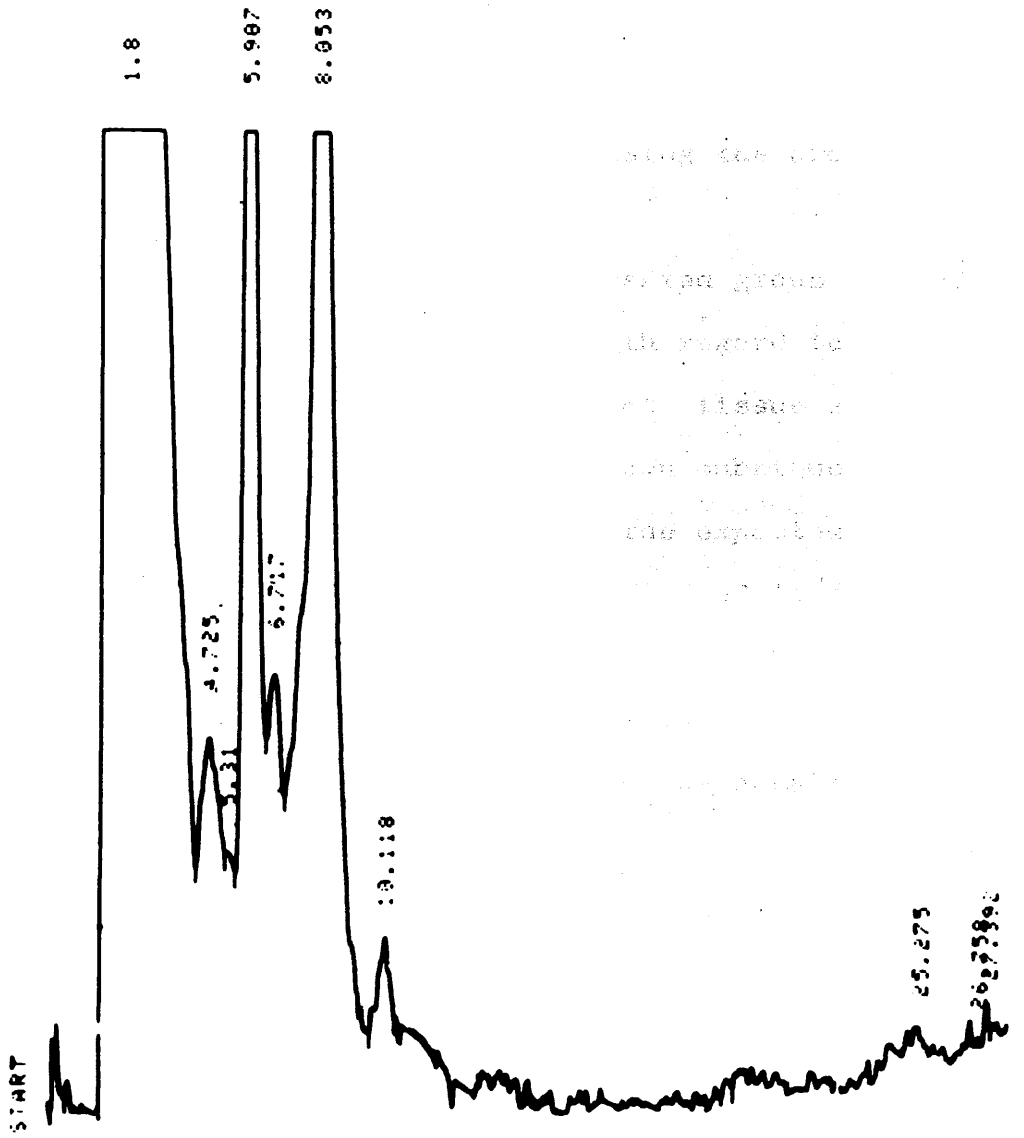


Fig. 54 Chromatogram of a human fetal brain sample obtained from a PGE2-terminated pregnancy.

determine the exact concentrations of NA present in the tissue being examined. It was eventually determined by rabbit experiments that it was the PGE_2 itself crossing the fetal blood brain barrier which was causing the problems in biogenic amine analysis.

The brain tissues from the PGE_2-treated group of rabbits did not yield any consistent results with regard to neurotransmitter levels (Fig. 47). When these tissue samples were applied to the HPLC column an unknown substance was detected at the electrode and obscured the expected neurotransmitter peaks. This interference occurred to some extent in all samples of PGE_2 treated-tissue (> 6). This unknown substance may have been PGE_2 itself, a metabolite of PGE_2 or a substance produced in the tissue in response to exposure to PGE_2. When PGE_2 itself was injected onto the HPLC column a similar but much more pronounced effect was seen to that when a PGE_2 exposed tissue sample was applied. This result would indicate that if tissues from PGE_2-terminated pregnancies are to be studied, the samples may have to be first prepurified (e.g. extraction of the neurotransmitters by absorption onto alumina (Odber, 1986).

It was not the birth process, as such, that was interfering with the amine measurements, as tissues from rabbit fetuses obtained from a spontaneous delivery at 29 days of gestation showed a normal pattern of peaks for the various neurotransmitters (Fig. 47).

Another possible source of variation could be due to the time delay in obtaining the specimens from PGE_2-terminated

pregnancies. However results from a study on the stability of monoamines in different regions of the brain once death has occurred suggest that post-mortem alterations would not be of great significance (Siddiqui, Clark and Gilmore, 1986).

Although measurement of the steady state level of a certain neurotransmitter provides an opportunity to understand some aspects of its neuroendocrine function, it only affords a limited insight into the specific activity of that neurotransmitter in the CNS. If a particular neurone or group of neurones employing a specific neurotransmitter alters its activity (i.e. its firing rate), this will not necessarily result in a change in the concentration of that neurotransmitter. For example, the ensuing change in the rate of synthesis may well be immediately matched by equivalent changes in the rate of release and catabolism of that neurotransmitter. Alternatively, the pre-existing store of a neurotransmitter may be released and retaken up at a new, but steady rate. In both cases the overall level of the neurotransmitter would not be observed to change, although its turnover rate would have been markedly altered. In other words, it is the turnover of a neurotransmitter which is more likely to reflect the functional activity of the neurones for which it is acting rather than its overall concentration (Korf, Sharman and Curzon, 1981). It could therefore be suggested that future research upon the role of neurotransmitters in brain sexual differentiation should focus upon the turnover rate to further elucidate the biological role of specific groups of neurones within the brain.

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