CONTROL OF OVULATION

D. B. CRIGHTON
N. B. HAYNES
G. R. FOXCROFT
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CONTROL OF OVULATION

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PREFACE

The Twenty-Sixth Nottingham Easter School in Agricultural Science entitled 'Control of ovulation' was held to review the many advances made since this topic formed part of the successful Thirteenth Nottingham Easter School held in 1966 and published under the title 'Reproduction in the female mammal'. It was a particular pleasure in 1977 to see delegates who had also been present in 1966 and it was therefore appropriate that Professor Bernard Donovan, who gave the first paper at the 1966 meeting, should open the 1977 discussions with an introductory lecture on 'The hypothalamo–pituitary–ovarian axis'. Professor Donovan's contribution preceded sessions on the basic physiological processes involved in ovulation – the involvement of the hypothalamus, extra-hypothalamic centres and the pituitary gland, the responses of the ovaries to stimulation, the roles played by the ovaries in modulating hypothalamus–pituitary activity and by the uterus in modulating ovarian activity.

The Easter School then continued with more applied aspects, preceded by an introductory lecture by Professor William Hansel on 'The application of ovulation control'. This was followed by a consideration of various situations which result in ovulation failure and sessions on the control of ovulation in the human and in domestic species. Finally, Dr. Wolfgang Jöchle addressed delegates on 'The symposium in perspective'.

The 1977 Easter School gave us the opportunity as co-organisers to bring together scientists from many countries, covering the whole spectrum from basic research to the practicalities of ovulation control. As co-editors who are involved in both the teaching and research aspects of animal physiology we are very aware of the necessity both to increase our knowledge of the fundamental mechanisms that control physiological activity in animals and to explore ways of applying this knowledge effectively in both veterinary and medical practice. Whatever was achieved, we ourselves had the satisfaction of renewing old friendships and of learning a great deal from our colleagues. While this volume cannot reflect the personal contacts made and discussions held at the Easter School, we hope that it will make a useful contribution to knowledge in the rapidly expanding and often perplexing field of ovulation control.

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ACKNOWLEDGEMENTS

The Twenty-Sixth Nottingham Easter School in Agricultural Science owed much of its success to the efforts of all those who presented papers and took part in the discussions. Our gratitude goes to them and particularly to Professor B.T. Donovan whose paper was prepared at very short notice and who has kindly provided a summary for this book.

We were delighted that Professor B.C.L. Weedon, Vice-Chancellor of the University of Nottingham, was able to welcome delegates and to open the Easter School. Grateful thanks are due to him and to those who acted as chairmen of the sessions: Professor B.T. Donovan, Professor H. Karg, Professor W.R. Butt, Dr. D.B. Crighton, Professor E.M. Symonds and Professor W. Hansel.

The University of Nottingham wishes to express its gratitude to the following organizations whose financial assistance contributed to the expenses of selected speakers from overseas:

Imperial Chemical Industries Ltd
Hoechst U.K. Ltd
Upjohn Ltd

Finally we should like to renew our thanks to staff of the Faculty for their invaluable assistance in organizing the Easter School, and to those other members of staff and postgraduate students who contributed in various ways to the success of the meeting.

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INTRODUCTION TO PARTS I, II and III

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THE HYPOTHALAMO–PITUITARY–OVARIAN AXIS

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In this introduction my purpose is to highlight general principles and to draw attention to matters in need of review.

To begin with the hypothalamus. In recent years, the so-called two-level concept of the control of hypophysial gonadotrophic function has gained currency. In the two-level concept the median eminence is considered to be concerned with tonic hormone secretion and is in turn driven or influenced by the preoptic area, which promotes cyclic gonadotrophin secretion and triggers ovulation. This view summarizes the results of much work in the rat, but has defects in its wider applications. Thus, sexual differentiation of the hypothalamus is presumed to be produced neonatally in the rat by the action of androgen upon the preoptic area. However, so far as the control of gonadotrophin secretion is concerned, this concept is not applicable to the rabbit, monkey, or, probably, man. The evidence is also much less conclusive in the sheep and guinea-pig, where ovulation still recurs at irregular intervals in females treated with androgen during fetal life. Although the effect of androgen upon the adult pattern of gonadotrophin secretion is much less striking in the latter species, it is evident that the hormone continues to exert a multiplicity of actions, hence the effects of androgen treatment during development extend far beyond the pituitary–gonadal axis. Changes are seen, for example, in sexual behaviour, growth and, in primates, intelligence. Neonatal androgen treatment may reduce the sensitivity of various end organs to oestrogen in the rat, but not in the monkey or sheep, and it seems somewhat myopic to focus attention on the hypothalamus of the rodent in view of the well known reduction in responsiveness to oestrogen of the pars distalis, uterus and vagina, to cite but three organs.

The cessation of oestrous cycles in the rat after the surgical isolation of the hypothalamus, or division of the anterior connections between the preoptic area and median eminence, provides important evidence in favour of the two-level concept of hypothalamic activity, but the argument is not fully convincing, since the position of the cut can be important in both the rat and the guinea-pig. With an anterior cut caudal to the suprachiasmatic nuclei, ovulation can still occur in both species, whereas more anterior cuts cause persistent oestrus. Clearly the suprachiasmatic area itself possesses the capacity to modulate gonadotrophin secretion, because the preoptic area is disconnected from the mediobasal hypothalamus in both experimental situations. The response would not be expected to vary if the preoptic area were the prime controller.

Three separate processes seem to be involved in the control of gonadotrophin secretion: the feedback actions of gonadal hormones; the mechanism involved in the episodic release of gonadotrophins and other pituitary hormones, and the generator necessary to produce the surge of gonadotrophin needed for ovulation. The feedback action of gonadal hormones upon gonadotrophin secretion and

consequently the effects of gonadectomy and of steroid administration, have been known for many years, but it is now realized that gonadotrophins are discharged in a discontinuous and episodic fashion, with the amplitude of the pulses being influenced by gonadal steroids. Here a major question concerns the nature of the driving force, for the pulses are not synchronous for all pituitary hormones and show individual variation. Does this mean that several motors must be sought? Some genetic programming may be involved, for a remarkable parallelism in the episodic discharge of hormones in twin boys and twin bulls has been reported.

The surge generator is coming to be equated with the positive feedback action of oestrogen, or the process by which oestrogen elicits, rather than inhibits, the secretion of luteinizing hormone. If oestrogen is of prime importance in inducing gonadotrophin secretion, as clinical and primate evidence indicates, then where does oestrogen act? Is it upon the hypophysis, upon the mediobasal hypothalamus, or at both locations? How does the usual negative feedback action of oestrogen become reversed? One suggestion refers to the dual action of the gonadotrophin-releasing factor in promoting gonadotrophin synthesis and release and argues that oestrogen favours the factor-induced synthesis, but impedes the release. At ovulation there may be an increased output of the releasing factor, favoured by the action of oestrogen upon the hypothalamus, and an overflow of gonadotrophin from the synthetic pool to the release pool. This idea also illustrates the current enthusiasm for two-pool concepts in describing the control of hormone release, as well as the tendency to presume that there is but a single gonadotrophin-releasing factor, a presumption that may well prove premature.

Recent work on gonadal function after isolation of the hypothalamus in the monkey, which has shown that spontaneous ovulation can occur, and can be induced by oestrogen, has been taken to indicate that the main function of the hypothalamus in some species is largely to provide the factor necessary for the enhancement of gonadotrophin release. However, as Ellendorf and Karsch and his colleagues show in the following chapters, there is much more to the hypothalamic story. The influence of other parts of the brain, and in particular the limbic system, has to be taken into account; the interactions between the adrenergic, cholinergic, serotoninergic and the novel peptidergic neuronal inputs have to be explored and evaluated; and attempts have to be made to interpret the changing sensitivity of all these components to gonadal hormones.

It is unrealistic to expect the hypothalamus of the rat to act in the control of ovulation exactly like that of the guinea-pig, sheep or monkey, and of course there are differences. It is much less easy to induce ovulation by electrical stimulation of the hypothalamus in the guinea-pig than in the rat, and close analysis of the changes in gonadotrophin secretion in the ovariectomized guinea-pig shows that luteinizing hormone (LH) secretion rises, while that of follicle-stimulating hormone (FSH) is little altered. By comparison, in the ferret, stimulation of the hypothalamus by exactly the same procedure produces a surge in both LH and FSH secretion. Unexpectedly, the oestrous ferret is idiosyncratic in being much less responsive to hypothalamic stimulation than it is during anoestrus, possibly because the ovarian hormones depress the sensitivity of the pituitary gland to gonadotrophin-releasing factor.

It is in facilitating comparisons of the kind just noted, as well as extending them to the mare and other farm species in the hope of developing understanding, that this Easter School can be expected to be productive – as it will.

I

THE HYPOTHALAMUS

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EXTRA-HYPOTHALAMIC CENTRES INVOLVED IN THE CONTROL OF OVULATION

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Introduction

Ovulation is preceded by a multitude of changes which occur during sexual differentiation and development as well as during the oestrous cycle. It is triggered by a surge of pituitary gonadotrophins, which in turn is initiated by the secretion of hypothalamic hormones. Humoral feedback from the ovary and possibly the adrenal gland, neurochemical signals, input from other than hypothalamic central nervous structures, and environmental stimuli, are the determinants of hypothalamic hormone secretion. The amygdala (Koikegami *et al.*, 1954), the hippocampus

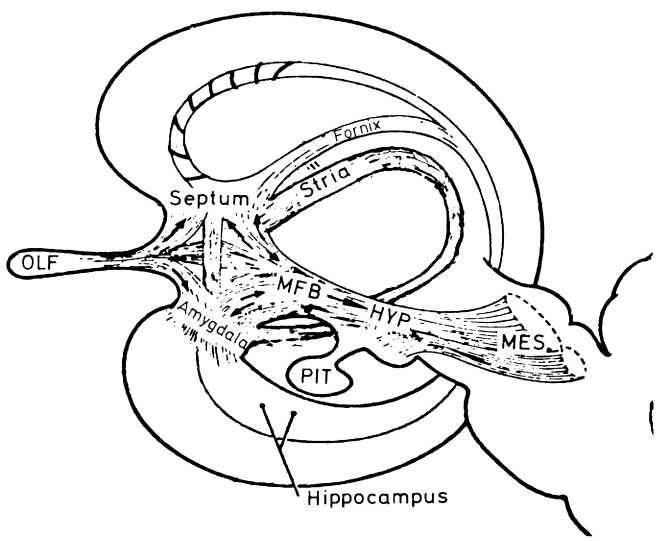

Figure 1.1 Diagram of the limbic system and major pathways. HYP, hypothalamus; MES, mesencephalon; MFB, medial forebrain bundle; OLF, olfactory bulb; PIT, pituitary; Stria, stria terminalis. (After MacLean, 1974)

(Riss *et al.*, 1963; Velasco and Taleisnik, 1969a) and the mesencephalon (Critchlow, 1958; Carrer and Taleisnik, 1970) are the most prominent extra-hypothalamic areas (*Figure 1.1*) implied in the control of ovulation (for reviews see de Groot, 1965; Raisman and Field, 1971a; Sawyer, 1972; Zolovick, 1972; Taleisnik and Carrer, 1973; Gorski, 1974; Ellendorff, 1976).

The Setting of Extra-hypothalamic Control Mechanisms for Ovulation

The prenatal development of extra-hypothalamic control mechanisms for ovulation in the adult rat has largely escaped investigation. In the rat the sexual differentiation of neural control mechanisms is not terminated at birth (for reviews see Barraclough, 1967; Gorski, 1971; Gorski *et al.*, 1975). Neonatal treatment of females with testosterone propionate results in endocrine males, while neonatal castration of genetic males creates endocrine females or feminized males (FALES). In adult FALES ovarian transplants into the anterior chamber of the eye can display cyclic activity and ovulation. One site of androgen action within the brain may be the preoptic area (POA), since implantation of testosterone into the POA of the newborn female suppresses ovulation during adulthood (Wagner *et al.*, 1966). Further morphological studies show that the arrangement of the neuropil in the strial part of the medial POA differs between males and females and between androgenized females and castrated males, that is, more non-strial synapses are found on dendritic spines in females than in males (Raisman and Field, 1971b, 1973) (see *Figure 1.2*).

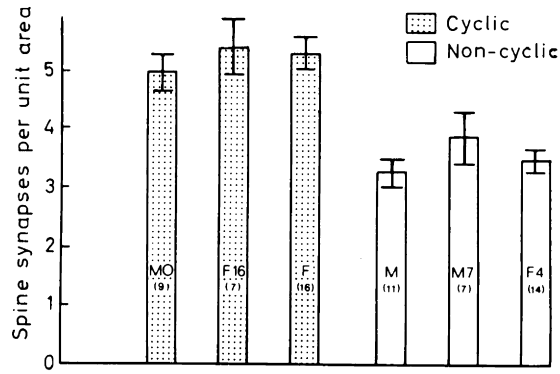

Figure 1.2 Mean incidence of non-strial synapses. (Error bars represent one standard deviation). M, normal males; MO, M7, males orchidectomized within 12 h or on day 7 after birth respectively; F, normal females; F4, F16, females treated with testosterone propionate on day 4 or 16 respectively. (Raisman and Field, 1973)

Since a stria-mediated amygdaloid input can be excluded, the difference lies within the POA or elsewhere outside the POA. The first possibility has been challenged. Electrochemical stimulation of the POA induced ovulation even in the androgenized female (Terasawa *et al.*, 1969). On the other hand, electrical stimulation of extra-hypothalamic structures revealed differences between androgenized and normal adult females: stimulation of the medial amygdala (AMY) failed to increase serum luteinizing hormone (LH) and did not induce ovulation in the androgenized rat, while stimulation of the intact female elevated plasma LH. When the hippocampus was stimulated electrically, plasma LH increased in the androgenized rat, although it never increased in the normal adult female (Kawakami and Terasawa, 1972).

Further experiments on a functional dichotomy between endocrine males and females involved electrophysiological analysis of the amygdala–preoptic–mediobasal hypothalamic relation (Dyer *et al.*, 1976). Recordings were made from single POA neurons with all possible combinations of responses to electrical

stimulation of the cortico-medial AMY or mediobasal hypothalamus (MBH) (*Figure 1.3*). POA neurons with axons both to the amygdala and the MBH were included (Ellendorff *et al.*, 1976). In those neurons with a direct projection only to the hypothalamus, significantly more synaptic connections with the amygdala were recorded in the male than in the female or the neonatally castrated male.

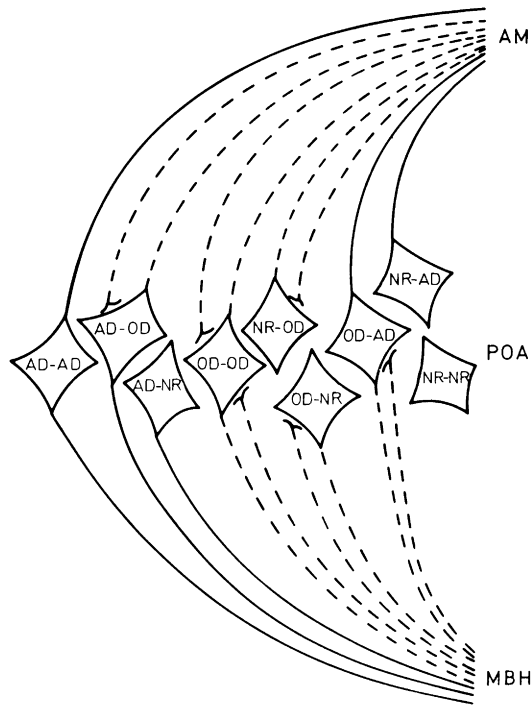

Figure 1.3 Schematic presentation of possible connections of the amygdala–preoptic–hypothalamic axis. AD, antidromically identified; OD, orthodromic response; NR, no response. The MBH–POA relation is given first followed by the POA–AMY relation. (After data from Dyer et al., *1976)*

An intermediate position was taken by neonatally testosterone-treated females (*Figure 1.4*). These data do not conflict with Raisman and Field's observation that the male–female difference is of non-strial origin. The functional difference that we found is located in a more dorsal part of the POA and we cannot make a conclusive statement whether the amygdala–preoptic interaction occurred via the stria terminalis or via a different route. Calculations on the conduction velocities of the 'bipolar' neurons suggest that the sexually differentiated input to the POA is not via the stria terminalis. Neurons not connected to the MBH fired twice as fast in females as in males.

It is likely that sexually differentiated neurons reside within the AMY with axons reaching the POA otherwise than via the stria terminalis; yet it is still possible that the sexual difference resides within the POA and the amygdaloid input would then only activate or inhibit sexually differentiated elements within the POA. It has been demonstrated that males have a 'slightly' larger mass per neuron in the POA than females. Neonatally castrated males resembled the

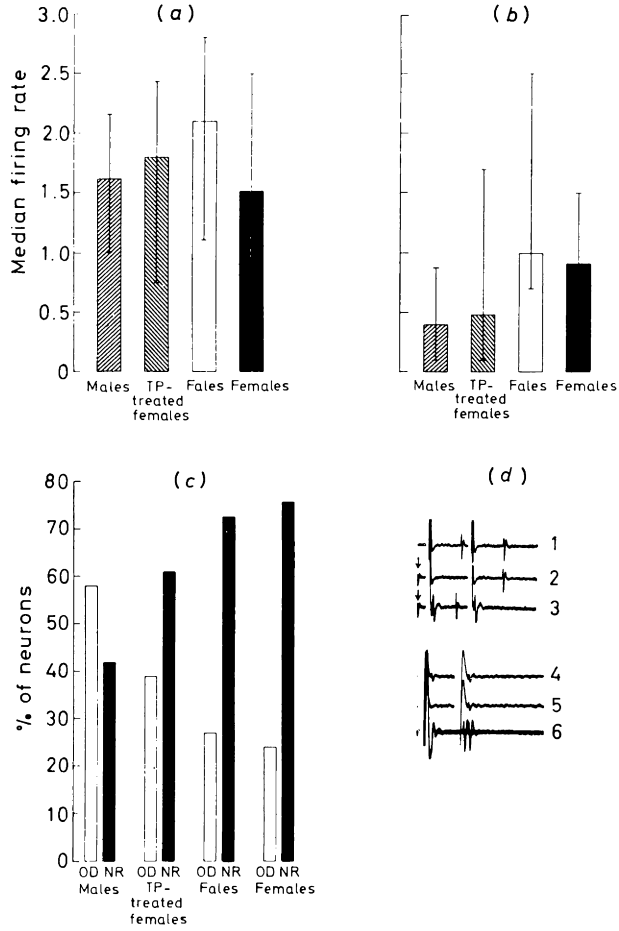

Figure 1.4 (a) Median firing rates (with 95% confidence limits) for all spontaneously active cells recorded from four groups of rats; (b) histogram showing the statistically significant ($P < 0.05$) difference between endocrine males and females, in the firing rates of spontaneously active cells which did not respond to MBH stimulation; (c) comparison of responses of preoptic neurons which project to the mediobasal hypothalamus to stimulation of the CMA. Note the reciprocal relationship between response (OD) and non-response (NR) in the series male, TP-treated female, fale and female; (d) 1 and 2, 4 and 5 confirm antidromic identification of neurons, 3 and 6 are neurons driven by the amygdala. (After Dyer et al., *1976)*

female pattern and androgen-treated females resembled the male pattern. When testosterone was added to a culture medium, preoptic anterior hypothalamic neuronal tissue responded with proliferation (Toran-Allerand, 1976). On the other hand, considerable testosterone and oestradiol uptake was demonstrated not only in the POA and hypothalamus but also in the AMY (Sheridan *et al.*, 1974; McEwen *et al.*, 1975) during the 'critical period' of neonatal sexual differentiation (*Figure 1.5*). All the evidence presented would then suggest that the AMY is one sexually differentiated extra-hypothalamic structure.

Following sexual differentiation further sexual development can be altered by interference with the extra-hypothalamic function (see Gorski, 1974; Gorski

et al., 1975; Ellendorff, 1976 for reviews). It has been suggested that limbic structures may affect the time of vaginal opening and the onset of ovulation reciprocally, with the AMY usually inhibiting (Elwers and Critchlow, 1960; Baum and Goldfoot, 1975) and the hippocampus enhancing the onset of events (Zarrow *et al.*, 1969; Döcke, 1974). The experimental approaches taken to reach such conclusions may not be adequate to imitate the normal sequence.

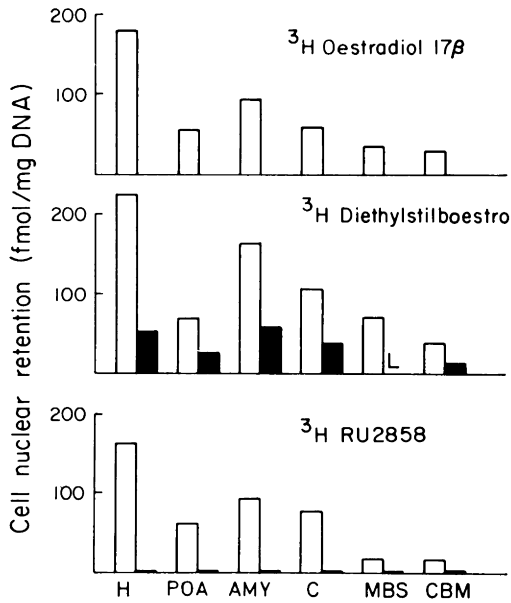

Figure 1.5 Regional distribution of cell nuclear binding sites in the brains of 3-day-old female rats. ^{3}H-oestrogens were given subcutaneously. Each bar is a single experiment with tissue pooled from 4 to 6 identically treated rats each for control and CI628 groups. Open bars, control ^{3}H-oestrogen uptake; filled bars γ-nitromifene citrate (CI628) given 15 h before ^{3}H oestrogen. In the ^{3}H-oestradiol experiment there was no group receiving CI628. L, lost sample; H, hypothalamus; POA, preoptic area; AMY, amygdala; C, cerebral cortex; MBS, midbrain plus brain stem; CBM, cerebellum. (From McEwen et al., *1975)*

For example, the success or failure of the enhancement of vaginal opening and ovulation by AMY lesions is dependent on the time at which animals were lesioned in relation to the time of puberty. The observation that at different phases of prepubertal life LH responses may occur or may be opposite to those found at other phases suggests that the AMY does not adopt a strictly inhibitory, stimulatory, or no-response role. Electrolytic lesions by stainless steel electrodes, which set an 'irritative' stimulus by iron deposition, advanced the onset of puberty, while platinum electrodes delayed vaginal opening (Velasco, 1972). This also argues against a strictly inhibitory nature of the AMY. Recent findings on mechanisms and the extent of action of iron deposits set a clear limit to the interpretation of results obtained from electrochemical stimulation (Colombo *et al.*, 1974; Dyer and Burnet, 1976; Dyball *et al.*, 1977).

Some endogenous factors have been related to limbic structures and puberty: oestrogens and the availability of oestrogen receptors are two of these factors. Thus, in the rat as in other species, plasma LH undergoes considerable changes

between birth and sexual maturation (Döhler and Wuttke, 1974) with very high oestradiol levels immediately after birth and lower but still irregular levels at later stages of sexual development (Rabii and Ganong, 1976). In the limbic system, notably the AMY, it is obvious that steroid uptake, steroid binding and the presence of protecting steroid binding proteins undergo changes during prepubertal phases (Plapinger and McEwen, 1973; McEwen *et al.*, 1975; McEwen *et al.*, 1976). It is very likely that such changes will influence the time of onset of ovulation and any experimental attempts to advance or delay the onset of first ovulation.

Extra-hypothalamic Control of Ovulation in the Adult

Classical studies which, by using micro-knives, separated the hypothalamus from the rest of the brain strongly suggested that signals must arrive from outside the hypothalamus to participate in the control of ovulation (Halász and Pupp, 1965; Halász and Gorski, 1967; Blake *et al.*, 1972). The origin and type of signals remained obscure. Numerous other studies have suggested involvement in the control of ovulation of various limbic structures, namely, the AMY and the hippocampus, but also the midbrain and, to a less obvious degree, other structures such as the cortex. Considerable attention has been given to sensory input, notably light and olfaction, as well as to tactile stimuli which are very important in those species which ovulate reflexly in response to mating.

It is the general consensus that in the adult female the AMY normally stimulates gonadotrophin secretion and ovulation. The major basis for this consensus is experiments involving either electrochemical stimulation or lesions of the AMY. We have recently discussed these experiments in detail (Ellendorff, 1976). The regulatory influences of the AMY, however, seem to be more subtle and apparently need reinterpretation for various reasons.

Firstly, the short-term effects of AMY or strial destruction must be separated from the long-term effects. While the immediate response of strial destruction is inhibition of ovulation, long-term disconnection of the AMY from the hypothalamus does not disturb cyclicity and ovulation (Velasco and Taleisnik, 1971; Brown-Grant and Raisman, 1972). This could indicate functional plasticity of amygdaloid function or an immediate potentially unspecific effect.

Secondly, we must distinguish results obtained in the acutely prepared and stimulated animal from those that have been obtained in the chronically prepared and fully awake animal. In the former case, ovulation may be inhibited after electrical stimulation (Ellendorff *et al.*, 1973) or stimulated after electrochemical stimulation (Velasco and Taleisnik, 1969b) while in the latter case it is not possible to inhibit ovulation by the use of electrical stimulation (Ellendorff *et al.*, 1973) (*see Table 1.1*).

Thirdly, the state of the oestrous cycle and/or circulating oestrogen levels determines the degree and possibly the direction of the AMY control over gonadotrophin secretion and ovulation. During any stage of the oestrous cycle other than pro-oestrus, electrical stimulation of the AMY fails to alter plasma LH levels (Kawakami *et al.*, 1973). The rat made persistently oestrus by constant illumination seems to be a particularly sensitive model which readily responds to either electrical or electrochemical triggering with ovulation (Bunn and Everett,

Table 1.1 Effects of electrical stimulation of the amygdala (AME/ACO) on ovulation in the pro-oestrous rat. (After Ellendorff *et al.*, 1973)

Group	*Drug*	*Electric stimulus*	*No. of rats*	*Number ovulating*[1]
Acute	Ether	—	8	8
	Ether	+	10	0
Chronic	Ether[2]	+	8	8
	—	+	5	5

[1] All rats that ovulated had shed seven or more ova.
[2] The contralateral amygdala was stimulated after two consecutive four-day cycles had intervened following the stimulation without ether.

1957; Arai, 1971). On the other hand, the ovariectomized (i.e. the oestrogen-deprived) rat reacted to electrical stimulation of the AMY with lowered plasma LH levels (Ellendorff *et al.*, 1973).

In neurophysiological approaches to amygdala–hypothalamic relations, any type of response, inhibitory, stimulatory, or no response may be recorded from single neurons in the POA when the AMY is driven by single electrical pulses (Fenske *et al.*, 1975; Dyer *et al.*, 1976) (*Figure 1.6*).

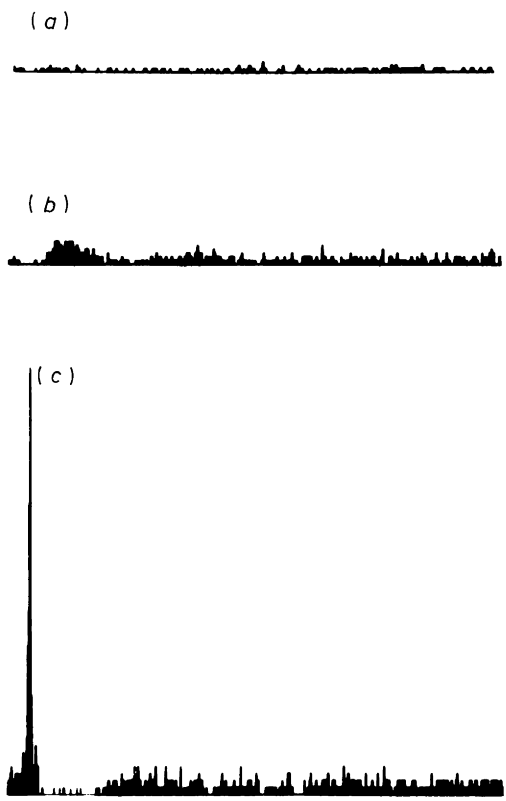

Figure 1.6 Responses of three different preoptic neurons to amygdala stimulation: (a) 3 mA, no response; (b) 600 μA, primary inhibition followed by excitation; (c) primary excitation followed by inhibition (F. Ellendorff, W. Wuttke and M. Fenske, unpublished observations)

Simultaneous recording from two adjacent neurons can produce inhibition in one and stimulation in the other neuron when an electrical impulse is given to the AMY (Fenske *et al.*, 1975). Amygdala–hypothalamic relations have also been reported by Renaud (1976). Tuberoinfundibular neurons are excited or inhibited by single pulses from the AMY. The connection can be monosynaptic (Ono and Oomura, 1975). A further observation is of interest, namely, that ventromedial-arcuate neurons can be invaded antidromically from the AMY (Renaud and Hopkins, 1977) but largely via non-strial systems. Thus the AMY modulates the activity of the hypophysiotropic area via the POA and possibly via other collaterals. The AMY can also receive direct afferents from the hypophysiotropic area of the hypothalamus. Dioestrus–pro-oestrus differences in the number of inhibitory or no responses of POA neurons after AMY stimulation can be deduced from two different experimental series which we carried out. In pro-oestrous rats, about half of the recorded POA neurons reacted with primary excitation to single pulse stimulation of the AMY, 16% did not respond (Fenske *et al.*, 1975). In dioestrous females and feminized males, which both responded in the same manner, however, about 50% of the recorded POA neurons did not respond, while about 30% were primarily excited (Dyer *et al.*, 1976). Apparently excitation of POA neurons by AMY stimulation is easier during pro-oestrus than during dioestrus (Dyer, 1973). A number of other indications suggest pro-oestrus–dioestrus differences in AMY activity. The seizure threshold of the medial part of the AMY is at its nadir during pro-oestrus (Terasawa and Timiras, 1968). Increased activity of protein synthesis can be observed in the POA and in the AMY 15–18 h prior to the LH surge (ter Haar and MacKinnon, 1973). The oxygen consumption of the AMY is more avid during oestrus than in dioestrus (Schiaffini *et al.*, 1969).

It seems obvious that the differences observed between intact and ovariectomized animals or between dioestrous and pro-oestrous animals are due to gonadal steroids, in particular to oestrogens which are specifically accumulated in the AMY of various species (e.g. Pfaff and Keiner, 1973; Keefer and Stumpf, 1975). Functional aspects of the effects of gonadal steroids have been reviewed in detail (Sawyer, 1972; Ellendorff, 1976). In summary, the AMY must be considered as one site where steroids, e.g. oestrogens and possibly progesterone (Kalra and McCann, 1975), initiate changes in brain activities which are part of a sequence of activities controlling the oestrous cycle and ovulation.

There are several ways that oestrogens may exert this function via the AMY. Sawyer (1972) has suggested the coexistence of two groups of cells: '(1) cells inhibitory to gonadotrophic function in general and (2) cells facilitatory to the ovulatory surge of pituitary LH-release. Oestrogen may simultaneously suppress group 1 and facilitate group 2'. So far this hypothesis has remained unchallenged. It is likely that the effects of oestrogens, whether inhibitory or excitatory, are brought about by alteration of the levels of enzymes specifically found in the AMY (Luine *et al.*, 1975). We have recently suggested another path by which oestrogens may alter plasma LH via the AMY. Metabolites of oestrogens – catecholoestrogens – that interfere with catecholamine metabolism cause changes in plasma LH levels (Parvizi and Ellendorff, 1975). Although this has been shown in the male, there is no reason why it should not take place in the female and influence ovulation.

The hippocampus has been included among the possible sites that participate in the modulation of hypothalamic–pituitary–ovarian functions. Stimulation

and lesion experiments as well as transection of hypothalamic afferents originating from the hippocampus show that the hippocampus can be triggered to inhibit the pre-ovulatory surge of LH and ovulation (Velasco and Taleisnik, 1969a, 1971; Kawakami *et al.*, 1972). Interestingly enough, the ventro-medial nucleus and the arcuate nucleus-multiunit activity was found to be elevated due to hippocampal stimulation (Gallo *et al.*, 1971). On the other hand, largely inhibitory responses were recorded from single units in the basal hypothalamus in response to electrical stimulation of the hippocampus (Mandelbrot and Feldman, 1972). The cyclic decrease in stimulatory threshold during pro-oestrus or after oestradiol benzoate treatment (Terasawa and Timiras, 1968) suggests some association with the level of circulating gonadal steroids (*see Figure 1.7*).

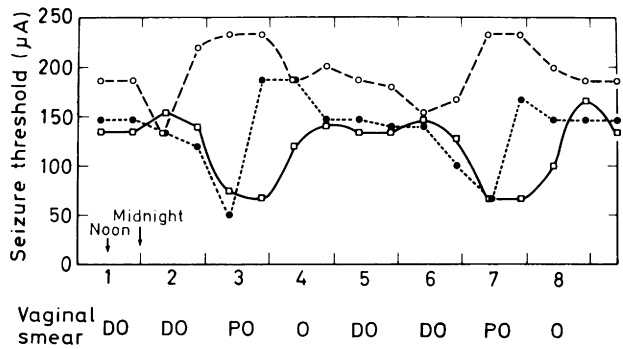

Figure 1.7 Comparison of localized seizure threshold curves of three portions of the limbic system during two oestrous cycles. ——□, dorsal hippocampus; - - - - •, medial part of the amygdala; — — —○, lateral part of the amygdala. Data for hippocampus and lateral part of amygdala from one rat; data for medial part of amygdala from another rat. D, dioestrous day; PO, pro-oestrous day; O, oestrous day (from Terasawa and Timiras, 1968)

In some parts of the hippocampus 'essentially unlabelled' (Stumpf, 1970) to 'reliable' (Pfaff and Keiner, 1973) radioactive labelling was observed after oestrogen administration. This points either to little involvement of oestrogens in the hippocampal function or to the highly efficient use of oestrogens. Despite these indications of hippocampal participation in the control of ovulation, several questions need to be answered. Does the hippocampus participate in gonadotrophin release and ovulation under non-experimental conditions? Which steroids and possible neurotransmitters are part of the regulatory system and how?

The mesencephalon is considered to exert a dual control over gonadotrophin secretion. This concept has been advanced by Carrer and Taleisnik (1970) on the basis of mapping the mesencephalon with the aid of electrochemical stimulation. Ovulation was induced by stimulation of parts of the ventral tegmental nucleus. Stimulation of other areas, such as the dorsal tegmentum, elevated LH levels in ovariectomized oestradiol-primed rats. A pathway is proposed since strial transection prior to stimulation prevented the ovulatory response: mesencephalon–amygdala–preoptic area–basal hypothalamus. The medial forebrain bundle is suggested as the mesencephalon–amygdala link (Taleisnik and Carrer, 1973).

Apart from stimulatory effects of parts of the mesencephalon, inhibition of ovulation has been observed from a number of structures such as the medial raphe nucleus, the periaqueductal grey and the ventral tegmental area.