

AN URGE TO EXPLAIN THE INCOMPREHENSIBLE: Geoffrey Harris and the Discovery of the Neural Control of the Pituitary Gland

G. Raisman

The Norman and Sadie Lee Research Centre, Division of Neurobiology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, United Kingdom

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ABSTRACT

Geoffrey Harris is responsible for our view that the brain controls the endocrine system by an exquisitely regulated pattern of synthesis and release of individual members of a family of peptide hormones. These hormones are carried through a portal vascular system that passes from the hypothalamus to the pituitary gland, where they selectively regulate the secretion of the six anterior pituitary hormones.

This family of hypothalamic hormones is highly conserved in all vertebrates, including humans. They are essential for all aspects of reproduction—courtship, mating, pregnancy and young rearing—and they are responsible for the seasonal regulation of breeding. The hypothalamic control mechanism for reproduction is sexually dimorphic, with a basic female pattern that becomes masculinized under the influence of specific steroid hormones acting during development. Other members of the hypothalamic hormone family specifically regulate the secretion of pituitary growth hormone and the anterior pituitary hormones controlling the functions of the thyroid and adrenal glands. The secretion of the hypothalamic hormones is itself regulated by the feedback of the target gland hormones (such as estrogen and progesterone), which concurrently act on the brain to elicit appropriate behavior patterns.

The hypothalamo-hypophysial axis plays a crucial role in the struggle for the survival of the species. By bringing the endocrine system under the control of the brain, it allows access to external environmental inputs, learned behavior patterns, and the whole of the central integrative machinery needed for the bodily

functions to be sensitively and optimally adapted to the ever-changing challenges and opportunities in the outside world.

Introduction

It is 25 years since the death of Geoffrey Harris, and the existence of hypothalamic peptides controlling anterior pituitary secretion has become just another section in an undergraduate textbook. In a reductionist world, where molecular biological techniques are revealing an ever-accelerating number of new genes, the broader picture of the whole animal and its integration with its environment can be something we take perilously for granted. This review attempts to reconstruct some of the mystery and drama of those earlier times, and to show the combination of tenacity and insight that went into the hard-won discovery of what now seems commonplace.

Seasonal Influences on Breeding

Introducing his Croonian Lecture on "Sexual periodicity and the causes which determine it" to the Royal Society in 1936, Francis Marshall, one of the great pioneers of reproductive physiology, observed:

The great majority of animals, both vertebrate and invertebrate, not to mention plants, have a more or less definite season of the year at which they breed. There is no month of the year at which some species does not have its breeding season, and yet for the particular species in question the season is most regular. In view of the general correlation between the seasonal and the sexual cycles it must be assumed that these stand in the relation of cause to effect (Marshall 1936).

The persuasive simplicity and the breadth of Marshall's postulate lead at once to the question of mechanism: How do the changing seasons determine the time when an animal breeds? What parts of the body are involved in the recognition of these environmental signals? What are the anatomical and physiological channels for their transmission? And which molecules carry these messages? These questions occupied the life's work of Geoffrey Harris, from his first contact with Marshall in Cambridge in 1936, to his death in 1971.

Electrical Stimulation: A Laboratory Model of Environmental Events

To examine the complex natural control mechanisms postulated by Marshall, the first step was to devise a laboratory situation that could mimic the effects of environmental events on reproduction. "Marshall had already formed the opinion," Harris wrote in his classical 1955 monograph, *Neural Control of the Pituitary Gland*, "that sensory stimuli act through reflex pathways in the central nervous system to affect the secretion of the anterior pituitary gland, which acts as a liaison organ between the nervous and reproductive systems" (Figure 1)

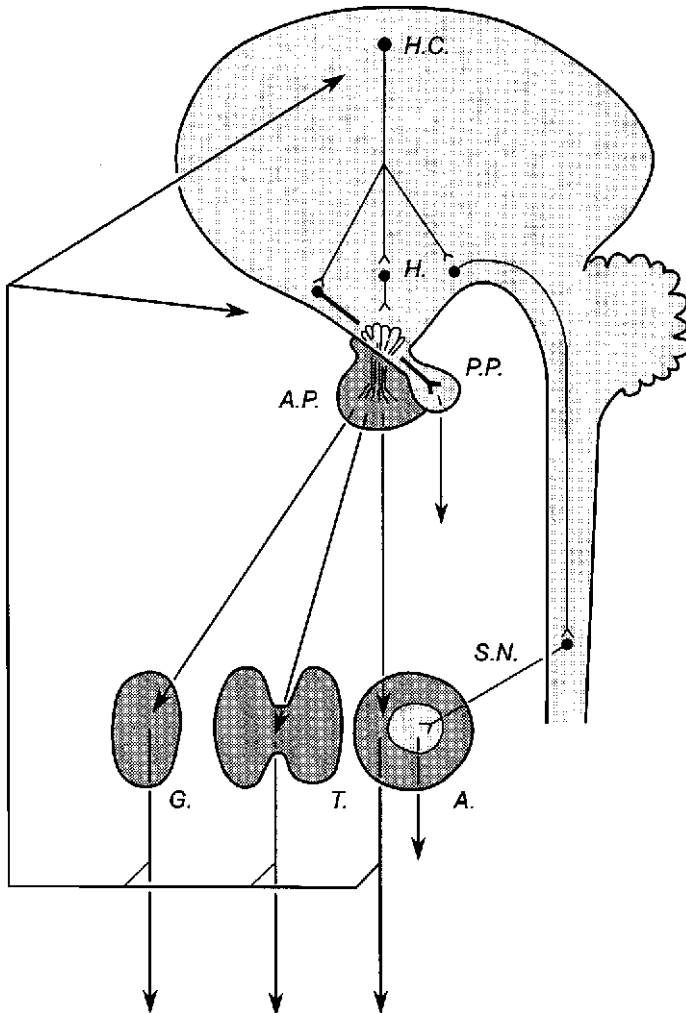


Figure 1 Neuro-endocrine integration. This figure, adapted from Harris's monograph of 1955, already shows the global theory of the reciprocal relationship between the CNS and the endocrine system in terms not different from our present concepts. The "higher centres" (H.C., a term that Harris also puts in quotation marks) influence the hypothalamus (H.), from which the portal vessels carry controlling factors to the anterior pituitary (A.P.), whose hormones in turn separately regulate the secretions of the gonad (G.), the thyroid (T.), and the cortex of the adrenal (A.). The hormones of these target glands are shown feeding back at hypothalamic and higher CNS levels. In addition, two nervous control pathways are shown: (a) the supraoptic and paraventricular projections through the pituitary stalk to the posterior pituitary (P.P.) and (b) the projection from the spinal preganglionic neurons through the splanchnic nerves (S.N.) to the adrenal medulla.

(Harris 1955). Experimental investigations of this liaison began with attempts to simulate environmentally induced events by electrical stimulation.

In rabbits the stimulus of mating induces ovulation. Both ovulation and the maintenance of corpora lutea were known to depend on the secretory function of the anterior pituitary gland (e.g. Smith 1927). In 1936, Marshall and Verney had made the seminal observation that in the absence of mating, diffuse electrical stimulation to the head of female rabbits was sufficient to trigger the mechanism for ovulation and that the ruptured ovarian follicles developed into functioning corpora lutea able to maintain a pseudopregnancy (Marshall & Verney, 1936).

Harris's first work was carried out in the Department of Anatomy at Cambridge when he was a medical student. Following Marshall and Verney, he applied similar diffuse stimulation to the head of rats (Harris 1936). This species, unlike the rabbit, normally ovulates spontaneously, but unless mating occurs, the ruptured ovarian follicles do not develop into functioning corpora lutea. The effect of the electrical stimulation was that the next ovulation was delayed, but then was followed by a pseudopregnancy. Harris concluded that "excitation of some neural structure had occurred, and this in turn had stimulated the anterior pituitary." But which structure had been excited?

"Marshall suggested to me," Harris wrote in his Dale Lecture, published posthumously in 1972, "that it would be interesting to apply precisely localized electrical stimuli to different regions of the hypothalamus of estrous female rabbits to see if it were possible to obtain evidence concerning the reflex pathway normally involved in post-coital ovulation" (Harris 1972).

During the next year, Harris reported that stimulation in the hypothalamus evoked ovulation in the rabbit, and noted (with what he later called "the brashness of the young": "There is no reason to believe that the thyrotropic, adrenotropic, lactogenic, parathyrotropic and growth hormone are not similarly controlled" (Harris 1937). Like Luther, he had nailed his articles to the church door, and by the end of his life, he would have demonstrated the truth of some, but by no means all of his postulates.

Over the next 10 years Harris set about devising a technique to apply electrical stimulation to precisely localized areas, but under conditions sufficiently noninvasive that the endocrine system would continue working normally. Taking advantage of the wartime advances in electronics that had been needed for the development of radar, Harris designed a stereotaxically placed, chronic in-dwelling electrode attached to a small secondary induction coil that was inserted under the scalp and activated by a large primary induction coil surrounding the cage (Harris 1947, 1948a,b). These electrodes were able to deliver highly localized stimuli. The electrodes could be used for chronic stimulation in freely moving, unanesthetized rabbits. The method of remote stimulation avoided the stress caused by ether or other anesthetics, which themselves can interfere

with ovulation or induce pseudopregnancy and which affect the secretion of adrenocorticotrophic hormone (ACTH) and several other anterior and posterior pituitary hormones.

Over the years, this meticulously developed and surgically demanding technique would serve Harris well. He was to use it to study the control of the release of the anterior pituitary hormones—luteinizing hormone (LH, involved in ovulation), prolactin (involved in pseudopregnancy, pregnancy, and lactation), ACTH, and thyrotrophic hormone (TSH)—and the posterior pituitary hormones—oxytocin and vasopressin.

Because of the precision of localization, Harris was able to show that the crucial position for inducing an ovulatory response was when the electrode tip lay in the hypothalamus, and he could now confirm the observations of Markee et al (1946) that the pituitary gland itself was nonresponsive (Harris 1948a). This led to the important conclusion that the ovulatory signal generated by stimulating the CNS must have passed from the hypothalamus to the pituitary. But the nature of the pathway from the hypothalamus to the anterior pituitary was unknown: The search was now on for the link between the hypothalamus and the pituitary gland. Harris was on the threshold of his first major breakthrough.

The Neurovascular Link

The most obvious link from the nervous system would have been by means of nerve fibers, and for several other endocrine organs a nervous link was already known. Thus, as Harris noted, “the adrenal medulla has a direct nerve supply, which controls its secretion; the posterior pituitary also has a direct nerve supply” (Harris 1955). Harris used his method of localized electrical excitation to show that unlike the anterior pituitary, the posterior pituitary does respond to localized electrical stimulation by release of vasopressin (causing antidiuresis and an increase in urinary chloride concentration), and oxytocin (causing increased uterine motility, as measured by a balloon) (Harris 1947). Thus the presence of a nerve supply passing down the pituitary stalk from the brain to the posterior pituitary is correlated with the ability to induce secretion by electrical stimulation.

The paradox is that the anterior pituitary, which is crucially involved in the response to environmental, seasonal, and mating stimuli, is clearly under the control of the CNS, but despite considerable efforts to find one, it has scant or no innervation. “The pars distalis of the pituitary,” Harris wrote, “may be described as a gland under nervous control but lacking a nerve supply” (Harris 1948b). How then is the nervous system able to control the release of the anterior pituitary hormones?

Not for the last time, chance was to play an important part in Harris’s life. Harris’s work in the Department of Anatomy at Cambridge coincided with the

visit of Professor Gregory T Popa, of the Department of Anatomy at Jassy in Romania. Popa & Fielding (1930, 1933) had shown that the human pituitary stalk is clothed by a system of hypothalamo-hypophysial vessels, which were named portal (by analogy to the hepatic portal circulation) because they were connected at both ends to a capillary plexus—above to the capillary plexus of the median eminence on the under surface of the midline hypothalamus, and below to the capillary plexus of the anterior pituitary gland (Figure 2). In a striking series of comparative anatomical studies of a large range of different vertebrate species, John D Green, Harris's close friend and fellow demonstrator in anatomy, demonstrated the phylogenetic constancy of the hypophysial portal circulation and the capillary loops in the median eminence (Green 1951). This degree of evolutionary conservation suggested that such an anatomical arrangement must have a very important functional role. In fact, as Harris was to show, the messages carried through this unique area of specialized vasculature were vital for all aspects of reproduction, which from an evolutionary point of view, is the most basic function needed for survival of the species. In 1947, Green & Harris suggested "that the central nervous system regulates the activity of the adenohypophysis by means of a humoral relay through the hypophysial portal vessels" (Green & Harris 1947).

However, for some time after their original discovery, the functional importance of the portal vessels remained obscured by uncertainty about the direction of blood flow within them. Popa & Fielding (1930, 1933) believed that the blood flowed from pituitary to brain, whereas Wislocki (1937, 1938) believed that it flowed from brain to pituitary. In amphibia, several investigators had directly observed in living animals that the portal blood flow was from the hypothalamus to the pituitary (e.g. Houssay et al 1935, Green 1947).

Harris initially accepted the view of his visiting senior colleague, Popa, that the blood flow was from the pituitary to the brain (Harris & Popa 1938). But finally, by a combination of india ink perfusion techniques and direct microscopic observation in the living rat, Green & Harris (1949) confirmed Wislocki's finding (1937, 1938) that the portal blood flows from the hypothalamus to the pituitary. This was the first demonstration of portal blood flow in a living mammal. [Later work has shown that in fact there is also a system of vessels with blood flowing in the opposite direction as well (Bergland & Page 1978).]

The portal vessels were, therefore, in a position to provide an anatomical pathway to convey the messages by which electrical stimulation of the hypothalamus could induce the secretion of hormones by the anterior pituitary, and it was therefore possible (as others had speculated, e.g. Hinsey & Markee 1933, Friedgood 1950) that the neural control of pituitary secretion could involve a humoral agent carried through this vascular link. This would explain why

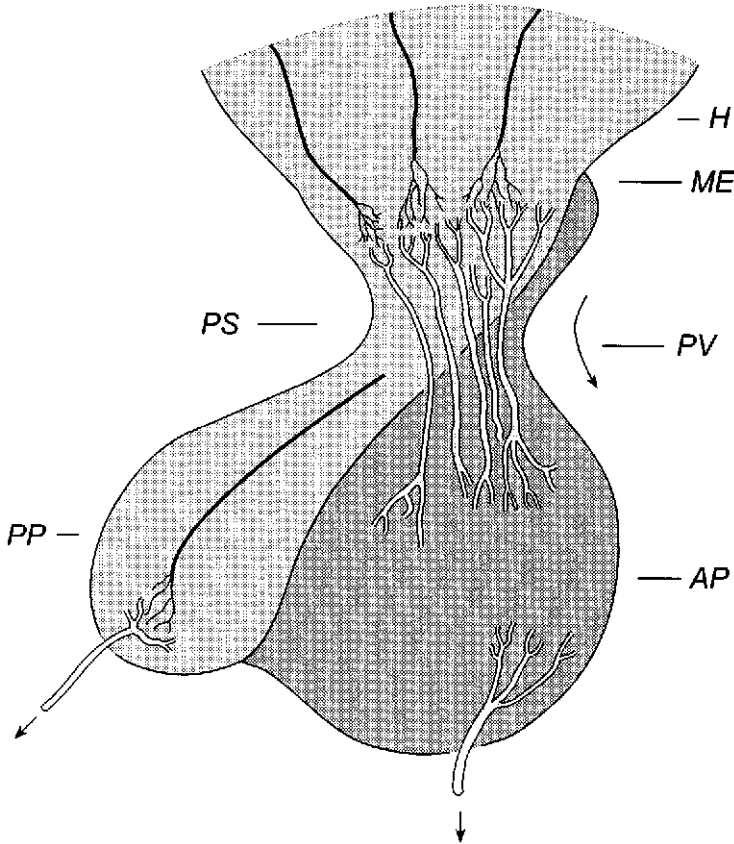


Figure 2 The portal vascular link. Hypothalamic (H) nerve endings are shown arborizing in the median eminence (ME), where they release their secreted factors into the primary plexus of the portal vessels (PV), which carry them down the pituitary stalk to be released at a secondary plexus in the anterior pituitary (AP), where they regulate the secretion of the anterior pituitary hormones into the general circulation. In contrast, the posterior pituitary (PP) is directly innervated by hypothalamic fibers passing down the pituitary stalk (PS) and releasing their secretions directly into the general circulation. (Adapted from Fawcett et al 1968.)

electrical stimulation could elicit an ovulatory response from the hypothalamus but not from the anterior pituitary.

Cajal (1911) had described hypothalamic nerve fibers that terminated on the capillaries of the median eminence. In his 1955 monograph, Harris suggested that these nerve endings might contain “some humoral substances which are liberated into the capillaries of the primary plexus of the median eminence, and

carried by the portal vessels to excite or inhibit the secretion of the gland cells in the anterior pituitary” (Harris 1955). Later electron microscopy (reviewed in Harris & Campbell 1966) showed that these capillaries are of the specialized fenestrated type found in other secretory and absorptive organs, with a double basal lamina separated by a narrow collagen-containing space. The blood-brain barrier is here permeable to the transmission of larger molecules. The nerve fiber terminals on the outer layer of the basal lamina contain synaptic vesicles and a variety of larger dense-cored vesicles.

The problem for Harris was how to prove that this anatomical arrangement of the hypothalamo-hypophysial portal vascular system did, in fact, constitute the functional link in the hypothalamic control of anterior pituitary secretions. “Sufficient evidence is not available,” Harris wrote, “to prove the neurohumoral control of the adeno-hypophysis, but we feel this theory has much to support it” (Green & Harris 1947).

In Harris’s opinion (1955), the definitive proof of a functional link would require observations that were, at that time, technically impossible, viz collection and analysis of the portal capillary blood, identification of the secreted hypothalamic substances (named releasing factors, according to the proposal of Saffran et al 1955) with the required functional specificity, and demonstration that appropriate changes in these factors actually occurred in vivo under the relevant physiological situations (such as ovulation) and that the changes in the releasing factors were both necessary and sufficient to cause functionally effective changes in the rate at which anterior pituitary hormones were secreted into the general circulation. It was only toward the end of Harris’s life (see below) that George Fink, working in Harris’s laboratory, was able to confirm that these criteria were valid for the hypothalamic factor needed to trigger the release of an ovulatory quota of LH from the anterior pituitary.

The Role of the Hypothalamo-Hypophysial Portal Vessels in Reproduction

As a first step to validating his proposal that the hypothalamo-hypophysial portal vascular link was essential for the control of anterior pituitary functions, Harris carried out a series of experiments with pituitary stalk section and pituitary transplantation that were designed to show that the normal control of the secretions of the anterior pituitary could occur only if the gland was vascularized from the capillaries of the median eminence.

It had long been known that the pituitary gland was essential for gonadal function. But up to that time, the extensive literature on the effects of pituitary stalk section was conflicting and did not exclude the possibility that the pituitary might function when no longer in contact with the brain. As Harris now showed, this confusion had arisen because of the failure to recognize the regenerative

capacity of the portal vessels. In his initial work with Gregory Popa in the rabbit (Harris & Popa 1938) and later in the rat (Harris 1950) and the monkey (Harris & Johnson 1950), Harris developed surgical techniques for cutting the pituitary stalk and demonstrated that cut portal vessels rapidly regenerate, revascularize the pituitary, and restore gonadal function. But if regeneration is prevented by insertion of a waxed paper plate, the gonads remain atrophic (Harris 1950, Fortier et al 1957). This, then, provided a clear indication that the portal vasculature was essential for the gonadotrophic function of the anterior pituitary, but it still remained to be shown that the failure of pituitary secretion after stalk section with prevention of portal vessel regeneration was not due to a general ischemic compromise, but was caused by deprivation of specific secretion-regulating hypothalamic factors.

This task was accomplished by a series of transplantation experiments, carried out after Harris's move to the Department of Physiology at Cambridge. In collaboration with Dora Jacobsohn of the University of Lund in Sweden (Harris & Jacobson 1950, 1952), Harris provided the definitive proof of the functional importance of the hypothalamo-hypophysial portal vascular system in regulating anterior pituitary secretion. It had already been shown (see Harris 1955) that while endocrine glands (such as the testis, adrenal cortex, ovary, and thyroid) can function in a regulated fashion when transplanted into a part of the body remote from their original site (e.g. under the kidney capsule), the anterior pituitary gland does not. Using the surgical parapharyngeal hypophysectomy approach [devised by Philip Smith (1927) to avoid damage to the hypothalamus or any part of the brain], Harris & Jacobsohn (1950, 1952) showed that although anterior pituitary tissue does not function normally if transplanted away from its original site, it will function if transplanted back under the midline hypothalamus within reach of the host portal vessels.

In an extensive physiological analysis of the criteria for successful return of multiple anterior pituitary functions in the rat, Harris & Jacobsohn (1950, 1952) demonstrated that neonatal or adult pituitary donor tissue of either sex could restore cyclic ovulation, mating, pregnancy, and parturition, as well as adrenal weight and functional thyroid histology. Subsequent histology showed that these functions were only resumed in cases where the transplanted pituitary had been revascularized from the primary capillary plexus of portal vessels in the median eminence. Transplants located on the adjacent ventral surface of the brain outside the portal vascular field survived, and even though they became equally well revascularized by nonportal vessels from the temporal lobe, they still did not function. However, pituitary transplants that had been grafted away from their normal site and allowed to establish there in a nonfunctioning state would resume function if retransplanted back into their normal site under the median eminence (Nikitovich-Winer & Everett 1958).

From such observations, Harris proposed that the median eminence is the final common pathway for transducing neural signals into releasing factors, which are carried in the portal vessels to the anterior pituitary, which in turn controls the separate functions of the distant target organs through secretion into the general circulation, thus bringing a wide range of endocrine glands under control of the CNS (Figure 1).

Posterior pituitary functions, however, were not fully restored in these transplants. After delivery of the young, the mothers produced milk as normal, and the mammary glands became engorged, but there was no let-down of milk, and therefore the litters did not survive. This defect could be remedied by administration of oxytocin, suggesting that the let-down of milk may require a reflex release of oxytocin from the posterior pituitary gland that is induced by the stimulus of suckling (Figure 3). With his graduate student Barry Cross, Harris confirmed this by showing that electrical stimulation of the supraoptic-hypophysial tract (i.e. the pituitary stalk) induces milk ejection (measured by cannulating a mammary duct) (Cross & Harris 1950) and that this effect is prevented by lesions of the tract (Cross & Harris 1952).

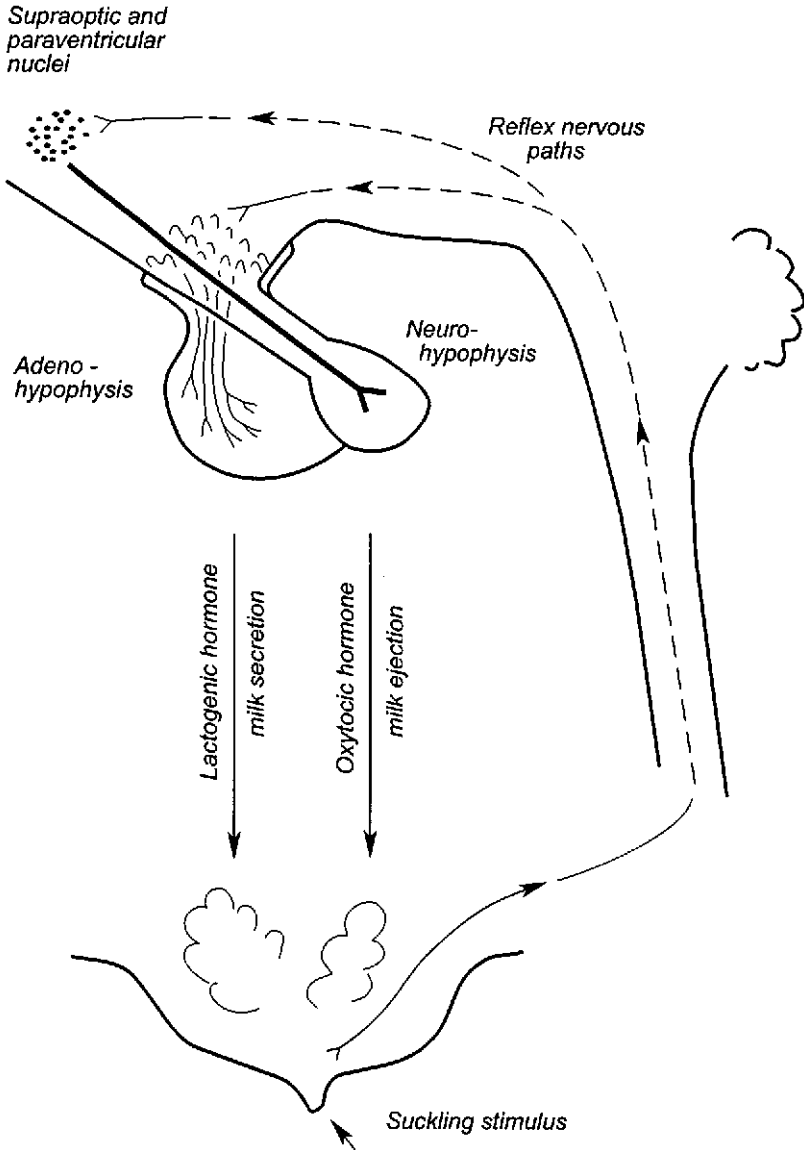
In his transplantation experiments, as in the case of the remote control electrical stimulation he had previously devised, Harris's holistic concern with the investigation of a fully integrated range of bodily functions in healthy animals was the distinctive hallmark of his experimental approach and enabled him to identify the overall significance of highly specific local events. An excellent example is the rapid onset of reproductive functions observed in adult hosts with neonatal pituitary tissue (Harris & Jacobsohn 1952). Because at this time the donor pituitary tissue age was still considerably pre-puberal, Harris reasoned that the delay in onset of reproductive function that occurs in normal development (i.e. puberty) is due not to an inability of the immature pituitary to respond, but to the time needed for maturation of the CNS (Harris 1955).

Hypothalamic Control of the Adrenal Cortex

"It is clear," Harris wrote in his monograph of 1955 (Harris 1955),

that environmental factors exert a profound effect on the secretory activity of the endocrine glands, but the mechanism by which these effects are produced is known only in outline.

Figure 3 Dual, neural, and endocrine control of lactation. The two postulated neuro-hormonal reflexes underlying the stimulating action of suckling on milk secretion. During pregnancy, the cumulative action of the lactogenic hormone (prolactin) from the anterior pituitary causes a buildup of milk, which is, however, retained in the breast until the stimulus of suckling activates neural pathways. This activation leads to the discharge of posterior pituitary oxytocic hormone, which triggers milk ejection (the "let-down" reflex). (Adapted from Figure 46 in Harris 1955.)



For example, trauma to a limb may result in a flexor reflex response in that limb, and also lead to a discharge of hormones from the adrenal cortex. Much could be said of the sequence of events starting in trauma and ending in contraction of the flexor muscles, but until recently very little could be said about the mechanism linking trauma and discharge of adrenal cortical hormones.

The mechanism of the control of adrenocortical secretion has always presented one of the most complex problems for analysis. This is because of the extreme lability of adrenal cortical hormone secretion in response to a wide variety of stressful and other factors, acting both immediately and over the long term, and also because the adrenal cortex can maintain a basal level of secretion in the absence of the pituitary (Vogt 1951).

Anyone attempting today to disentangle the immense number of conflicting papers and the mass of data published at that time (see for instance Selye 1950) might marvel that Harris had the insight and clarity of thought to extract the central fact, which seems so simple to us now with hindsight, that the control of the secretions of the adrenal cortex depends on central nervous integration of stressful and other inputs. As in the case of the anterior pituitary control of reproduction, this control is exerted by modulating the secretion of what were at that time unidentified factors passing through the hypothalamo-hypophysial portal vessels to regulate the release of ACTH from the anterior pituitary.

In favor of a central nervous control of pituitary ACTH release, Harris quoted the observation (Sayers 1950) that after removal of their adrenals (to avoid the complications due to feedback both from the cortex and the medulla of the adrenal), animals respond to stress by an increased output of ACTH. In their transplantation experiments, Harris & Jacobsohn (1952) found that adrenal atrophy was only avoided if the pituitary grafts were revascularized by the portal vessels of the median eminence. This indicated a role for the hypothalamo-hypophysial neurovascular axis in the control of the adrenal cortex.

With Jack de Groot, Harris used the lymphopenic response to the release of adrenal glucocorticoids to show that the pituitary release of ACTH could be evoked by remote control electrical stimulation of the posterior hypothalamus (de Groot & Harris 1950), and that the lymphopenic stress response of rabbits to restraint was abolished by hypothalamic lesions, hypophysectomy, or cutting the pituitary stalk under circumstances where regeneration of the portal vessels was prevented by inserting a plate of waxed paper (Fortier et al 1957).

Harris concluded that although the negative feedback of adrenal cortical hormones "sets a ... base-line level of secretion against the background of which other factors adjust pituitary activity according to the needs of the organism" (Harris 1955), the CNS plays the key role in the response to stressful physical and psychical events in the environment, and it does so by means of factors that are channeled through the hypothalamo-hypophysial portal vascular system to

control the secretion of ACTH by the pituitary gland. [They also, as we now know, control the circadian sleep/activity rhythm of ACTH (e.g. Moore & Eichler 1972, Raisman & Brown-Grant 1977).]

Hypothalamic Control of the Thyroid

Harris & Jacobsohn's transplantation experiments (1952) had demonstrated that, as in the case of the gonads, the maintenance of the functional status of the thyroid gland (on histological criteria) also depended on the grafted pituitary tissue becoming revascularized by the hypothalamo-hypophysial portal vessels.

Extending this work with Keith Brown-Grant, a recent Cambridge medical graduate, and Sy Reichlin, a young American internist from St. Louis, Missouri, Harris used the rate of release of organically bound radioiodine from the thyroid gland (over a period of days) to measure thyroid activity. By this method, they demonstrated that if stalk section is carried out in rabbits, with precautions to prevent portal revascularization, the basal level of thyroid activity decreases (Brown-Grant et al 1954). In addition the acute, stress-induced inhibition of thyroid secretion is abolished.

Remote control electrical stimulation of freely moving rabbits, with the electrode tip in the median eminence, caused a consistent increase in thyroid activity, provided the complicating effects of the associated induced adrenal hypersecretion were eliminated by adrenalectomy (Harris & Woods 1958). A similar pattern of thyroid response could be elicited by a single injection of anterior pituitary thyrotrophic hormone (TSH) (Campbell et al 1960), thus suggesting that electrical stimulation of the median eminence had produced its effect by releasing a TSH-releasing factor that was carried through the portal vessels to the anterior pituitary gland.

The Mechanism for Neural Control of the Anterior Pituitary

It was because his investigations encompassed multiple aspects of the physiological and behavioral status of the individual animal that Harris was able to formulate a general mechanism for the neural control of the whole endocrine system. He proposed that specific hypothalamic-releasing factors carried through the local portal vascular system could activate the anterior pituitary in situ or a pituitary transplanted into the portal vascular field but that they would not be present at high enough concentrations in peripheral blood to influence anterior pituitary tissue transplanted away from the portal vascular field. The anterior pituitary in effect "amplifies" the effect of local releasing factor signals by secreting pituitary hormones at levels that are effective through the peripheral blood and can therefore control distant target endocrine organs, which will, as a result, function wherever they are transplanted.

Putting together his own and others' data from localized stimulation and lesion studies, Harris assembled a tentative map of the hypothalamic regions involved in control of the different anterior and posterior pituitary hormones (Harris 1955). A comparable, but less well known map was constructed by Halász and coworkers (Szentágothai et al 1968) on the basis of the novel approach of studying the histological and functional status of anterior pituitary tissue grafted into various positions in the ventro-basal hypothalamus.

Endocrine Control of Behavior

In 1952, at the invitation of Sir Aubrey Lewis, Harris moved from the Department of Physiology at Cambridge to the specially created Fitzmary Chair of Physiology in the Institute of Psychiatry at the University of London. This provided an intellectual environment where he was to develop his lasting interest in the psychiatric aspects of clinical endocrine disorders, and to set up experiments investigating how the effect of hormones on the brain influences behavior.

Up to now Harris's published work had concentrated on the way in which the hypothalamo-pituitary axis controls the peripheral target organs: gonads, adrenal, thyroid. For the integration of the system into a full adaptive behavior pattern, the organism needs not only to regulate glandular secretion (and ovulation), but also to display appropriate behavior patterns. In reproduction, for example, ovulation must be coordinated with behavioral receptivity (estrus), mating must be followed by preparation of the uterine endometrium for implantation, and pregnancy and parturition must be accompanied by the induction of maternal behavior such as nest-building and nursing. This integration is accomplished by a return feedback of target gland hormones that inform the CNS of the state of readiness of the endocrine system (Figure 4).

In a preliminary study at Cambridge, Harris had attempted to elicit sexual behavior by implanting estrogens directly into the brain of ovariectomized rabbits, but the appearance of positive responses in the controls had prevented firm conclusions (reviewed in Harris 1964). Now, in collaboration with Patricia Scott and a young psychiatrist, Richard Michael, Harris demonstrated that the implantation of crystals of stilboestrol esters in the hypothalamus of ovariectomized cats elicited full mating behavior even though the genitalia remained atrophic (Harris & Michael 1964). These animals were, therefore, estrous from the point of view of the CNS, and anestrous from the point of view of the endocrine periphery. They established the concept that the brain was a target for the specific feedback action of gonadal steroids to elicit mating behavior.

The Hypothalamo-Hypophysial Axis and Seasonal Onset of Reproduction in the Ferret

While Marshall's interest had focused on the effect of seasonal and other exteroceptive factors on reproduction, Harris's work so far had involved the use of

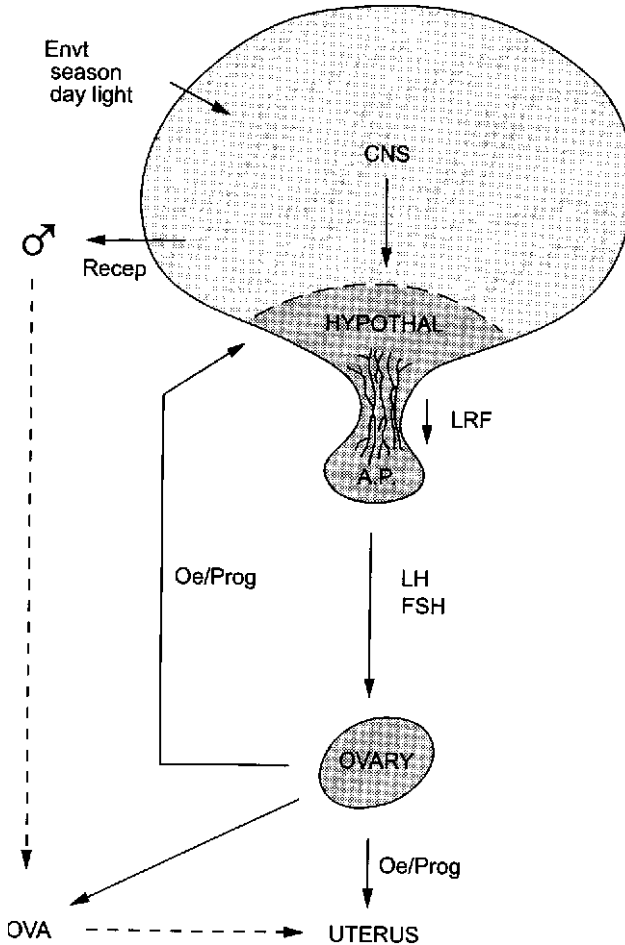


Figure 4 Control of reproduction. A schematic representation showing the pivotal role of the hypothalamo-hypophysial link in the integration of the neural and endocrine systems to produce a coordinated response to the environmental stimuli that signal the onset of breeding. The maturation of the gonads and ovulation are stimulated by environmental inputs such as the seasonal increase in day length. This input is translated by the CNS into stimulation of the hypothalamic neurons, leading to an increased secretion of luteinizing hormone–releasing factor (LRF), which is carried to the anterior pituitary (AP), where it elevates the secretion of the gonadotrophic hormones LH and FSH. Under their influence the ova mature and are released, and the ovary increases the secretion of estrogen and progesterone (Oe/Prog) to prepare the uterine endometrium for implantation. At the same time, the ovarian hormones also feed back into the CNS to trigger receptivity to the male. This system ensures that mating occurs when ripe ova are available, and fertilization is coordinated with the endometrial changes needed for implantation. [Outline adapted from Harris & Naftolin (1970).]

artificial factors, such as localized electrical stimulation, as laboratory models for the natural control of the release of anterior pituitary hormones. With his graduate student Bernard Donovan, Harris now turned his attention to the analysis of the mechanism of action of a natural, exteroceptive factor—the induction of estrus in sexually quiescent (winter) ferrets (Donovan & Harris 1954). For this they used Marshall's demonstration that precocious estrus could be induced in the laboratory by extending the daily animal house lights-on period to simulate the natural seasonal lighting effect (Marshall 1940).

Donovan & Harris (1954) reported that, in the ferret under conditions where regeneration of the portal vessels was prevented by the insertion of a waxed paper plate between the base of the brain and the pituitary gland, section of the pituitary stalk prevented the light-induced onset of estrus. This observation brought them into direct conflict with Solly (later Lord) Zuckerman, one of the most politically powerful UK scientists of the day, and a person who might well have impeded Harris's coveted election to the Royal Society. Based on two cases, Thomson & Zuckerman (1953) had made the claim that pituitary stalk section did not prevent light-accelerated induction of estrus in the ferret. One can therefore imagine with what feelings Harris insisted, as politely as he could (see p. 88 in Harris 1955), that Thomson and Zuckerman had been misled by the fact that the quality of their india ink perfusion technique was inadequate to exclude regeneration of the portal vessels.

Harris was elected to the Royal Society in 1953, and in a review in *Nature* of Harris's seminal monograph of 1955, Zuckerman contented himself with describing Harris's theory as “an edifice of speculation .. that has been erected because of an urge to explain the incomprehensible” (Zuckerman 1956). What would finally vindicate Harris's position would be the isolation of his proposed hypothalamic factors from extracts of the median eminence, the demonstration of their regulated secretion into the portal vessels, their functional effects on the anterior pituitary gland, and their molecular nature. But these were to be a long time coming. In fact it was to be 14 years from the publication of his 1955 monograph before the first of Harris's postulated hypothalamic hormones (the TSH-releasing factor, TRF) was finally identified (see below).

The First Steps in the Search for the Hypothalamic-Releasing Factors

Harris's earliest attempts to identify the putative releasing factors were based on screening the then known neurotransmitters and neuromodulators—adrenergic [as originally suggested by Friedgood (1950)], cholinergic, or histaminergic—for effects on the control of anterior pituitary gonadotrophic or adrenocorticotrophic secretion (Harris 1955, Donovan & Harris 1956). It was well established that central cholinergic and adrenergic blockers inhibited ovulation

(Sawyer et al 1949). Experimentally, these substances were valuable in providing ovulation blockers that could be used to dissect the time course of the events induced by an ovulatory stimulus (e.g. Everett & Sawyer 1950, Nikitovitch-Winer 1962, Everett 1964). Harris assumed, correctly, that these blockers were not acting as direct antagonists of the putative releasing factors, but were acting upstream, i.e. by modulating neural activity in the hypothalamus.

The germ of the idea that the releasing factors might be peptides perhaps came to Harris from his earlier experiments on the posterior pituitary at Cambridge (Harris 1947, Cross & Harris 1952). In 1949, Bargmann had used the Gomori stain to demonstrate that the posterior pituitary is directly innervated by perivascular terminals of the axons of the neurosecretory neurons lying in the magnocellular hypothalamic nuclei (Bargmann 1949). The Gomori-stained substance was later shown to be the precursor of the posterior pituitary polypeptide hormones (Livett et al 1971), which are synthesized in the supraoptic and paraventricular neurons and carried down along their axons (which form the neural component of the pituitary stalk) to their release sites on the outer layer of the double basement membrane surrounding the fenestrated capillaries of the posterior pituitary gland.

In 1955, Du Vigneaud had received the Nobel Prize for the elucidation of the nonapeptide structures of the posterior pituitary hormones vasopressin and oxytocin and the synthesis of vasopressin (references in Harris 1955). A similar recognition might well greet the discovery of the structure of the releasing factors that Harris had postulated to control the secretion of the anterior pituitary hormones.

The pituitary nonapeptides of terrestrial animals arise by evolutionary divergence from a single fish nonapeptide gene (Sawyer 1964). Fish have a median eminence, but no separate posterior pituitary. As the enlarged posterior pituitary of terrestrial animals develops, it becomes separated from the rest of the median eminence by the development of the pituitary stalk. Harris suggested that “secretions of the median eminence are perhaps chemically related to the posterior pituitary polypeptides (which may be evolved from the median eminence principles)” (Harris & Campbell 1966). For the different anterior pituitary releasing factors, Harris envisaged a spectrum of related polypeptides, possibly with overlapping physiological activities.

In 1955, both Guillemin (Guillemin & Rosenberg 1955) and Schally (Saffran et al 1955) had started the search for ACTH-releasing activity in hypothalamic extracts. In 1961, Campbell, Feuer, Garcia, and Harris reported the preparation of a crude acidic (polypeptide) extract of median eminence (Campbell et al 1961). In over 200 rabbits, they applied a meticulous technique based on very slow infusion over 2 h (to simulate the natural time course) through chronically fixed intrapituitary cannulae whose position was monitored by X ray. The

LH-releasing activity of the extracts was confirmed by induction of ovulation in these rabbits (Campbell et al 1964), as well as in rats in which spontaneous ovulation had been blocked by Nembutal, a drug acting on the CNS (Nikitovitch-Winer 1962). This finding indicated that the effect was exerted at the level of the pituitary gland, i.e. downstream from the hypothalamus. The intrapituitary route of administration was considerably more effective than intravenous injection into the peripheral blood stream. This is consistent with the view that the extracts contained factors normally produced in such minute amounts that they had to be sequestered into the localized hypothalamo-hypophysial portal mini-circulation in order to achieve high enough concentrations to affect the anterior pituitary.

Starting a year earlier, McCann et al (1960) had also prepared median eminence extracts, and reported stimulation of pituitary LH secretion, as demonstrated by the Parlow bioassay of ovarian ascorbic acid depletion in the rat (Parlow 1958), and similar results were reported by Courier et al (1961). What Wade (1981) called the "Nobel Duel" had begun, but for different reasons, neither Harris nor McCann were to be among the finalists.

Sexual Differentiation of the Brain

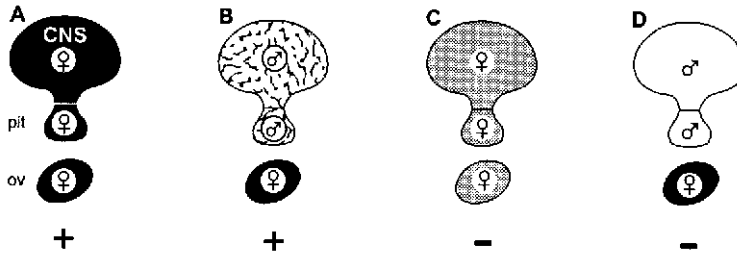
Harris & Michael's work (1964) on the induction of cat estrous behavior by the action of gonadal steroid hormones on the adult brain was the prelude to a remarkable series of studies showing the dramatic and permanent effects produced by these same steroids on the developing brain of the rat (Figure 5).

That the control mechanism for ovulation is present in female animals but not in males was well known: An ovary transplanted into a gonadectomized adult female is able to ovulate (Marshall & Jolly 1907), whereas an ovary transplanted into a gonadectomized adult male is not (Figure 5D) (Goodman 1934). These observations apply to adult, sexually mature animals. For some time, however, there had been an interest in how the different male and female sexual patterns developed and what controlled them. In the second edition of his classical textbook, *The Physiology of Reproduction*, Marshall (1922) proposed the issue in terms that continue to resonate in our understanding of sexuality to the present day:

While the chromosome constitution may determine sex at fertilisation, in some instances this is clearly overridden during subsequent development, and this results in the production of "somatic" males or masculinised females. The mechanism by which the female is transformed is one which acts through the internal secretion of the gonads.

If it be true that all individuals are potentially bisexual, the dominance of one set of sexual characters over the other may be determined in some cases at an early stage of development in response to a stimulus which may be either internal or external. The observations upon animals of many different kinds point even to the possibility that sex may be reversed after it has once been established.

Pfeiffer



Harris

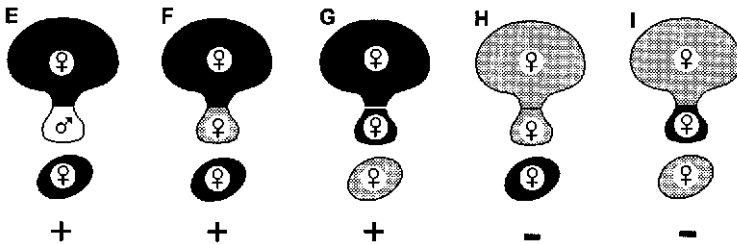


Figure 5 Sexual differentiation. (Upper row) Ovulation (+) occurs in normal genetic female rats (A), but not (-) in ovaries (ov) transplanted into normal genetic males (D). Pfeiffer (1936) demonstrated that the later development of ovulation in the adult is prevented by transplantation of a testis into a newborn female (C), and that castration of the newborn male allows the development of a mechanism by which the adult can induce ovulation in a transplanted ovary (B). From this experiment it remained possible that sexual differentiation resided in the pituitary (pit), or in the CNS. (Lower row) The work of Harris & Jacobsohn (1952), Harris & Levine (1965), and others (for a summary see Harris & Campbell 1966) demonstrated that sexual differentiation resided in the CNS and not in the pituitary. They obtained androgen-sterilized females by a single neonatal injection of testosterone. They showed that a normal female will ovulate if her pituitary is replaced by that of a male (E) or an androgen-sterilized female (F). In addition, the brain/pituitary axis of an ovariectomized normal female host is able to induce ovulation in an anovulatory ovary transplanted from an androgen-sterilized female (G). Thus, both the pituitary and the ovary of androgen-sterilized females can maintain cyclic function. Conversely, a normal female ovary will not ovulate if transplanted into an androgen-sterilized female (H), and replacement of the androgen-sterilized pituitary with a normal female pituitary transplanted into an androgen-sterilized female (I) will not induce ovulation. This combination of experiments proves conclusively that sexual differentiation is a property of the CNS. Black, normal female; grey, androgen-sterilized female; white, normal male, speckled, neonatally castrated male. ♀ indicates genetic females; ♂ indicates genetic males. +, ovulation; -, ovulation does not occur.

When once we admit the existence of latent sexual characters in individuals in which the characters of one sex are dominant, and that under certain circumstances those of the latent sex can develop at the expense of the dominant ones, we are compelled to acknowledge also that the sex of the future individual is not always predetermined in the gametes or even in the fertilised ovum, but may be called into being at a later stage in life.

But although Marshall had raised the possibility of the hormonal control of sexual differentiation, little was known about the mechanism by which it might be brought about in normal development, and few would have suspected that the crucial target for sexual differentiation would be found to be the brain.

Pfeiffer's Experiments

In 1936, Pfeiffer showed that neonatal ovariectomy did not prevent the female rat from developing into an adult capable of maintaining cyclic ovulation in a transplanted ovary (Pfeiffer 1936). On the other hand, the implantation of testes into female rats at birth caused a permanent loss of the ability to ovulate (Figure 5C). Thus in the genetic female, the development of the ability to ovulate does not require that an ovary be present during development, but the presence of a testis can prevent it.

Pfeiffer also showed that genetic males castrated at birth develop into adults in which the presence of an intact ovulatory control mechanism could be revealed by transplantation of an ovary (Figure 5B). That this was a permissive effect due to absence of the testicular secretion was demonstrated by the fact that testes immediately transplanted back into the neck of a neonatally castrated male were sufficient to suppress the development of the ovulatory mechanism.

Pfeiffer concluded that both females and males have the potential to develop a female-type cyclic ovulatory mechanism. If gonadectomy was performed at birth in either genetic sex, the resultant adult developed a female-type ovulatory mechanism. Testes present in rats of either genetic sex during the neonatal period induce a male-type noncyclic mechanism.

One possible interpretation of these observations was that the anterior pituitary gland was the target for sexual differentiation. Subsequently, however, the work of Harris and others established a compelling case that the mechanism for triggering ovulation resides in the CNS, e.g. stimulation of the hypothalamus induces ovulation (see above), hypothalamic lesions, cutting the pituitary stalk, or transplantation of the pituitary to a site away from the portal vascular field, all prevent ovulation, and drugs acting on the CNS block ovulation (reviewed in Harris & Campbell 1966). This raised the possibility that sexual differentiation also might occur not at the pituitary level, but in the CNS (Everett et al 1949).

Harris's Experiments

In their transplantation experiments published in 1952, Harris & Jacobsohn (1952) had found that not only neonatal pituitaries of either sex, but also adult

male pituitaries can support estrous cycles, mating, pregnancy, and milk production when transplanted into hypophysectomized adult female hosts (Figure 5E). This established that the pituitary was not sexually differentiated, and further favored the view that the sexually differentiated cyclic ovulatory mechanism was located in the CNS.

By 1960, it was known that the sterilizing effect of a testis transplanted into neonatal females could be obtained more conveniently by a single injection of the long-lasting testosterone propionate ester, and that the critical period for obtaining this effect was limited to the first week after birth (for references, see Barraclough 1961). Using this method, Harris & Seymour Levine (1962, 1965) reported a series of studies that conclusively showed that the sexually differentiated mechanism lay in the CNS.

With Harris's characteristic thoroughness, they analyzed the mechanism of the anovulatory defect induced in adult female rats by administration of testosterone propionate during the critical postnatal period. They noted that the ovaries of these adult genetic females were able to maintain a state of persistent vaginal cornification (which requires the secretion of estrogen) but that they did not ovulate. High doses of exogenous estrogen caused ovarian atrophy, and ovariectomy caused castration cells to appear in the anterior pituitary. These observations showed that the negative feedback of ovarian steroids on the anterior pituitary is functionally intact in androgen-sterilized females. However, the positive feedback of estrogen—i.e. induction of the ovulatory surge of LH secretion—is absent. The animals did not show female behavior (enticing and lordosis) under conventional testing conditions with a normal male, and they expressed male behavior (mounting and intromission) when tested with a receptive female.

That the failure of ovulation in androgen-sterilized females is not due to an impairment of the ovaries was confirmed by showing that when the anovulatory ovaries of an androgen-sterilized female were transplanted under the kidney capsule or [using the technique of Goodman (1934)] into the anterior chamber of the eye in an ovariectomized normal adult female rat (Figure 5G), they ovulated and maintained 4- or 5-day vaginal cycles in the host (Harris 1964, 1970; Harris & Levine 1965; Harris & Campbell 1966). Therefore the ovaries had retained the ability to ovulate when stimulated by appropriate pituitary gonadotrophin secretion. On the other hand, normal ovaries transplanted into the anterior chamber of the eye of androgen-sterilized rats did not ovulate (Figure 5H) (Harris 1964), and normal pituitaries transplanted into hypophysectomized androgen-sterilized rats were not able to reverse the anovulatory status of the hosts (Figure 5I) (Adams Smith & Peng 1966).

When transplanted into the sella turcica (Segal & Johnson 1959) or the subarachnoid space of the median eminence region (Adams Smith & Peng 1966) of hypophysectomized normal females, the pituitaries from androgen-sterilized

rats (as with male pituitary tissue) (see Harris & Jacobsohn 1952, above) could maintain ovulatory cycles, mating, pregnancy, and parturition (Figure 5*F*). Therefore the pituitaries of androgen-sterilized rats remained able to drive cyclic ovulatory function, provided they had access to a competent hypothalamic mechanism. From this, Harris concluded that neonatal androgen sterilization was due to an effect, not on the pituitary or the ovary, but upstream of the pituitary, i.e. on the CNS.

In 1962, Harris moved from the Institute of Psychiatry to succeed Sir Wilfrid Le Gros Clark as Dr Lee's Professor of Human Anatomy at Oxford, where he also set up the Medical Research Council Neuroendocrinology Unit. Here he extended his studies on sexual differentiation to the feminization of neonatally castrated genetic male rats (Harris 1964). Following Pfeiffer's observation of ovulation in a transplanted ovary (1936), Harris wished to know whether these males (Figure 5*B*) could actually maintain regular cyclic ovulation with the same periodicity as normal females. Yazaki (1960) had shown that such males transplanted with ovaries and a vagina showed 5-day cyclic vaginal changes and cyclic changes in running activity. Harris now confirmed these results for cyclicality in a transplanted vagina and for running activity, and by direct examination of ovaries transplanted in the anterior chamber of the eye, he was also able to observe at least the first two cycles of ovulation usually had 5 days duration. With appropriate hormone priming, the neonatally castrated males were bisexual, i.e. they showed both male and female behavior patterns. Subsequent work has confirmed the view, originally put forward by Marshall (see above), that the neural circuitry for both male and female sexual behavior is present in rats of both sexes (e.g. Beach 1971).

Thus, regardless of the genetic sex, the neural circuitry present at birth in both male and female rats can lead to development of a cyclic, female type of ovulatory control mechanism in the adult CNS. For both sexes, the basic pattern of development of this mechanism is female. In the normal life history of the male, the development of the cyclic ovulatory mechanism is prevented by his own testicular secretions during the critical neonatal period. The female is not exposed to adequate levels of steroids during the critical neonatal period, so her CNS develops the basic cyclic ovulatory generator. However, when exogenous androgen or other steroids (see references in Harris & Campbell 1966) are administered to the newborn female, the effect is the same as that of the endogenous testicular steroids of the male—the development of the adult CNS cyclic ovulatory pulse generator is prevented.

As Harris pointed out (1970), this sexual differentiation of the CNS is part of a wider picture. Thus, at an earlier stage of development, gonadal hormones also control the differentiation of the genital tract. Secretions from the embryonic testis cause regression of the female-type Mullerian duct system (Behringer

1995) and induce development of the male-type Wolffian duct system (Jost et al 1972). During this somatic and the later CNS development, the basic default pattern appears to be female. The switch to male-type absence of the CNS ovulatory pulse-generating mechanism is induced in normal development by exposure to endogenous testicular steroid secretions, and experimentally by administration of exogenous steroids. The common factor was later shown to be the conversion of these steroids to estrogens by a local aromatase mechanism that is present in the hypothalamus and limbic system during the period of sexual differentiation in the rat (Reddy et al 1974, Naftolin et al 1975).

Anatomical Evidence for Sexual Differentiation of the Rat Preoptic Area

Although Harris's work had established that the brain was sexually differentiated, there was no indication of whether this effect was localized, and if it was, to which region. The prime candidate for such localization would be the part(s) of the brain involved in generating the sexually dimorphic functions, of which the most obvious is ovulation. An accumulation of data from stimulation and lesion experiments (reviewed in Harris & Campbell 1966) had shown that the central ovulatory mechanism in the rat depends on the integrity of the preoptic or suprachiasmatic area. Thus the ovulatory signal from the preoptic area can be blocked by exposure to constant light or by administration of Nembutal at pro-estrus, and in these situations, ovulation can be induced by mating or by electrical stimulation of the preoptic area. If the preoptic area is destroyed, electrical stimulation does not induce ovulation. In the same way, electrical stimulation of the preoptic area does not induce ovulation in the androgen-sterilized female (Barraclough & Gorski 1961). This suggests that the effect of the neonatal testosterone on development is similar to that of a preoptic lesion in the adult, viz it irreversibly abrogates an ovulatory trigger mechanism located in the preoptic area.

In his 1966 review, Harris noted: "It would be of much interest to make detailed studies of this region with the electron microscope to see if any difference can be detected between the normal female on the one hand, and the androgen-sterilized female or normal male on the other" (Harris & Campbell 1966).

By chance, when Harris arrived at the Department of Human Anatomy at Oxford, Pauline Field and I were developing an electron microscopic sampling procedure to count the number of synapses in specific projection pathways in the CNS. We had used this approach to demonstrate the reinnervation (plasticity) of denervated postsynaptic sites in the adult septal nuclei (Raisman 1969), and we were currently examining the recently described projection of the amygdala to the hypothalamus (Raisman 1970) and comparing it with the amygdaloid projection to the preoptic area.

To our surprise, a comparison of individual rats consistently showed a bimodal distribution in the number of a particular category of spine synapses in that part of the preoptic area receiving amygdaloid input. When we showed this puzzling observation to Keith Brown-Grant, his immediate question was something at that time largely disregarded by neuroanatomists: "What was the sex of the animals?" Going back to the records, we discovered that indeed the animals with the fewest specific spine synapses were all males. This was the first demonstration of sexual dimorphism in numbers of synapses in the brain (Raisman & Field 1971). The possible relationship to the ovulatory mechanism was further supported by Velasco & Taleisnik's demonstration (1969) that stimulation of the amygdala induced ovulation and an increased pituitary LH secretion in female rats (but no increase in LH in castrated estrogen-primed male rats), and that these responses were blocked by lesions of the pathway leading from the amygdala to the preoptic area.

We therefore set up a developmental series of rats of both sexes. The females were treated with a single 1.25-mg injection of testosterone propionate (Barraclough 1961) either before or after the critical period for sexual differentiation. With Harris's direct instruction, and the gift of his own personal microsurgical instruments, the males were castrated either on the first day of life or after one week. The results were counted "blind." They showed that the anatomical sexual dimorphism in the preoptic area was differentiated in exactly the same way as Pfeiffer and Harris had described for the ovulatory mechanism, i.e. it depended not on the genetic sex of the animal but upon the presence or absence of gonadal steroid hormones during the critical period of the first week of postnatal life in the rat (Raisman & Field 1973).

Measurement of Luteinizing Hormone-Releasing Factor in Portal Blood

But now the wheel of Harris's life was coming full circle. In the introduction to his 1955 monograph, Harris had written: "The ultimate test by which the number of substances secreted by a given endocrine gland will be decided is the qualitative and quantitative analysis of the hormones in the venous blood from the gland." It was as though, two decades earlier, he had been given an intimation of the end point of his own life's work.

In collaboration with George Fink, a Nuffield Demonstrator from Monash University, Australia, Harris applied the method for collection of rat portal vessel blood described by Curtis Worthington (1966). Using the Parlow ovarian ascorbic acid depletion bioassay for LH, they were able to detect LH-releasing activity [luteinizing hormone-releasing factor (LRF), now called GnRH; see below] in samples (after inactivation of any LH in the sample). But they failed to detect the expected rise in LRF during the pre-ovulatory critical period of

pro-estrus (Fink & Harris 1970). With hindsight this was due to suppression of the pre-ovulatory surge by the anesthetic used. Electrical stimulation of the hypothalamus at a level sufficient to overcome Nembutal block of ovulation did cause a surge in LRF activity in the portal blood in all phases of the rat cycle except estrus (Harris & Ruf 1970, Fink & Sheward 1989).

George Fink persevered in improving the technique of portal vessel blood collection (Fink & Sheward 1989) and finally discovered a method of steroid-based anesthesia that showed unequivocally that the level of immunoassayable LRF increases abruptly immediately before the pre-ovulatory surge of anterior pituitary LH on the pro-estrous day of the rat cycle (Sarkar et al 1976). Harris's final criterion was thus fulfilled: The time of appearance of the active agent in the venous effluent from the hypothalamic gland was correlated with, and necessary for, its proposed physiological function.

The Race for the Identification of the Ovulatory Signal

But Harris had set himself yet another goal that, had he been successful, would have given him the molecular nature of the ovulatory signal released from the hypothalamus. As it was, other laboratories, building on the knowledge Harris had uncovered, but with greater resources and greater molecular expertise, were already moving ahead of him.

Around the world now, the search for the hypothalamic hormones was accelerating, and other groups were using techniques less physiological but far more rapid than those of Harris's induction of ovulation in rats or rabbits. Some years earlier, two papers (Guillemin & Rosenberg 1955, Saffran et al 1955) had reported tissue coculture assays that showed that hypothalamic tissue could induce the release of ACTH from pituitary tissue. In 1969, the groups of Schally and Guillemin, both of whom had previously been involved in a long and as yet unsuccessful search for the identity of the ACTH-releasing factor (CRF), independently worked out that the TSH-releasing factor (TRF) in hypothalamic extracts was the tripeptide pyro-glutamine-histidine-proline amide (Burgus et al 1969, Folkers et al 1969).

In the quest for LRF, Harris's main competitors were the groups of Don McCann in Texas (Ramirez et al 1964), Andrew Schally in New Orleans, and Roger Guillemin at the Salk Institute in San Diego. In 1964, Schally & Bowers, using both in vivo and in vitro assays, proposed a tentative 11-amino acid residue composition for bovine LRF peptide (Schally & Bowers, 1964), and the next year Guillemin proposed 9-amino acid residues for ovine LRF (Guillemin 1977a).

Like Dora Jacobsohn, Marthe Vogt was a refugee from Nazi Germany and a great admirer of Harris, both as a scientist and a hater of injustice. In the Royal Society Biographical Memoir of Geoffrey Harris, she wrote:

The race for the isolation of the luteinizing hormone releasing factor, LRF, had started in the USA with such a drive and with such vast financial resources that, seen retrogradely, there was little chance for Harris of being the first to arrive. Harris was too careful a worker to win a battle in which speed was decisive.

Compared with the highly directed research teams of Schally and Guillemin, with their major support from the US National Institutes of Health, Harris's style of associating with collaborators with independent scientific programs, and the terms of his arrangement with the UK Medical Research Council, did not provide him with the same scale of resources to assign to the purchase and large-scale purification of median eminence extracts. The meticulous surgical precision that had been such a strength of Harris's electrical stimulation work was a disadvantage when he attempted to use the all-or-none rabbit ovulatory response to intrapituitary infusion as an assay in the purification of the hypothalamic ovulatory hormone (Campbell et al 1964). His competitors were able to process material much faster using the quantitative Parlow ovarian ascorbic acid depletion test (Parlow 1958) and, subsequently, radioimmunoassay (Niswender et al 1968, Harris et al 1970, Harris 1972).

For the chemistry, Harris had the assistance of Harry Gregory, a distinguished ICI scientist who had previously solved the structure of gastrin but who was only permitted to spend part of his effort in the purification of LRF. By 1968, in collaboration with May Reed and Harry Charlton, they used 50,000 hypothalami to obtain a purified preparation of a putative LRF polypeptide, with a molecular weight of around 1000 to 1500, indicating between 9 and 13 amino acids (Gregory et al 1968). Harris was in sight of the goal.

In 1971, Naftolin, Harris, and Bobrow used the purest preparation of this putative LRF diagnostically to show that a pituitary LH response could be elicited in two human patients with Kallmann's syndrome (familial hypogonadism with anosmia) after priming the pituitary with an estrogen agonist (Naftolin et al 1971). This finding indicated that the pituitary was competent to respond to appropriate stimuli and therefore that the defect in the disease lay in the CNS. [It is now known to be due to failure of migration of the GnRH neurons (Schwanzel-Fukuda et al 1989).] "This work," Naftolin commented, "shows the physician in Harris." It foreshadowed the future diagnostic use of GnRH (see below).

But by 1971, the competition was over. In that year, Schally's group used the extracts of over a million pig hypothalami to purify a decapeptide with LRF activity, determined its amino acid sequence to be pyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂ (Matsuo et al 1971b), synthesized a molecule with the predicted structure, and demonstrated that it had biological activity indistinguishable from the natural factor and was effective in inducing ovulation at a threshold of 0.5 ng in rats (Matsuo et al 1971a). Their extracts induced a

rapid rise in LH in human pre- and post-menopausal females and males (Kastin et al 1970). They showed that the same hypothalamic molecule controlled the anterior pituitary secretion of both LH- and follicle-stimulating hormone (Schally et al 1971), and they renamed it gonadotrophin hormone-releasing hormone (GnRH), making it, as Wade (1981) said, “the hormone that governed the breeding behavior of pigs, and probably of man and all other mammals as well.”

In the same year, Schally’s group reported a tripeptide sequence for melanocyte-inhibiting factor (which regulates the secretion of the pars intermedia of the pituitary) (Nair et al 1971), and in 1973, Guillemin’s team published the structure of somatostatin, the hypothalamic hormone that regulates pituitary growth hormone secretion (Brazeau et al 1973).

At last the existence of Harris’s long-postulated hypophysiotrophic hormones was being proved, but all that now becomes part of another story. Harris’s is over.

In his 1971 Henry Dale Lecture, it was typical of Harris that he should have written: “The studies...especially of Schally and his co-workers in New Orleans, will undoubtedly stand as a milestone in the history of endocrinology.”

The lecture was published the year after Harris’s death (Harris 1972). For the elucidation of the nature of the chemical messages from the hypothalamus to the pituitary, Schally and Guillemin shared the Nobel Prize of 1977 with Rosalyn Yalow, who received it for her part in developing the technique of radioimmunoassay (see Fink 1977, Meites 1977).

Conclusion

Looking back over the 25 years since his death, the work of Geoffrey Harris towers over the surrounding landscape like a solitary mountain peak, standing clear of all around. In the First Geoffrey Harris Memorial Lecture of the International Society for Neuroendocrinology (Sawyer 1975), Charles Sawyer wrote,

Harris was not the first to suggest that the adenohypophysis might be controlled by a humoral mechanism involving the hypophysial portal system, but it was the force of his intellect, personality and multifaceted research approach to the problem in the late 1940’s and early ’50’s which really established the neurovascular concept.

Harris’s professional life started in the Cambridge Physiology Department, where the Nobel Prize winners Hodgkin, Huxley, and Adrian were heroes of the study of electrical transmission in the nervous system. Against the background of their achievements, Harris had to fight hard to establish the credibility of a control mechanism that required humoral transfer of neural information. Not only in Cambridge, but throughout the world at that time, the chemical theory

of neurotransmission was still hotly debated for the peripheral nervous system, let alone for the CNS (e.g. Feldberg 1945).

Harris's contemporaries were comfortable with the concept of the brain as the organ of thought, perception, memory, decision, and movement, but less so with the idea that the brain is also a gland. For the 14 years between his exchange with Zuckerman in 1955 and the demonstration of TRF in 1969, Harris defended his theory of the existence of specific hypothalamo-hypophysial hormones when no such hormone had yet been identified. "What Guillemin and Schally did," Wade wrote in *The Nobel Duel* (1981), "was to look for the entities Harris described in the places where he said they would be found."

Following Marshall's lead, Harris's work had consistently moved the endocrine control mechanism "upwards" from the early days of what he used disparagingly to call "pelvic endocrinology." His work also effectively disposed of the idea of the pituitary as the "conductor of the endocrine orchestra" by showing that the control mechanism actually lay in the brain itself. This was perhaps most unexpected for the demonstration of sexual differentiation.

Harris was the founder of the science of neuroendocrinology. As Yasumasu Arai put it: "Harris put the brain into the endocrine system." He also put the endocrine system into the brain. Subsequent research has shown that neuropeptides of major functional importance are present in all parts of the central and peripheral nervous system (Guillemin 1977b), far beyond the hypothalamus. Hypothalamic peptides such as LRF (Moss & McCann 1973) and CRF (Vaughan et al 1995) have major effects on brain as well as on pituitary tissue.

Harris's work also led to practical advances. GnRH came to be used for diagnostic purposes and therapeutically, not only in its primary, pulsatile role as a means to increase fertility (Mason et al 1984), but more often as a chronic pituitary suppressant (chemical hypophysectomy) in conditions such as prostatic carcinoma, precocious puberty, and endometriosis (Belchetz et al 1978, Crowley et al 1981, Walker et al 1983). But to pick out subsequent developments in the use of individual releasing factors would be to obscure Harris's major intellectual contribution to our present way of thinking, rather as if one were to ask of Darwin's theory: To what specific practical developments has the concept of evolution led?

Extending over nearly half a century, Harris's work forms a bridge from an earlier era. The time seems remote when Marshall could write: "Lord Latymer states that deer have been imported into New Zealand from many sources besides Scotland...but that now, wherever they come from, they all begin roaring about the third week in March" (Marshall 1936).

On the wall of the professorial study in Oxford, hung a framed black and white photograph of a nineteenth century gentleman in a suit of a continental cut, white shirt, and a cravat. Sometimes Harris would point to the photograph

and ask: “Do you know who that is?” From his seated position, the respectful visitor would be unable to read the signature crossing the lower corner of the portrait and would need to be told that it was Claude Bernard, the eminent French physiologist, famous for his postulate: “The constancy of the internal milieu is the condition of free life.” The ability to colonize widely different and changing external environments requires that the organism, like a high altitude airplane, be provided with a series of fail-safe control mechanisms to shield its internal environment from changes outside. Claude Bernard’s concept of homeostasis was central to the science of endocrinology, in which the internal secretions are regulated by feedback from circulating hormones. Harris set this in a wider context. He identified the hypothalamo-hypophysial system as the final common pathway for the integrated brain-endocrine response to environmental inputs (Figure 4).

The concept of the hypothalamo-hypophysial system has implications far beyond the laboratory. In the pigeon, the sight of another bird or even the reflected image in a mirror is sufficient to induce ovulation (Matthews 1939). The secretion of LRF into the portal vessels is the mechanism by which seasonal changes in day length in ferrets, diurnal lighting rhythms in rats, overcrowding or lack of food in mice, mating in rabbits, or the presence of a mate or eggs in birds regulate the balance between the positive forces of procreation and the negative, or protective, forces of aggression. During the life cycle of each individual, the hypothalamo-hypophysial axis is the route through which the developing brain signals the onset of the reproductive period (puberty). In the adult, it carries the messages by which trauma, stress, or disturbance of biorhythms affect the reproductive cycle (as in the human menstrual cycle). Through it the aging brain signals the cessation of the reproductive period (menopause). The brain-pituitary axis offers a potential route for planned human population control by interventions to reduce or to increase fertility. It will be a key player in the human struggle to adapt to the ever-increasing world shortage of food and space.

Our understanding of these vital neural mechanisms that are so important for our future is still in its infancy. The events Harris described were cyclic—the cycles of birth and growth, reproduction and death, and the passing of the generations. The combined cycles of individuals merge into those of populations and interact with the external cycles of the days and months, seasons and years.

Great research not only answers questions of its generation, but raises those of the next. One of the great mysteries is how the neural mechanism achieves its coordination of these internal and external cycles, so essential to the survival of the species. At a very basic level, how are signals from the circadian clock in the suprachiasmatic nucleus transmitted to the ovulatory center? How does the hypothalamus of a rat remember it has mated, and maintain ten days of

pseudopregnancy? Harris himself started the search for evidence as to whether the human male brain can maintain a monthly cycle of gonadotrophin release comparable to the female. Buried in our brains, and conserved through millennia of evolution, how far do these hidden biorhythms, beyond our conscious control, govern our lives?

Harris was an extremely determined scientist. He had a robust, outgoing character that could inspire colleagues. The list of the names of his students and coworkers forms a roll call of those who led and were to lead the field of neuroendocrinology. His writing was clear and easy to understand. His conclusions were always careful, at times cautious, and he was always aware of the human dimension of his research, the clinical aspects of neuroendocrine malfunctions, and the potential applications of his findings to them.

Harris's scientific life began with a question—how to explain the seasonal effects described by Marshall. Forty-five years later, his final message was also a question: “What benefits will this work and knowledge confer on human welfare?”

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