THE REGULATION OF MAMMALIAN REPRODUCTION

Edited by

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New York, New York

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WITH 132 EXPERT CONTRIBUTORS

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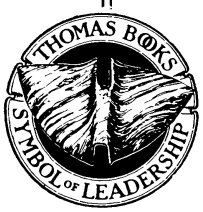

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Preface

The Center for Population Research was established within the National Institute of Child Health and Human Development to foster biomedical and social science research which may contribute to the solution of population problems. One aspect of this mission is the stimulation and support of research in reproductive physiology to provide the scientific basis for development of an array of new methods of fertility regulation. The biomedical programs of the Center include fundamental research in human and animal reproduction; development of new devices, techniques, and drug-delivery systems; screening and testing of potential antifertility drugs; and studies of the effects of contraceptives presently in use.

Attention is currently focused on the need for new methods of fertility control to help attain the Federal Government's goal that all couples everywhere may have control over their fertility. Present technology is improved over that generally available a decade ago, but no existing technique fits all the criteria of safety, efficacy, low cost, and acceptability. The development of such methods must await the gathering of a great deal more information concerning reproductive processes in humans and experimental animals.

Fundamental research is also needed as the basis for solution of problems as yet undefined. For instance, decisions or nondecisions for conception rely on a multitude of subtle and complex psychological and social factors, and we may find a decade from now that our specifications for the ideal contraceptive will radically change and the new methods which we have developed will not fulfill changing criteria. To be responsive to future needs, we will need more fundamental knowledge of reproduction.

When the John E. Fogarty International Center was established at the National Institutes of Health for the promotion of international cooperation in biomedical research, the Center for Population Research was asked to assist in the organization and sponsorship of a conference on population research. Since a comprehensive assessment of current knowledge in the field of reproductive biology had not been conducted since the Conference on Physiological Mechanisms Concerned with Conception, held at West Point, New York, in 1959, scientific interest in an NIH conference was high.

Center staff worked with Dr. Peter Condliffe and his colleagues of the Fogarty International Center to plan and conduct the conference. We were fortunate to enlist Sheldon Segal, Director of the Biomedical Division of the Population Council, to serve as Conference Chairman and Irving

Geschwind, Mario Burgos, M. C. Chang, Anne McLaren, Richard Blandau, and Eugenia Rosemberg as chairmen of the six individual sessions.

Governments of many nations and a number of international organizations have expressed concern over population problems, and the NIH conference, held September 27 to October 1, 1970, brought together experts in the field of mammalian reproduction from 23 countries throughout the world. The keynote address was delivered by Dr. M. G. Candau, Director-General of the World Health Organization, an agency currently engaged in expanding its support for research and training in the population field in many countries. The keynote address was introduced by Dr. Roger Egeberg, Assistant Secretary for Health and Scientific Affairs of the United States Department of Health, Education, and Welfare, representing commitment by the United States to this important field.

The conference reviewed a wide range of current studies in the physiological regulation of mammalian reproduction. It is hoped that the conference—by fostering the interchange of ideas among scientists in quite different areas and the publication of the papers and discussions in this book—will contribute to the advancement of knowledge in reproductive physiology and will facilitate development of new approaches to the regulation of human fertility.

PHILIP A. CORFMAN

Keynote Address

M.G. Candau

It is an honor and a pleasure for me to participate in this Conference on the Regulation of Mammalian Reproduction. The conference represents an important contribution of the Fogarty International Center and the Center for Population Research to the interchange and stimulation of ideas and developments which are of such vital concern to all of us throughout the world.

The organizers are to be congratulated for their selection of subjects for the program in which recent developments offer promise for significant advances in knowledge of mammalian and particularly human reproduction. The presence of internationally recognized authorities as speakers and discussion leaders is a guarantee that the meeting will bring forth new facts, contrasting viewpoints, and perhaps significant conclusions.

The program of the conference is a challenging one, both in terms of the individual subjects to be discussed and, even more so, because of the framework within which they are to be developed. To review at one conference and at one time the several structures and processes involved in reproduction should throw further light on the various general interrelationships and on the specific relevance of individual processes to one another. The simultaneous assessment of the state of current knowledge will reveal, as it always does, gaps in our understanding of reproduction and its regulation and will thus provide the basis and impetus for further research.

One of the things that has impressed me about research in reproduction in recent years is the tremendous progress that has been made, on the one hand, and the vast areas of ignorance that still remain, on the other. This point has been made repeatedly in the reports of the groups of experts convened by the World Health Organization who have summarized the current state of knowledge and made recommendations on research needs in the various aspects of reproduction. This juxtaposition of increased understanding and residual ignorance appears to hold true at all levels of knowledge—the fundamental, the clinical, the epidemiological, and the administrative. It is clear that the promotion of reproductive health, the provision of adequate means to regulate fertility, and the alleviation of reproductive disease require advances at each of these levels.

The primary focus of this conference is on reproductive physiology and the regulatory processes involved. Here again, I have been impressed by

the recurrent recommendations of WHO's meetings of experts as to the urgent need for intensifying fundamental research. Their reports repeatedly emphasize that a better understanding of the normal physiological mechanisms involved in the regulation of reproductive processes constitutes an essential prerequisite for the development of rational methods of therapy—whether they are directed to the control of fertility, the treatment of infertility, the improvement of the intrauterine milieu, or the optimal development of the fetus.

Fundamental research can be developed within a program aimed at the specific application of old or new knowledge, but it should allow for considerable flexibility, since it is often difficult to assess immediately the future or ultimate significance of one or a series of findings. What appears to be farfetched today may turn out to be completely down to earth and realistic tomorrow. There is little doubt, for example, of the great need to increase our knowledge of the reproductive biology of many more mammalian species so as to maintain and strengthen the support it can give to the study of human reproduction. And this applies to the physiological and biochemical processes as much as to the morphological aspects of comparative mammalian reproduction. The lack of suitable animal models constitutes a serious obstacle to developments in this field, including research into fertility-regulating agents.

The prospects for progress in reproduction research depend, to a large extent, on the appropriate formulation of research problems—one of the main objectives of this conference. It is often undoubtedly true that "the difficulty in most scientific work lies in framing the questions, rather than in finding the answers." Sometimes a ready-made problem presents itself, and such an opportunity should not be neglected. But in a field like mammalian reproduction, there is scope for creative and inventive thinking which avoids the familiar beaten paths. And, on the other hand, there is scope for a review of old investigations which were set aside and never brought to a conclusion. With the lapse of time and the availability of new techniques, new knowledge and new minds, the solution may present itself unforced.

In addition to these suggested activities, there is need for an increase in both the range and the intensity of research and inquiry over the whole field. The acceptance of these challenging opportunities will depend to a large extent on the recognition society gives to the importance of the health and social issues that human reproduction and its regulation pose.

Above all, I need hardly add, new discoveries are to a large extent a measure of the inspiration, perseverance, and dedication of the research scientists. But I am sure that much more can be done to further your purely scientific efforts by easing the day-by-day machinery of research in a number of ways.

Considerable investments of time and thought are required in the imaginative approach to the administration of research, in the coordina-

tion of research activities and in the arrangements for funding. Compared with other areas of biomedical investigation, research in reproduction receives a relatively small share of the total expenditure on research. Increased support of all kinds is required everywhere but especially in developing countries. This applies to facilities, to equipment, and to long-term support for research programs, and may also involve a reconsideration of the career pattern and an extension of career opportunities for senior and junior scientists and for technicians.

Existing research groups and institutions which have mammalian and, in particular, human reproduction as their primary interest should be strengthened and developed. At the same time it will be necessary to establish a variety of additional research groups in universities, in medical schools, in other research institutions, and in industry.

Many research problems require the attention of groups of multidisciplinary composition and appropriate size. This, in turn, postulates both the active recruitment of scientists from other disciplines besides those traditionally involved in reproduction research and the availability of sufficient funds. It may be expected that the multidisciplinary approach will bring fresh insight to bear and stimulate new ideas. In time, it should attract additional scientific manpower of high calibre. There remain the two important questions of interinstitutional research programs, both within countries and on an international basis, and of the exchange of personnel. The administrative arrangements which govern both these forms of collaboration need to be developed and extended.

One of the greatest limiting factors in reproduction research, as in many other areas of biomedical research, is the shortage of trained manpower. The number of able and experienced scientists engaged in this field is disproportionately small in comparison with the importance of its problems. Moreover, the geographical distribution of these scientists shows widespread inequalities and deficiencies in many parts of the world where the problems are most urgent.

The reasons for this lack of trained manpower are undoubtedly multiple, but I am sure you will excuse me if I do not discuss them here. On the other hand, the present intensified interest in the field of reproduction, including its control, dates back no more than a couple of decades, and the positive response to the still-limited opportunities for research training in reproduction suggests that a potential reservoir of talent does exist and has only to be tapped.

The Member States of the World Health Organization, from its beginning, have recognized the importance and indeed the necessity for the Organization to concern itself with research and training for research. This derived from the realization that progress in medicine and in public health depends very largely on the use that is made of research, of its operational processes and findings.

The promotion of research and research training were, in fact, among

the initial activities of the Organization when it concentrated its attention on the problems of human reproduction. Research still remains a very important and key component of both the reproduction and the total programs of the Organization, while advisory services, technical assistance, and the evaluation and training of health professionals add increasingly to our responsibilities in every field.

WHO has developed a variety of procedures for stimulating, coordinating, and supporting research in reproduction. The basis for the research program was established in 1963 and has been kept under continuous review in a series of meetings of scientific groups which have covered almost all aspects of human reproduction. The reports of the majority of these study groups have been published in the Organization's Technical Report Series and are no doubt familiar to you.

Research contracts and grants are awarded for studies and projects in physiological, chemical, epidemiological, and operations research. Research training grants have been made available to both senior and junior scientists. Some research training courses have been organized and assistance given to others. A network of national, regional, and international reference laboratories is being created with a view to establishing and maintaining standards of nomenclature, methodology, and reagents. Through the medium of this network, it will be possible to promote collaborative studies, to train research workers, and to cooperate in the monitoring and surveillance of the side effects of fertility-regulating agents.

We are attempting to assess systematically current and projected needs and resources for research throughout the whole field of the sciences concerned with reproduction. On the basis of the results of these wide-ranging activities, we should be able to formulate a strategy for the further development of research.

Scientific work and thought are, in essence, fluid and progressive, but advances in a given field tend to be related to a special constellation of factors. The pressures which certain problems exert on society may help to obtain and focus the necessary resources for their solution. Similarly, the availability of particular research techniques may permit rapid advances to be made. Research in reproduction requires inputs from both these sources at the present time. The challenging program of this conference and your inevitable response to it should serve as an important catalyst toward both these objectives.

Contents

THE REGULATION OF MAMMALIAN REPRODUCTION

SECTION 1

REGULATION OF PITUITARY FUNCTION

Chairman: Irving I. Geschwind

1

Interactions of Steroid Sex Hormones with Brain Tissue: Studies of Uptake and Physiological Effects

DONALD W. PFAFF

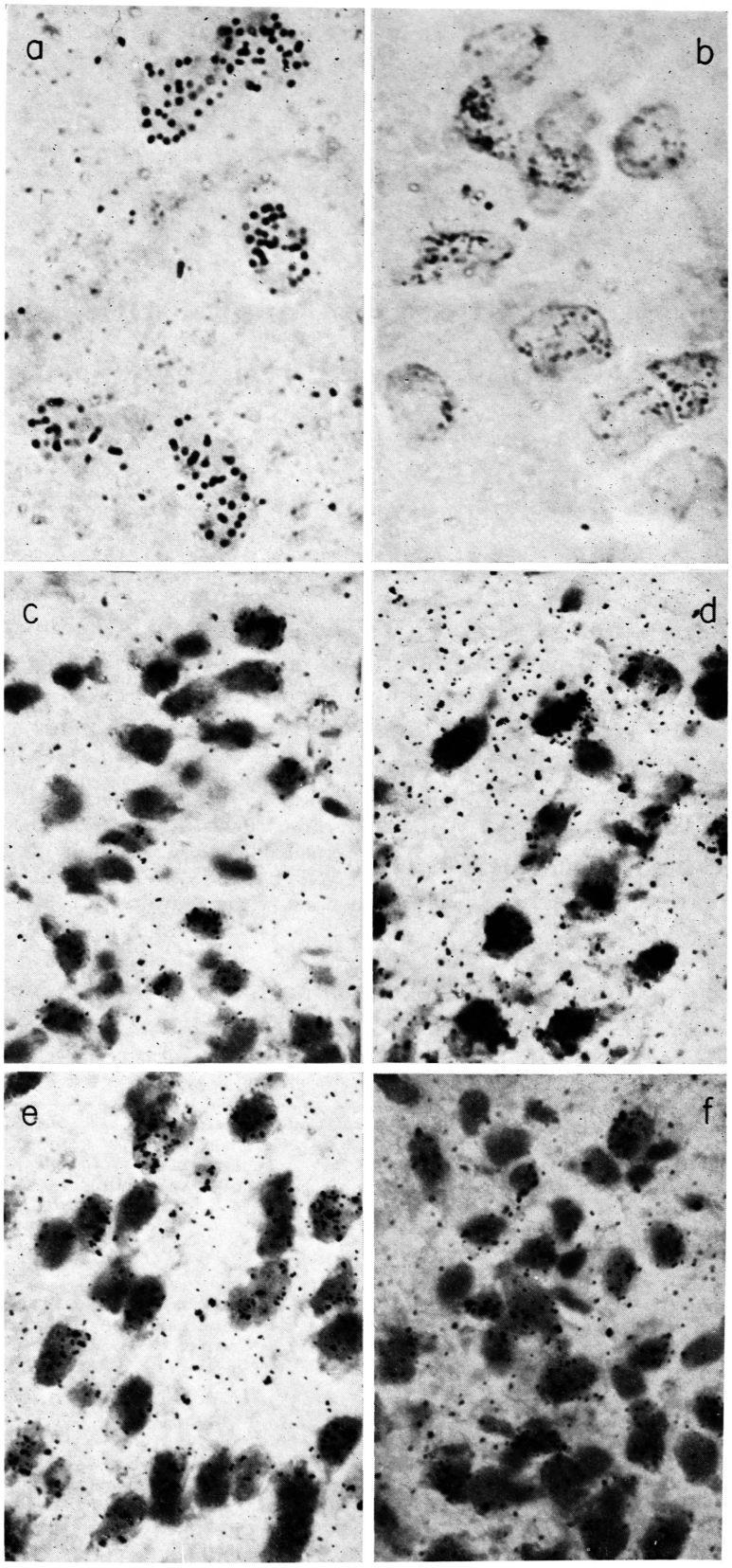
INTRODUCTION

Two functional results of sex steroid effects on brain tissue have been well established: alteration of pituitary gonadotropin release and facilitation of mating behavior. This chapter is not an attempt to review exhaustively the work on these topics, since several excellent reviews have appeared recently and are referred to in the appropriate sections below. Rather, I treat a few points regarding pituitary control and mating behavior, organized according to a simple scheme for the study of hormone effects on brain (Table 1–1).

Note: Preparation of this manuscript and the work reported were supported by NSF grant GB4198X, NIH grant NS-08902–01, and the Biomedical Division of the Population Council.

TABLE 1–1
STEPS IN THE PHYSIOLOGICAL ANALYSIS OF HORMONE EFFECTS ON BRAIN FUNCTION: ANALOGY TO HORMONE EFFECTS ON THE UTERUS

		Brain	
	Uterus	*Pituitary Control*	*Control of Mating Behavior*
1. Identify the hormone(s) involved.	Estradiol	Estradiol, testosterone progesterone	Estradiol, testosterone, progesterone
2. Determine if and where the hormone is taken up in the affected tissue.	Demonstrations of nuclear and cytoplasmic receptors (Jensen *et al.*, 1968, 1969; Shyamala and Gorski, 1969; King and Gordon, 1967; Talwar *et al.*, 1964).	Demonstrations of hormone concentration by brain cell bodies and cell nuclei. (see text)	
3. Search for and characterize physiological effects of the hormone in the target tissue.	Effects on RNA synthesis, protein synthesis, enzymatic activity (Hamilton 1968; Villee *et al.*, 1960; Hechter and Halkerston, 1965;Gorski *et al.*, 1965; Segal *et al.*, 1965).	Demonstrations of sex hormone effects on neuronal electrophysiological activity and on brain metabolic and chemical measures. (see text)	
4. Determine how detailed physiological effects are related to the overall functions of the hormone in the target tissue.	Relation of increased protein synthesis to estradiol-stimulated uterine growth.	So far, further characterization of sex steroid effects on pituitary function that are to be explained at a physiological level.	So far, further characterization of the sex hormone effects on mating behavior that are to be explained at a physiological level.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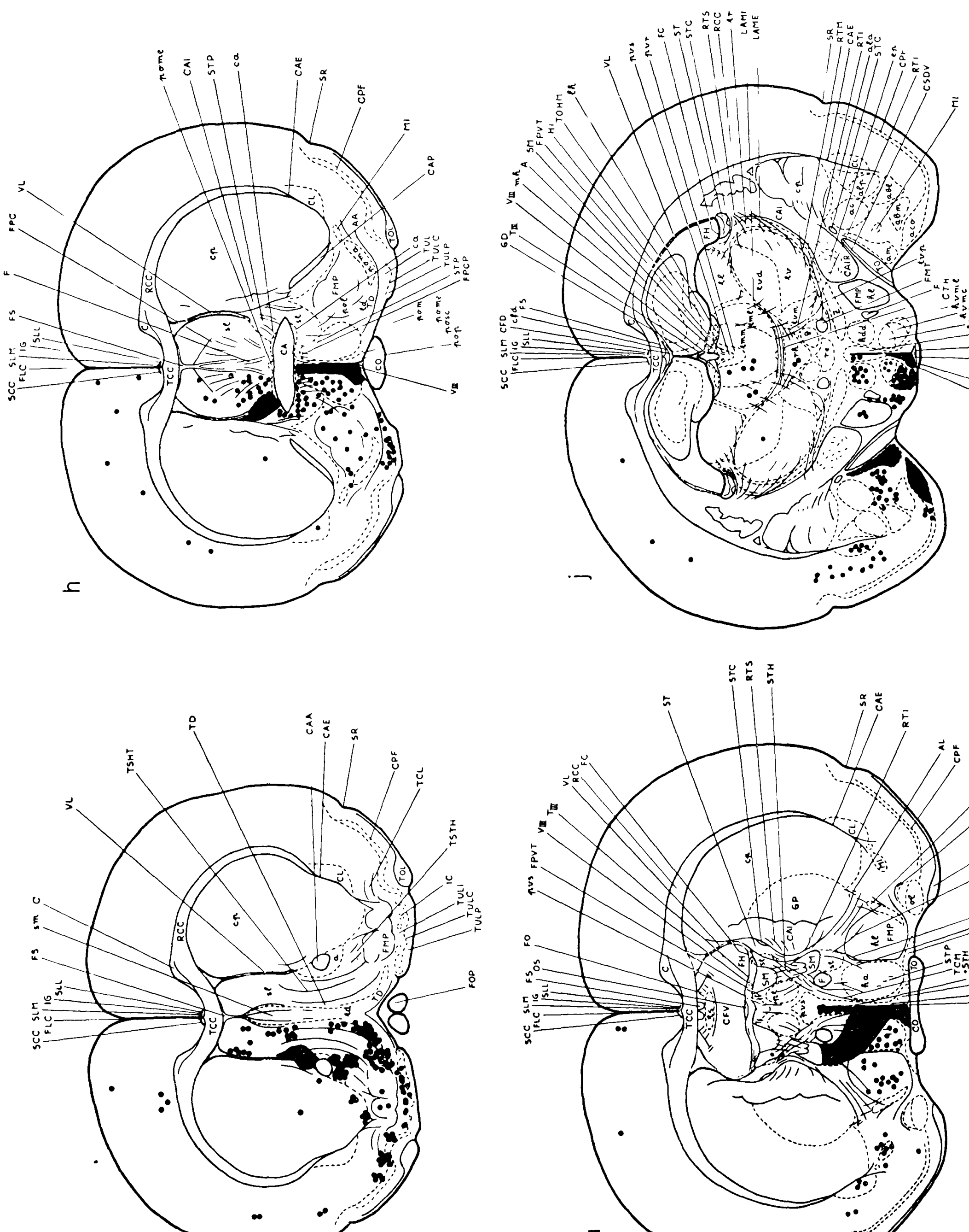
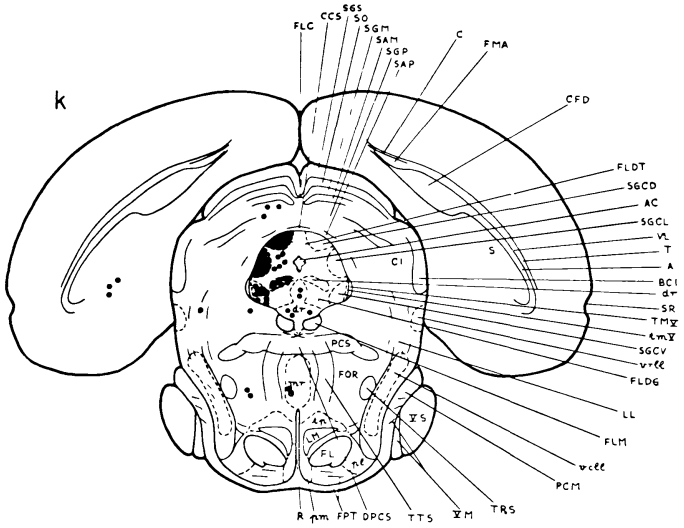
UPTAKE STUDIES

Radioactive estradiol-17β is highly concentrated in the ventromedial hypothalamus, preoptic area, amygdala, and septum, and is less highly concentrated in other brain regions, such as the cerebral cortex. This basic regional distribution has been determined both from scintillation counting of dissected brain regions (Eisenfeld and Axelrod, 1965; Green *et al.*, 1969; Kato and Villee, 1967a; McEwen and Pfaff, 1970; McGuire and Lisk, 1968; Michael, 1965; Michael and Glascock, 1961) and from autoradiographic description of estrogen concentration following systemic injections (Michael, 1965; Pfaff, 1968a). A high percent of the radioactivity retained in the brain is still in the form of estradiol (Kato and Villee, 1967a; McEwen and Pfaff, 1970). In all of these experiments, areas outside the septal-preoptic-hypothalamic axis showed quantitatively less uptake but were not devoid of radioactivity.

In our laboratory, two different autoradiographic procedures have yielded descriptions of estradiol-^{3}H retention which agree with each other and with scintillation-counting results. The first, using combined osmium tetroxide-formalin fixation of frozen brain sections (Pfaff, 1968a), showed estradiol concentrations in a limbic-hypothalamic system which includes the septum, amygdala, hippocampus, medial preoptic area, and medial hypothalamus (Fig. 1–1a). The second method, modified from the Anderson and Greenwald (1969) approach, avoids the fixation step by mounting unfixed brain sections directly from the cryostat knife onto emulsion-coated slides. This method gives results which agree with the first approach (Fig. 1–1c-k) and with the ranking of estradiol-concentrating structures derived from scintillation counting of dissected brain regions (McEwen and Pfaff, 1970). Three factors have been crucial for the successful autoradiographic description of estradiol-concentrating neurons in brain: (a) keeping the radiochemical in place during histological treatment by choosing a successful fixation procedure or by avoiding fixatives and other liquids, (b) cutting cross-sections of the entire brain throughout its anterior-posterior extent to achieve adequate sampling of all brain regions and accurate identification of anatomical sub-

≪ Figure 1–1*a, b.* Autoradiographs showing estradiol and testosterone uptake in rat brain, prepared from frozen sections fixed with combined osmium tetroxide-formalin method (Pfaff, 1968a, 1968b). Lightly stained with cresyl violet. *a,* Five labeled cells in preoptic area of ovariectomized female rat, two hours after intravenous injection of estradiol-^{3}H. Brain regions outside limbic and hypothalamic areas showed fewer average grains per cell. *b,* Several labeled cells in preoptic area of castrated male rat two hours after intravenous injection of testosterone-^{3}H. Preoptic area and surrounding region showed higher uptake than most other brain areas. *c, d, e, f.* Autoradiographs showing estradiol uptake in female rat brain, prepared from unfixed frozen sections mounted directly onto emulsion-coated slides (Pfaff, Keiner, and Warren, 1971, unpublished observations). Ovariectomized females were injected with physiological dose of ^{3}H-estradiol I.P. two hours before sacrifice. Cell bodies stained with cresyl violet. Labeled cells shown in c, the arcuate nucleus of the hypothalamus; d, ventromedial nucleus of the hypothalamus; e, medial amygdala; and f, medial preoptic area.





≪ Figure 1–1*g*, *h*, *i*, *j*, *k*. Maps from autoradiograms prepared as in *c-f*, showing estradiol-^{3}H uptake in brain of an ovariectomized female rat (Pfaff, Keiner and Warren, 1971, unpublished observations.) The five sections include regions of highest estradiol uptake and are superimposed on the rat brain atlas of König and Klippel (1963). Each dot shows the position of an estradiol-concentrating neuron. Where many dots would overlap, that area is filled in with solid black.

divisions; limitations at this step led to the temporary claim (Stumpf, 1968) that estradiol uptake was limited exclusively to the medial preoptic region and ventromedial hypothalamic region, and (c) allowing long exposure times of six months or more, achieving high enough autoradiographic sensitivity to see the complete distribution of estradiol-concentrating neurons.

Radioactive testosterone retention in the rat brain has been described using autoradiographic (Fig. 1–1b) (Pfaff, 1968b) and scintillation counting (McEwen *et al.*, 1970a, b) methods. Testosterone-^{3}H tended to be retained by cells in the same regions as estradiol-^{3}H, but from the scintillation counting results, testosterone appeared not to be concentrated from the blood to as great an extent as was estradiol.

Competition studies using preinjections of unlabeled estrogens have demonstrated limited-capacity retention in the preoptic area and hypothalamus (Eisenfeld and Axelrod, 1965, 1966; Kato and Villee, 1967b; McEwen and Pfaff, 1970; McGuire and Lisk, 1968). Nuclear isolation procedures (Zigmond and McEwen, 1970) and autoradiographic aproaches (Anderson and Greenwald, 1969; Stumpf, 1968; Warembourg, 1970) have demonstrated estradiol concentration by cell nuclei in these brain regions. In general, results with these procedures have magnified the difference between brain regions of relatively high and relatively low estradiol-uptake levels. However, even in these studies, it has not been possible to dismiss possibly significant estradiol retention outside the septal-preoptic-hypothalamic axis. For example, some competition effects of unlabeled estradiol were observed in the amygdala, hippocampus, and cerebellum (McEwen and Pfaff, 1970). Autoradiographic results from counting re-

duced grains over cell bodies agree with this finding in showing estradiol retention in limbic structures such as the amygdala and hippocampus similar to that in the hypothalamus (Pfaff, 1968a). Finally, isolated cell nuclei from amygdala and hippocampus contained four times or more the concentration of radioactive estradiol compared to whole homogenates from these regions (Zigmond and McEwen, 1970).

A question which arises is the meaning of the relatively low estradiol uptake in many structures outside the limbic-hypothalamic system described in Figure 1–1. Although estradiol action in the medial preoptic area and ventromedial hypothalamus provides the most convenient model for biochemical study of estrogen action in brain tissue (especially with reference to pituitary control), it is also necessary for neurophysiological study of brain function to have an accurate picture of estradiol uptake outside this region (especially for the study of mating-behavior control). Quantitative autoradiographic and scintillation counting results both show that there is not an *absolute* anatomical specificity of sex steroid hormone uptake for restricted cell groups in the medial preoptic area and hypothalamus but rather *quantitatively greater uptake* there than elsewhere in the brain. The quantitative difference between the relatively intense sex steroid uptake by some brain regions and relatively weak retention by other regions is not as great as the difference in estradiol retention, for instance, between uterus and skeletal muscle.

EFFECTS OF SEX STEROIDS IN BRAIN TISSUE

The electrophysiological and neurochemical effects of sex steroids reviewed below have been studied with the intention of understanding hormone effects on normal neural operations. However, no effect demonstrated to date includes proof that it is related to pituitary control or mating behavior control or both.

Electrophysiological

Effects of injected hormones on neuronal activity have been reported for several gonadal and adrenal steroids. This field of work has been pioneered by Sawyer and his colleagues (see e.g. Kawakami and Sawyer, 1959a, b), who described biphasic effects of progesterone on the electroencephalogram (EEG) arousal threshold (studied by electrical stimulation of the reticular formation) and the EEG after-reaction threshold (as can be induced by low-frequency stimulation in the basal forebrain). Kawakami *et al.* (1970a, b) have gone on to describe sudden increases in multi-unit activity in the medial basal hypothalamus on the afternoon of proestrus, which are susceptible to change by ovariectomy and estrogen or progesterone administration. Effects of injected progesterone on responses of individual hypothalamic neurons to peripheral stimuli were first described by Barraclough and Cross (1963). The existence of a progesterone effect has been replicated (Ramirez *et al.*, 1967; Komisaruk *et al.*, 1967; Beyer *et al.*, 1967; Lincoln, 1969b), but one salient question which has not been resolved for progesterone—nor has it been for other effects of steroids on neuronal activity—is the degree to which the hormone has *selective* effects on specific sex-related stimuli. A similar situation holds for estradiol ef-

fects, in which the existence of an effect is not disputed (Lincoln and Cross, 1967; Beyer, 1970), but the exact nature of the effect on responses to peripheral stimulation is not yet clear. What may be an estradiol effect (as opposed to an effect of more than one hormone) has been reported recently by Cross and Dyer (1970). On the day of proestrus, increased unit firing rates were observed primarily in the anterior hypothalamus, and to a smaller extent in the lateral hypothalamus, in a "hypothalamic island" preparation. In these experiments, the hormone effects must have been exerted directly on neurons within the "hypothalamic island," because connections to all other regions of the brain were removed.

Testosterone injected into castrated male rats influences the spontaneous activity and the responses to peripheral stimuli of individual neurons in the preoptic area, olfactory bulb, and mesencephalic reticular formation (Fig. 1–2) (Pfaff and Pfaffmann, 1969a). Direct testosterone administration to the preoptic area also influenced neurons there, increasing their responses to electrical stimulation of the olfactory bulb. Further experiments have been conducted comparing recordings in normal and castrated male rats to describe more fully the physiological nature of the androgenic influence. It appears that the *absolute* magnitude of a neuron's response to an individual odor is androgen-sensitive, but the *relative* magnitudes of a neuron's responses to different odors (measured by a "differential response analysis" (Pfaff and Pfaffmann, 1969b)) is *not* androgen-sensitive (Pfaff and Gregory, 1971a). Finally, the degree of correlation between levels of preoptic-hypothalamic neural activity and the state of the cortical EEG, observed in several laboratories during recording from urethane-anesthetized rats (Komisaruk *et al.*, 1967; Ramirez *et al.*, 1967; Lincoln 1969a, b; Pfaff and Pfaffmann, 1969a), is different between normal and castrated male rats: normals show a significantly higher proportion of cells which are related to the EEG state (Pfaff and Gregory, 1971b), and this increase is accounted for by units behaving more like reticular formation cells (Schlag and Balvin, 1963; Pfaff and Pfaffmann, 1969a), i.e. in this respect increasing firing rate during EEG activation.

A new technical development in this field is the use of an FM telemetry system to record single units in freely moving rats. This technique reduces encumbrance on the animal's movement, eliminates anesthesia and electrical artifacts from wire movement, and allows long-term experiments. The telemetry system has been used to show opposite effects of corticosterone and adrenocorticotropic hormone (ACTH) on hippocampal units in rats (Pfaff *et al.*, 1971a, b): corticosterone inhibited and ACTH excited unit activity in hypophysectomized female rats (cf. Sawyer *et al.*, 1968).

Neurochemical

A self-consistent set of effects of sex hormones on hypothalamic monoamine levels has been reported. Norepinephrine levels increase in the anterior hypothalamus after ovariectomy or castration (Stefano *et al.*, 1965), are decreased again following estrogen and progesterone treatment (Donoso and Stefano, 1967), and in the normal female rat, are minimum at estrus after a peak at proestrus (Stefano and Donoso, 1967). These changes in norepinephrine levels complement the results of Kobayashi and his colleagues from measurements of monamine oxidase

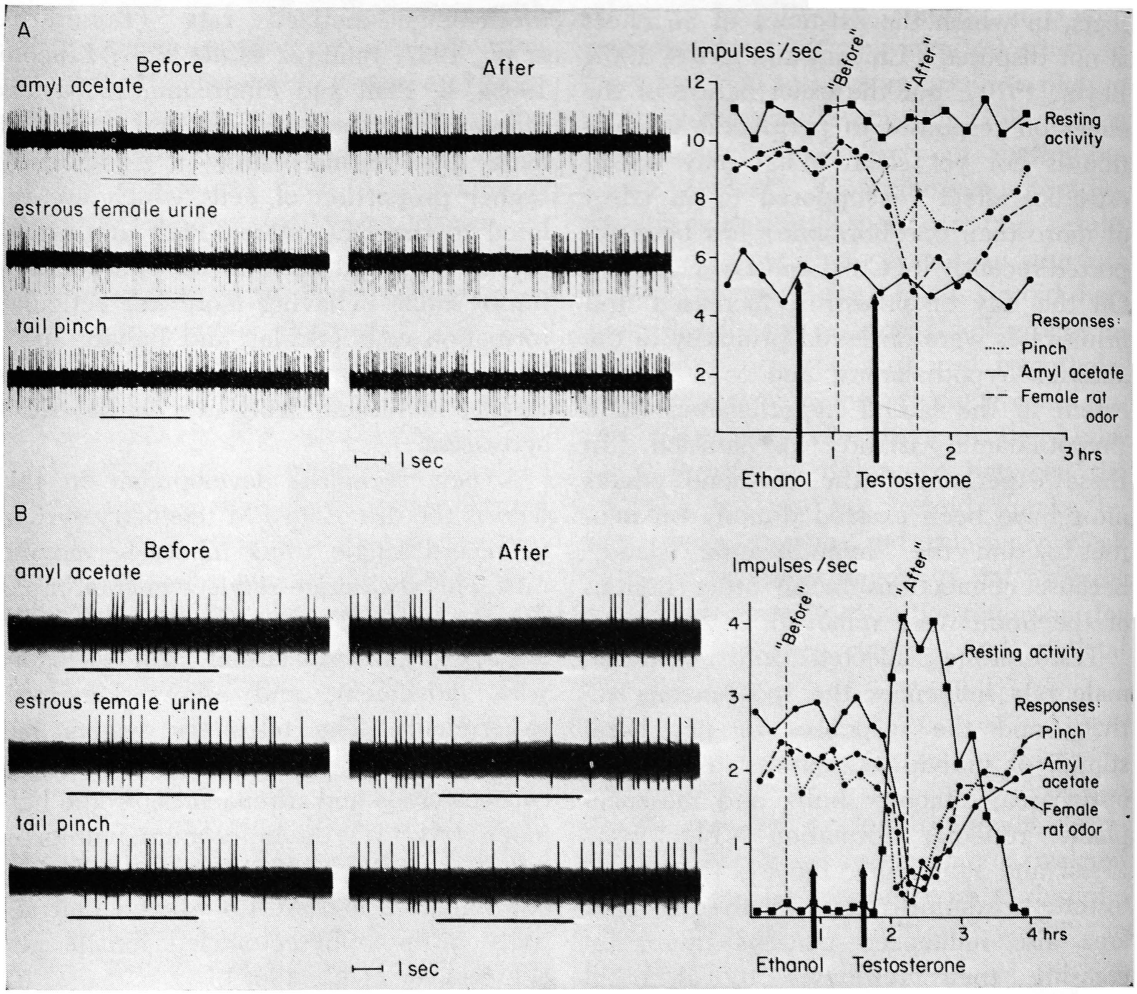


Figure 1–2 *A, B.* Effects of intraperitoneal testosterone injections on the electrical activity of units in the preoptic area. The records on the left were selected from times in the experiments shown in the graphs by dotted vertical lines. Testosterone and ethanol (control) injections are indicated by arrows. *A.* Inhibitory responses to estrous female urine odor and to painful pinches were increased after testosterone injection (i.e. fewer spikes per second during response), while resting activity and responses to amyl acetate odor were not affected. *B,* after testosterone injection, single-unit resting activity increased and responses to all three stimuli changed from excitations to inhibitions (Pfaff and Pfaffmann 1969a).

(MAO) levels (Kobayashi *et al.,* 1963, 1964a, b, 1966): they found that hypothalamic MAO activity increased after ovariectomy, decreased again after treatment with estrogen and was highest in proestrus. After ovariectomy, hypothalamic choline acetylase changes were the reciprocal of MAO changes (Kobayashi *et al.,* 1963). Zolovick *et al.* (1966) found MAO-level variations throughout the rat estrous cycle similar to those found by the Kobayashi group except for activity which remained high in estrus as well as in proestrus.

Concentrations of chemicals in brain do not give complete information about pos-

sible hormonal effects because changes in concentration could be due to changed synthesis or changed utilization (breakdown) or both. Indeed, changes in turnover rate can occur without any change in concentration. Anton-Tay and Wurtman (1968) found, in fact, that ovariectomy or castration increased the whole-brain turnover rates of norepinephrine, even though there were no large changes in norepinephrine concentration. A brilliant approach to these questions has been begun by Fuxe, Hokfelt, and their colleagues (Fuxe and Hokfelt, 1969a, b), using the histochemical fluorescence technique for localization of monoamines in brain tissue in conjunction with pretreatment with an inhibitor of amine synthesis. These investigators have reported increases in the utilization of dopamine in the median eminence after low doses of estrogen or testosterone in castrated rats and possible variations in median-eminence dopamine during the estrous cycle. They have also found changes in the tuberoinfundibular dopamine system during pregnancy and lactation (Fuxe and Hokfelt, 1967; Fuxe, Hokfelt, and Nilsson, 1967).

Moguilevsky and his colleagues have demonstrated changes in hypothalamic oxidative and anaerobic metabolism following sex hormone variations. Endogenous oxygen uptake by hypothalamic tissue was highest during proestrus and estrus in cycling female rats (Moguilevsky and Malinow, 1964; Moguilevsky, 1965) and was significantly depressed in male rats by castration (Moguilevsky *et al.*, 1966). Castration also depressed the anaerobic metabolism of anterior and posterior hypothalamus in male rats (Moguilevsky *et al.*, 1967).

FURTHER CHARACTERIZATION OF HORMONE EFFECTS ON BRAIN FUNCTIONS

Pituitary Control

The understanding of sex steroid effects on pituitary control (via brain mechanisms) at this time does not yet include detailed understanding at the neurophysiological or cell biological level. Perhaps the most mechanistic approach so far has involved description of the hypothalamic releasing factors controlling gonadotropin secretion (reviewed by McCann and Porter, 1969). Follicle-stimulating hormone (FSH) releasing factor has been found in the median eminence and arcuate nucleus, while luteinizing hormone (LH) releasing factor has been found in those two places plus several other hypothalamic and preoptic sites (reviewed by Mess, 1969). Excellent reviews are also available describing experiments on the control of ovulation and on steroid feedback in the female and the male (Everett, 1969; Gorski, 1968; Davidson, 1966b; Flerko, 1966). An increasing amount of evidence supports the concept of a negative feedback effect of sex steroids on LH or FSH release (Brown-Grant, 1970; Bogdanove, 1967; Bogdanove and Gay, 1967; Yamamato, Diebel and Bogdanove, 1970), and sophisticated classifications of different kinds of feedback relations have been proposed (Yates and Brown-Grant, 1969).

It seems likely that the action of estradiol includes a positive feedback effect on LH (Ramirez, 1969), including the advancement of puberty (Smith and Davidson, 1968), and that progesterone shows a positive or biphasic feedback effect (Caligaris, Astrada and Taleisnik 1967, 1968) in stimulating ovulation, acting through the preoptic area (Barraclough *et al.*, 1964).

Another new development concerns the sites of action in the brain through which steroids may control gonadotropin release. The simplest initial idea was that estrogen or testosterone exerted a negative feedback effect on a single brain site. However, new data from the use of a small bayonet-shaped knife to cut around the borders of the hypothalamus—achieving a "deafferentation" of the hypothalamus—suggest that there may be at least two levels of neural control over the anterior pituitary (Halasz 1969a, b). The hypothalamus, inside the circumferential cut, is able to produce releasing factors which maintain tonic basal levels of gonadotropic hormone and ACTH secretion (Halasz, 1969a). However, the deafferentation procedure separates from the hypothalamus the brain sites through which certain influences on tropic hormone release are exerted, including the effect of stress and of the daily light cycle on ACTH and the stimulation for the ovulatory surge of LH (Halasz and Gorski, 1967; Koves and Halasz, 1970; Halasz *et al.*, 1967a, b). Halasz and his colleagues also found that the effect of a complete circumferential cut could be achieved by a cut interrupting only the afferent pathways coming through the anterior hypothalamus. This fits well with anatomical findings showing that a very high percentage of afferent fibers to the ventromedial hypothalamic nucleus do, in fact, come from or through the anterior hypothalamus (Chi, 1970). Taken together, these findings support the early suggestion of Barraclough and Gorski (1961) of a dual control over gonadotropic secretion—a prediction based on work with neonatally androgenized female rats.

Perhaps the closest approach so far to understanding pituitary control in terms of demonstrated sex-steroid effects on brain comes from the use of drugs which affect pituitary gonadotropin output (reviewed by Sawyer, 1969). For example, sex steroids alter monoamine levels (see above), and drugs which deplete the brain of monoamines (in particular, of norepinephrine) block ovulation (Coppola *et al.*, 1966; Meyerson and Sawyer, 1968). Therefore, it is tempting to suggest that one way that estradiol and progesterone regulate ovulation is through the alteration of norepinephrine and other monoamine levels.

Control of Mating Behavior

At this time there is no sure understanding of how the effects of sex steroids on the brain, summarized above, may constitute the brain mechanisms of sex-steroid effects on behavior. The most frequently used approach has been to characterize further the effects of sex hormones and brain manipulations (including brain hormone implants) on mating responses (see reviews by Phoenix *et al.*, 1967; Lisk, 1967a; Beach, 1967). One promising aspect of the search has been the use of drugs to mimic hormone effects. Meyerson (1964a, b) has shown that reserpine and certain other amine depletors can be substituted for progesterone in the hormonal induction of estrous behavior in female rats. Since the reserpine effect is obtainable in adrenalectomized rats (Meyerson, 1964c), release of adrenal progesterone following reserpine cannot be the sole means by which the effect is produced, although it probably enhances the magnitude of the effect. One possible implication of this work is that if sex steroids alter monoamine levels (see above) and drug-induced monoamine changes release sex behavior, then perhaps hormone effects on sex behavior are, at least in part, mediated by monoaminergic mechanisms.

Experiments with brain hormone im-

plants have provided the important evidence that estradiol placed in the hypothalamus or preoptic area of an ovariectomized female cat or rat is sufficient to elicit feminine mating behavior (Harris and Michael, 1964; Michael and Scott, 1964; Lisk, 1962), and that testosterone placed in the brain of a castrated male rat is sufficient to elicit masculine mating behavior (Davidson, 1966a; Lisk, 1967b). From this evidence, it was initially assumed that testosterone or estradiol directly triggered nerve cells controlling mating behavior. However, it was also possible that the effects of the sex steroids on mating behavior were mediated via their effects on the pituitary. In this case, gonadotropins would be the actual substances affecting the "trigger neurons" for mating behavior. This question was settled by manipulating gonadal steroids and pituitary hormones separately in hypophysectomized gonadectomized rats (Pfaff, 1970a). It was shown that in the hypophysectomized rat, testosterone has a direct triggering action, independent of the pituitary, to increase male mating behavior, and estradiol does the same for female mating behavior.

Other experiments have provided systematic tests of the specificity of sex-hormone effects on mating behavior in rats (Pfaff, 1970b). Tests included both male and female test animals, measured for both masculine and feminine behavior frequencies during periods of estrogenic, androgenic, and control treatments. Results showed that the same rats performing the most frequent mating responses under one hormone condition also did this under other hormone conditions. This consistency in individual rats' mating behavior across hormone conditions was replicated in an independent experiment using rats with various neonatal treatments (Pfaff and Zigmond, 1971). Finally, the results of the main experiment (Pfaff, 1970b) indicated that the stimulation of female behavior in female rats by estradiol is the strongest, most specific hormone-behavior link among all the possible causal combinations. That is, while the effect of testosterone on male behavior in male rats could be mimicked using testosterone injections in females or estradiol injections in males and females, the estradiol effect on female behavior could not be successfully mimicked using male rats or simple testosterone injections. The results, overall, conformed quite closely to the scheme proposed by Beach (1948, p. 220). The greater specificity of the estradiol effect on female behavior (vis-à-vis testosterone effects) is matched by the greater specificity of radioactive estradiol (vis-à-vis testosterone) uptake in brain (McEwen and Pfaff, 1970; McEwen *et al.*, 1970a, b; Zigmond and McEwen, 1970). Thus, it seems heuristic to concentrate for further physiological analysis on the estradiol stimulation of female behavior in female rats, due to the strength and specificity of estradiol uptake and effects.

The estradiol effect on female behavior has not been thought to create neural circuits for the execution of that behavior where none existed before hormone treatment. In fact, ovariectomized female rats untreated with any hormones are commonly observed to exhibit lordosis (the female's receptive posture), although they do so much less frequently than normal or estrogen-treated animals. Moreover, Bard (1940) was able to observe sexual reflexes in brain-transected female cats, whether or not they were in estrus. The classical idea, therefore, has been that sex hormones alter thresholds for the release of mating behavior reflexes and in particular may release forebrain inhibition of mating reflexes governed by lower brain centers (Beach, 1967). One possible example of