activity in the adult is higher than that in the young animal (Fig. 1c). The detection of a daily rhythmicity in rat liver TT activity so soon after birth emphasizes the extreme care with which future investigations of developmental changes in enzyme activity should be carried out.

EVA HONOVA, SANFORD A. MILLER
RICHARD A. EHRENKRANZ, ANNA WOO
*Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge 02139*

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## Estradiol-Concentrating Neurons: Topography in the Hypothalamus by Dry-Mount Autoradiography

Abstract. *Serial section autoradiograms were prepared of different planes of the hypothalamus and diencephalon of immature female, immature male, and ovariectomized mature rats injected with 6,7-$^3$H-estradiol-17 $\beta$. Known causes of diffusion and redistribution of the label, such as fixation, embedding, and thawing, were eliminated by the use of an autoradiographic technique based on the dry-mounting of freeze-dried sections. Neurons that concentrate estradiol exist in distinct and definable anatomical areas that are independent of the sex and hormonal state of the animals. Distribution of these neurons follows known terminations of the stria terminalis, which supports the concept of an endocrine amygdaloid-hypothalamic-hypophysial axis.*

Hohlweg and Junkmann (*1*) first provided evidence for neuronal control of pituitary function and postulated the existence of a "sexual center." Numerous attempts have since been made to determine its localization; the methods used include destruction of specified regions, stimulation, ovarian and hypophysial grafting, hormone crystal implantation, measuring of nuclear or nucleolar diameter of neurons, determination of regional uptake of radioactively labeled estrogens, and autoradiography. Although evidence suggests that the basal hypothalamus, the anterior hypothalamus, and the preoptic region influence gonadotropin concentrations (*2*), the precise anatomical representation of the areas involved is in question, largely because of limitations inherent in the technical approaches used, that is, lack of resolution, lack of differentiation between effects on cells or nerve-fibers, and diffusion of applied hormones in vivo or redistribution during tissue processing. The latter is most conspicuous in the controversial and misleading data obtained by autoradiography with $^3$H-stilbestrol (*3*) and $^3$H-estradiol (see *4*).

A precise anatomical determination of the areas involved in the feedback control, important for the understanding of the physiology and pathology of hormone regulation and the pharmacology of treatment with hormones and antihormones, particularly antifertility drugs, requires techniques that provide a cellular and subcellular resolution. Mapping of hormone-concentrating sites in the hypothalamus may also help to determine whether feedback control for different endocrine glands occurs in separate centers or in one nucleus.

The autoradiographic method used (*5*, *6*) was adapted for the study of brain tissue and excludes all the known sources of translocation artifact. It is based on (i) simultaneous freezing and mounting of small blocks of tissue (ii) low-temperature cryostatic sectioning and freeze-drying of 2.0-$\mu$ sections, and (iii) dry-mounting of the unfixed and unembedded sections on photographic emulsion (*6*).

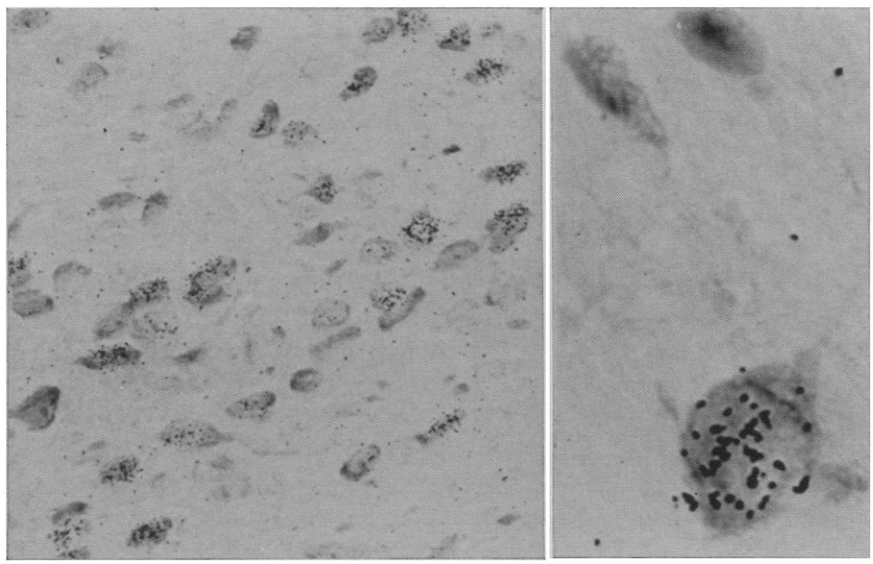
Three immature female Sprague-Dawley (SD) rats, 25 or 29 days old, one immature male SD rat, 24 days old, and two ovariectomized mature female SD rats, 65 and 101 days old, ovariectomized 21 and 3 days, respectively, before the experiment, were injected with 6,7-$^3$H-estradiol-17$\beta$ (*7*) (0.09 to 0.4 $\mu$g) dissolved in isotonic saline. The animals were decapitated 1 to 2 hours after the injection, the hypothalamus or diencephalon was excised and frozen, and 2-$\mu$ serial sections were cut in a cryostat at $-40°$ to $-50°$C. The sections were freeze-dried by cryosorption-pumping (*6*) and then dry-mounted onto photographic emulsion-coated slides (Kodak NTB 3) by pressure with a Teflon support. After exposure for 3 to 8 months at $-15°$C, the slides were processed and stained with methyl green–pyronin. Photomicrograms and sketches were prepared from microscopic slides and used for schematic drawings adapted to the sagittal and coronal planes of the atlas of König and Klippel (*8*).

In the ovariectomized rat, the autoradiograms encompass the hypothalamus, preoptic region, septal region, and anterior and medial thalamus; in the immature female and male, the studies were limited to the hypothalamus, the caudal half of the preoptic region, and the ventral thalamus. Radioactivity concentrated in nuclei of neurons (Figs. 1 and 2), but not in glial and ependymal cells, which are located in distinct and definable areas (Figs. 3 and 4); neurons of other areas remained unlabeled. In the hypothalamus and preoptic region studied the localizations of estradiol-concentrating neurons were not different in ovariectomized mature, immature female, and immature male rats.

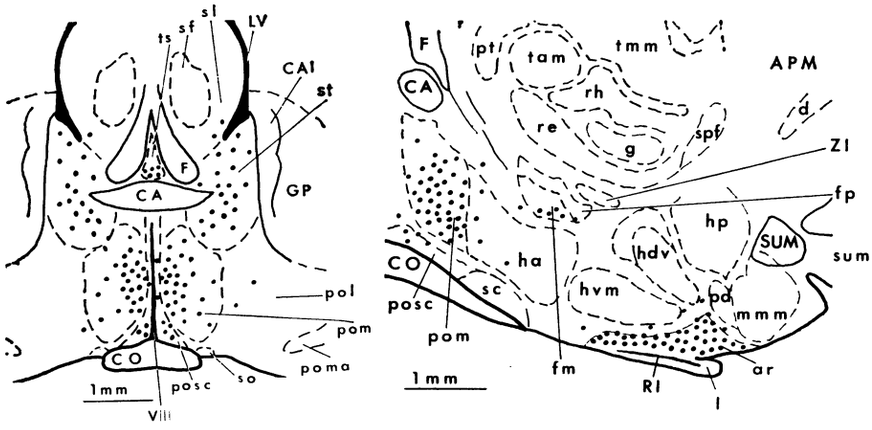
Labeled neurons were concentrated in the nucleus arcuatus and the pars lateralis of the nucleus ventromedialis, including less densely packed neurons in its lateral and caudo-lateral vicinity. Krieg, who described five subunits of the nucleus ventromedialis in the rat, did not attach "especial significance to the differentiation of these parts" (*9*). His meticulous description, however, facilitates the recognition of the now apparent differences. The neurons of the nucleus hypothalamicus lateralis, characterized as large and stellate, with several long tapering processes (*9*), were not labeled. The nucleus periventricularis with its outgrowth of the nucleus paraventricularis parvicellularis contained neurons with a higher proportion of labeling in the anterior hypothalamic and preoptic region than in its more caudal part. The magnocellular portion of the nucleus paraventricularis remained, except for a few interspersed smaller neurons, unlabeled. A group of elongated, medium-sized cells located

ventrally to the magnocellular portion of the nucleus paraventricularis, with which it is closely associated, was also labeled. This labeled subunit of the nucleus paraventricularis could be identified in all the animals in which this area was studied.

The anterior hypothalamus contained only a few widely dispersed labeled neurons concentrated toward the heavily labeled areas of the nucleus preopticus medialis, nucleus preopticus suprachiasmatis, and nucleus interstitialis striae terminalis. Labeled neurons were also found in nuclei bordering the nucleus striae terminalis, that is, the nucleus accumbens and nucleus septi lateralis. Furthermore, a concentration of labeled neurons existed in the nucleus triangularis septi and in the organon subfornicale.



Figs. 1 and 2. Autoradiograms of the nucleus ventromedialis, pars lateralis (Fig. 1, left) and the nucleus arcuatus (Fig. 2, right), frontal plane, showing concentration of radioactivity in nuclei of neurons; prepared 2 hours after subcutaneous injection of 0.4 or 0.3 $\mu$g of $^3$H-estradiol into mature female rats ovariectomized 36 hours before injection. Exposure times, 117 days (Fig. 1) and 70 days (Fig. 2). Stained with methyl green–pyronin for DNA and RNA. (Fig. 1, × 200; Fig. 2, × 1500)




Figs. 3 and 4. Selected schematic drawings prepared after serial-section autoradiograms from immature female (Fig. 3, left) and immature male (Fig. 4, right) rats 1 or 2 hours after the injection of 0.4 or 0.093 $\mu$g of $^3$H-estradiol, respectively. Figure 3, coronal section; Fig. 4, sagittal section, about 240 $\mu$ lateral from the midline. The black dots represent areas of concentration of neurons labeled with radioactivity, while in the blank regions neurons remained unlabeled. Abbreviations: *APM*, area pretectalis medialis; *ar*, nucleus arcuatus; *CA*, commissura anterior; *CAI*, capsula interna; *CO*, chiasma opticum; *d*, n. Darkschewitsch; *F*, columna fornicis; *fm*, n. paraventricularis magnocellularis; *fp*, n. paraventricularis parvicellularis; *g*, n. gelatinosus; *GP*, globus pallidus; *ha*, n. anterior hypothalami; *hdv*, n. dorsomedialis ventralis; *hp*, n. hypothalamicus posterior; *hvm*, n. ventromedialis; *I*, infundibulum; *LV*, ventriculus lateralis; *mmm*, n. mamillaris medialis, pars medialis; *pd*, n. premamillaris dorsalis; *pol*, n. preopticus lateralis; *pom*, n. preopticus medialis; *poma*, n. preopticus magnocellularis; *posc*, n. preopticus suprachiasmatis; *pt*, n. paratenialis; *re*, n. reuniens; *rh*, n. rhomboides; *RI*, recessus infundibuli; *sc*, n. suprachiasmatis; *sf*, n. septalis fimbrialis; *sl*, n. septi lateralis; *so*, n. supraopticus; *spf*, n. subparafascicularis; *st*, n. interstitialis striae terminalis; *SUM*, decussatio supramamillaris; *sum*, n. supramamillaris; *tam*, n. anterior medialis thalami; *tmm*, n. medialis thalami, pars medialis; *ts*, n. triangularis septi; *VIII*, ventriculus tertius; and *ZI*, zona incerta.

Furthermore, a concentration of labeled neurons existed in the nucleus triangularis septi and in the organon subfornicale.

Single labeled cells were observed in the vicinity of clusters of labeled neurons and were found occasionally in parts of the nucleus ventromedialis outside of its pars lateralis, and at the borders between the nucleus arcuatus and nucleus premamillaris ventralis, as well as the nucleus arcuatus and nucleus suprachiasmatis. The neurons of the mamillari nuclei, including the nucleus premamillaris ventralis and dorsalis, were not labeled. Also unlabeled were the nucleus supraopticus; nucleus suprachiasmatis; nucleus ventromedialis partes ventralis, centralis, dorsalis, and medialis; the nucleus dorsomedialis ventralis and dorsalis; and the nucleus hypothalamicus posterior.

An interpretation is suggested when the distribution pattern of the labeled neurons is compared with the areas of termination of the stria terminalis reported in the literature. The stria terminalis, which originates in nuclei of the amygdala, divides into four or five components at the anterior commissure and terminates in certain areas of the hypothalamus, the preoptic region, and the anterior thalamus (*9*, *10*), which are largely identical with those areas in which labeled neurons were found.

This topographic relationship supports earlier reports on the involvement of the amygdala in the control of gonadotropin secretion (*11*) and promotes the concept of an endocrine amygdaloid-hypothalamic-hypophysial interrelationship. It remains to be established further whether this autoradiographic localization represents the "feedback" receptor sites and whether there are two neuronal centers for ovulation within the diencephalon (*12*), or if what has been proposed as preoptic-suprachiasmatic cyclic center for ovulation reflects only the effect of the interruption, inhibition, or stimulation of stria terminalis fibers rather than that of the destruction of specific estrogen-concentrating neurons.

Nuclear concentration of $^3$H-estradiol exists in areas of feedback control, that is, pituitary cells and certain neurons, identical with the subcellular localization

in peripheral target tissues (*13*, *14*). The neuronal nuclear concentration of the label may, therefore, in analogy to the estradiol induction of a specific genetic expression in uterine nuclei (*15*), represent the site of a positive "feedback" rather than that of a direct inhibition.

WALTER E. STUMPF

*Department of Pharmacology,*
*University of Chicago,*
*Chicago, Illinois 60637*

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## Rana pipiens Complex: Mating Call Structure and Taxonomy

Abstract. *Geographic variability in call structure indicates that four largely allopatric populations of leopard frogs are present in the central United States. These forms appear to maintain their distinctness in narrow zones of sympatry, and most adult males can be separated morphologically. It is suggested that the four forms represent distinct species and that the idea of gradual clinal variability in one wide-ranging species is wrong.*

Attempts to classify leopard frogs (*Rana pipiens*) by adult morphology and artificial diploid hybridization tests have led to general acceptance of the idea of one wide-ranging, variable species, with levels of genetic incompatibility between populations directly related to geographic separation (*1*, *2*). The example has since become widely used as a classical model for geographic speciation in which the effect of distance on rates of gene flow and the consequent development of reproductive isolation are shown (*3*). More recent studies (*4*, *5*) found further evidence of geographic variability but left the evolutionary interpretation unchanged. Other studies (*6*, *7*) recognized two distinct, largely allopatric forms (the CF and DF complexes) in leopard frogs of northern Colorado with a narrow zone of overlap, about 5 miles (8 km) wide.

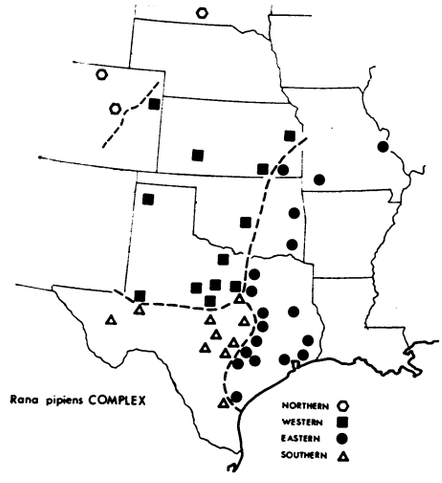


Fig. 1. Distribution of call types of the *Rana pipiens* complex in the central United States and approximate positions of replacement zones.

In 1963 Oldham found two distinctive mating call types (*8*), (Western and Southern), which overlap and maintain their distinctness in a narrow east-west zone across north-central Texas. In 1966 Littlejohn found a third distinctive call type (Eastern) in central Texas. This form is largely allopatric to the other two but has a narrow north-south zone of overlap with the Southern form in central Texas. In Johnson County, Texas, all three populations occur in a mosaic distribution, with two forms at some breeding sites.

Finally, mating calls of leopard frogs in the northern United States are also distinctive. Field work with D. Pettus and D. D. Post (Colorado State University) enabled us to establish that this fourth (Northern) call group is equivalent to the CF complex, and the Western call race to the DF complex. Figure 1 shows a provisional map of the distribution of the four call types in the central United States.

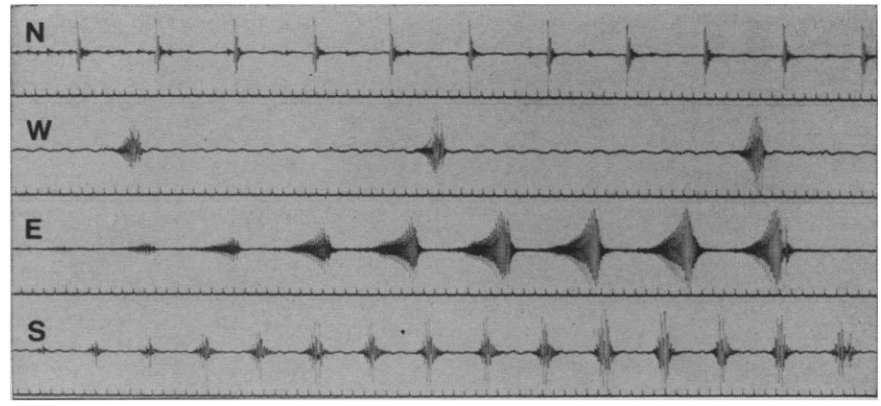


Fig. 2. Oscillograms of the mating calls of the *Rana pipiens complex*. (*N*) Northern (incomplete call): Larimer County, Colorado, effective temperature 12.5°C; (*W*) Western: Cowley County, Kansas, effective temperature 17.2°C; (*E*) Eastern: Johnson County, Texas, effective temperature 17.5°C; (*S*) Southern: Johnson County, Texas, effective temperature 16.3°C. Time marker indicates 10-msec intervals.