Journal of Steroid Biochemistry, 1976, Vol. 7, pp. 1163–1170. Pergamon Press. Printed in Great Britain

STEROID HORMONE TARGET SITES IN THE BRAIN: THE DIFFERENTIAL DISTRIBUTION OF ESTROGEN, PROGESTIN, ANDROGEN AND GLUCOCORTICOSTEROID

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SUMMARY

Nuclear concentration of steroid hormones or their metabolites exists in neurons in selective areas of the mammalian brain. The distribution of estrogen target cells in different mammalian species appears to follow a similar pattern that involves selective nuclear groups in the forebrain, midbrain and lower brainstem. The distribution of androgen target cells in the rat brain largely corresponds to the distribution of estrogen target cells, with differences existing, however, in certain structures, such as lateral septum, ventromedial hypothalamus, hippocampus, cortex, epithalamus, lower brainstem and spinal cord. The distribution of progestin in the guinea-pig hypothalamus seems restricted to the preoptic and infundibular region, where it overlaps with estrogen localization. Corticosterone target cells are accumulated in extrahypothalamic regions, and not in the hypothalamus, in contrast to the sex steroid. The synthetic "glucocorticosteroid" dexamethasone, however, is found in neurons and glial cells throughout the rat brain, differing from the natural glucocorticosteroid corticosterone. Localization of different hormones in neurons of identical regions suggests that the same neuron may be addressed by different hormones. On the other hand, the differential distribution of the various types of hormones is likely to reflect differences in their action.

Both estrogens and androgens appear to act on neural structures that are identical to or closely associated with (1) various sensory pathways and (2) ventricular recess organs, constituting the periventricular gland. Androgens, in addition, stimulate selectively neurons of the somatomotor system and circuits of aggression.

In general, the wide existence of hormone target cells in the central nervous system reflects the multiple actions of the steroid hormones on endocrine regulation, autonomic functions and behavior.

The identification of steroid hormone target cells in the brain has been accomplished through the use of dry-mount and thaw-mount autoradiography [1]. The topographical definition of sites of accumulation of hormone "receptor" cells has led to the introduction of what is called *hormone-architectonics of the brain* [2].

Currently data are available for different steroid hormones, such as estrogen, androgen, progestin, and glucocorticosteroid, which has been covered extensively in *Anatomical Neuroendocrinology* [3]. While mainly rodents were studied, estrogen has also been localized in the brain of representatives of other vertebrate classes and was found to exist throughout phylogeny. In addition, estrogen target cells have been identified in neonatal [4] and embryonic [5] brain. From this information, clues can be obtained as to topographical relationships among target areas of the different hormones and between hormone target neurons and neurotransmitter producing neurons. The present paper briefly reviews and compares the distribution of steroid hormone target cells based on the results obtained in our laboratory.

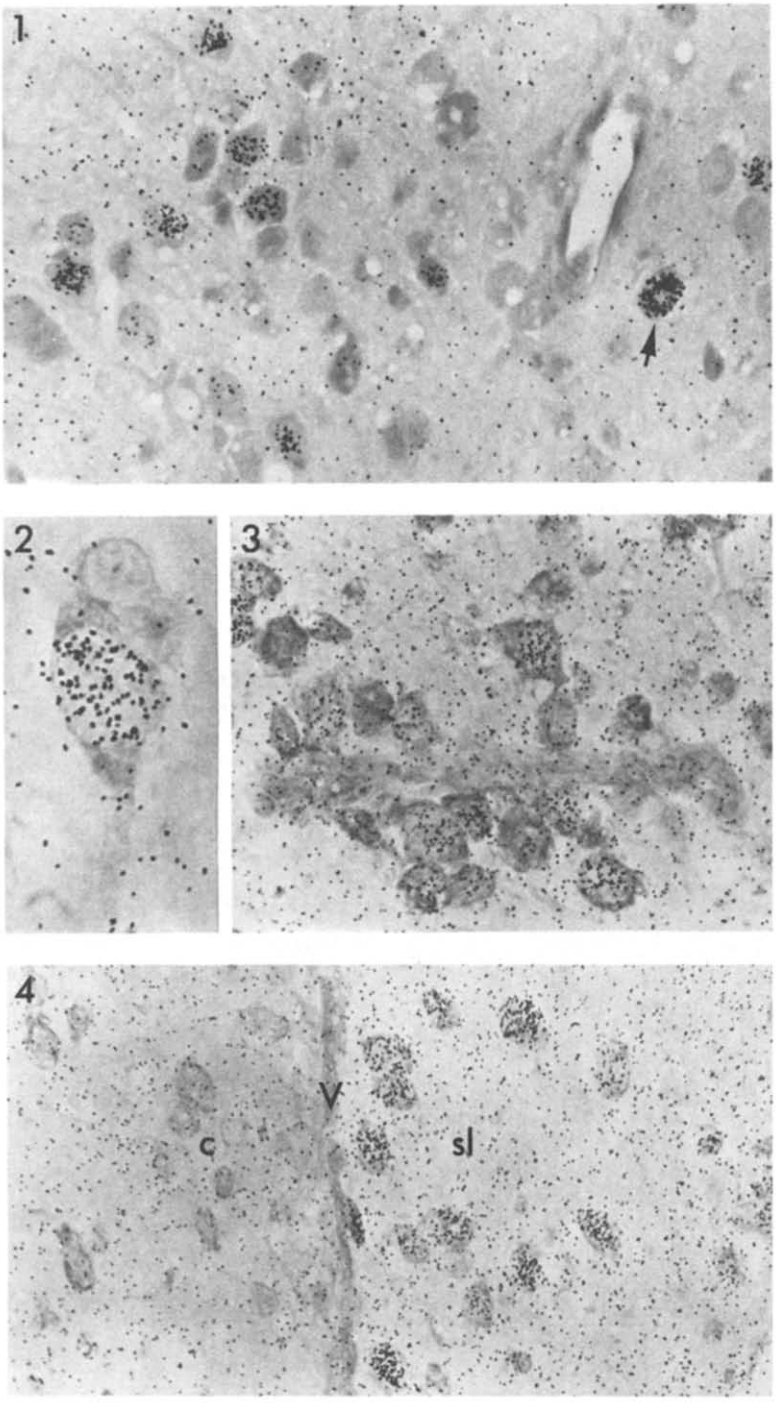
Autoradiograms were prepared after intravenous or subcutaneous injection of tritium labeled steroid hormone with a S.A. between 40 and 100 Ci/mM. The dose used per 100 g bw was for [^{3}H]-estradiol-17β 0.1–1.0 μg, for [^{3}H]-testosterone, [^{3}H]-dihydrotestosterone, [^{3}H]-progesterone, [^{3}H]-corticosterone and [^{3}H]-dexamethasone 0.5–1.0 μg. The steroid was dissolved in ethanol–isotonic saline. The animals were killed at 15 min to 2 h after the injection, depending on the compounds used. In order to establish specificity of the localized radioactivity, competition studies with unlabeled agonists and antagonists were performed. Details have been published elsewhere [6].

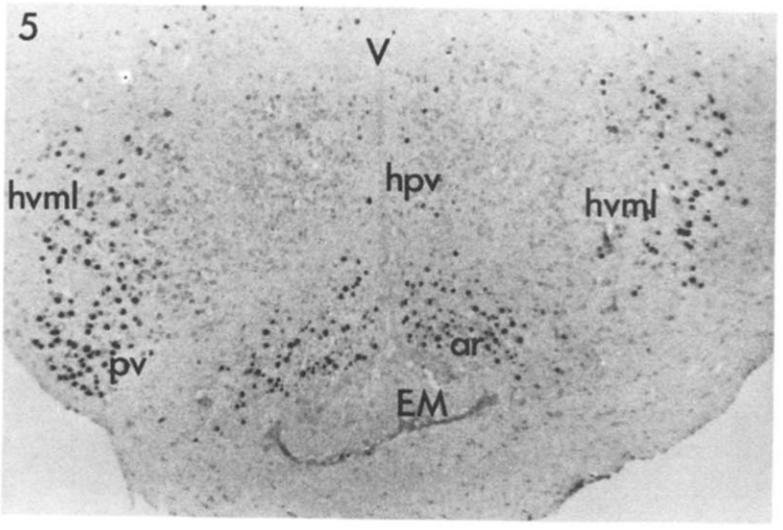
Among the various steroid hormones studied most detailed information is available on [^{3}H]-estradiol. This is probably due to the early availability of highly specifically labeled estrogen and the high binding affinity in target cells, together with relatively low levels of metabolites produced. All of the steroid hormones studied in our laboratory except dexamethasone seem to enter the brain readily without any barrier. Typically, radioactivity concentrates in nuclei of cells with varying intensity. The differences in intentisy of nuclear labeling are apparent between different neuronal regions and also within a given group of labeled neurons. Target cells are recognized and defined by the concentration and retention of labeled hormone in nuclei above cytoplasmic and extracellular radioactivity. The topographical distribution of radioactivity within labeled cell nuclei is not uniform: the nucleolar region, if visible in the autoradiogram, is always unlabeled (Fig. 1). Glial and ependymal cells are generally unlabeled, except in specialized regions,

for instance, in or around ventricular recess organs. Figure 2 shows a labeled neuron together with two unlabeled satellite glial cells. Labeled neurons occasionally are observed in contact with capillaries, as demonstrated in Fig. 3 in the medio–lateral preoptic region, suggesting neurosecretion directly into brain capillaries.

The separation of areas of accumulation of target and non-target cells may be distinct as shown between the nucleus septi lateralis and the nucleus caudatus (Fig. 4) on opposite sites of the lateral ventricle. Frequently, the transition is gradual as demonstrated in Fig. 5. In the hypothalamus, occasionally labeled neurons are observed within the ependymal lining and also within the ventricular lumen (so-called supraependymal cells).

Figure 6 provides an overview of the distribution of estrogen target cells in the rodent brain. Accordingly, the anatomical distribution of steroid hormone target neurons is extensive and involves many regions





Figs. 1–5. Autoradiograms of hypothalamus, obtained 1 h after injection of [^{3}H]-estradiol-17β (Fig. 1 and 5) or [^{3}H]-dihydrotestosterone (Figs. 2–4) [7]. Figure 1: guinea-pig, arcuate nucleus, showing typical nuclear concentration of radioactivity. The area of the nucleolus (arrow) is free of radioactivity. Figure 2: rat, preoptic area, showing association of unlabeled glial cells with androgen target neuron. In Fig. 3 androgen target neurons of rat preoptic region are seen in close proximity to capillary wall, suggesting neurosecretory activity. Figure 4 demonstrates androgen concentrating neurons in the nucleus septi lateralis (sl), separated by the lateral ventricle (v) from unlabeled neurons in the nucleus caudatus (c). Figure 5 is an autoradiogram of a frontal section of mouse central hypothalamus (slightly oblique cut), showing the topographic relationship of labeled (black) and unlabeled (grey) neurons. This survey autoradiogram corresponds to a portion of the schematic drawing of Fig. 7c. Labeled neurons are seen in the nucleus arcuatus (ar), the pars lateralis of the nucleus ventromedialis (hvml), the nucleus premammillaris ventralis (pv) which is cut only on the left side, and the nucleus periventricularis (hpv). The neurons of a large portion of the nucleus ventromedialis, such as its pars centralis and pars medialis, show no or only weak labeling. V = IIIrd ventricle, EM = median eminence. Magnification: ×540, Figs. 1, 3 and 4; ×1080, Fig. 2; ×70, Fig. 5.

of the phylogenetically old periventricular brain. This suggests strongly that the concept of the "hypophyseotrophic area" needs to be revised.

Selected schematic drawings of cross-sections show the topographical distribution of target cells in rodent brain for estrogen (Figs. 7a–d and 8a–e), androgen (Figs. 9a–e) and natural glucocorticosteroid (Figs. 10a–e). A comparison of related levels, for instance, Fig. 7a, 9a and 10b reveals that there is overlap between the target regions for estrogen, androgen and glucocorticosteroid, with a considerable similarity for estrogen and androgen. Androgen target neurons, however, appear more frequently than estrogen target neurons in the lateral septum, the piriform cortex and the periallocortex, under the conditions of the experiments. Similarly, comparing Figs. 7c and 9c, androgen target neurons are found throughout the hippocampus and throughout the hypothalamic ventromedial nucleus, in contrast to the absence or low intensity of labeling of these neurons with estrogen. While in the caudal colliculus, estrogen (Fig. 8a) and androgen (Fig. 9e) concentrating cells appear distributed in a similar fashion, neurons of the nucleus pontis, as shown in the same drawings, are unlabeled after [^{3}H]-estradiol injection, but extensively labeled after [^{3}H]-testosterone or [^{3}H]-dihydrotestosterone injection.

More recent data from our laboratory [7; in part unpublished] indicate that in the brain stem and spinal cord, after [^{3}H]-dihydrotestosterone injection, nuclear concentration of androgen exists not only in the regions where estrogen localization is found, but also in motor neurons, for instance, of the fifth, the seventh and the twelfth cranial nerve, as well as in the ventral horn of the spinal cord and Purkinje cells of the cerebellum. Thus, it appears that estrogens and androgens address commonly cells of the perventricular gland and of sensory pathways, while androgen stimulates selectively neurons that are associated with circuits of aggression and somatomotor activities.

Midbrain, pons and medulla oblongata are still incompletely investigated. Best studied is the distribution of [^{3}H]-estradiol, or metabolites of it, in the mouse lower brain stem [9]. Figures 8a–e represent selected levels, showing heavy accumulation of estrogen target neurons in the central gray of the colliculus caudalis and the nucleus raphes dorsalis, all surrounding the collicular recess of the fourth ventricle. In this region nuclei of ependymal cells of the outer ventricular wall as well as the wall of the lingula

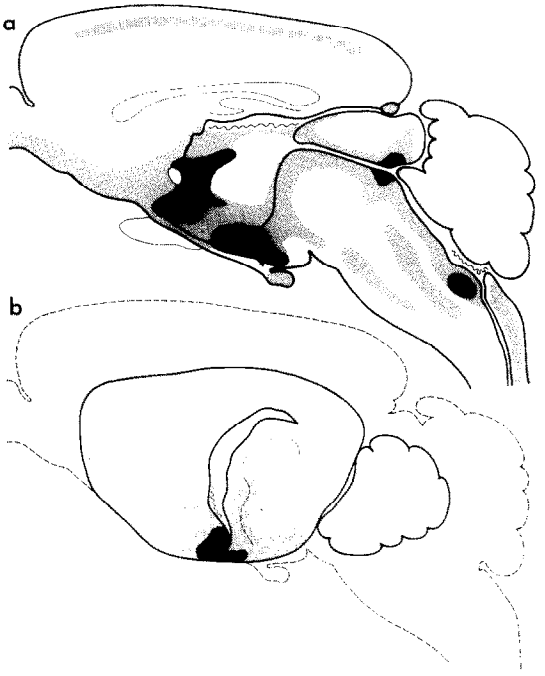


Fig. 6a,b. Estrogen target cell distribution in rodent brain based on the autoradiographic localization of [^{3}H]-estradiol-17β; projected on sagittal planes and showing relationships to the third and fourth ventricles and aqueduct (Fig. 1a) and the lateral ventricle (Fig. 1b). The dark and light stippling corresponds to high and intermediate–low concentration of steroid hormone in nuclei of nerve cells. The extensive pattern reflects endocrine–neuronal circuits and areas of neurosecretion.

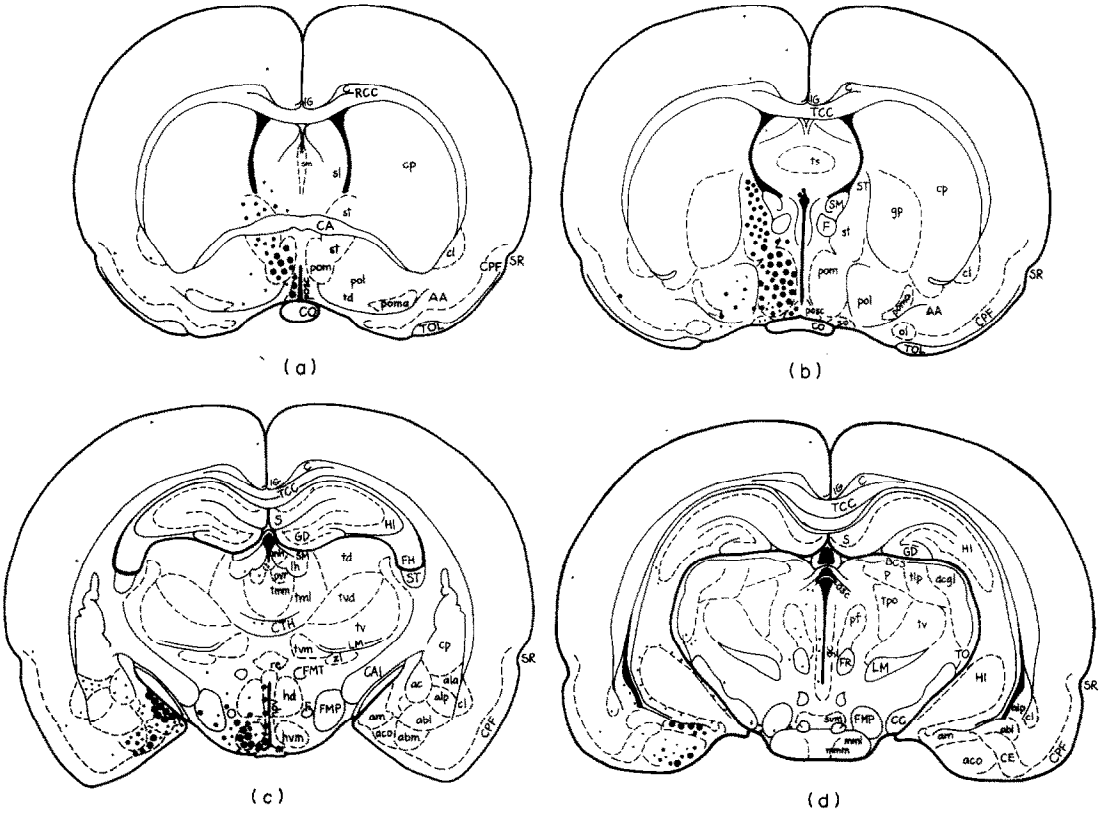


Fig. 7a–d. Schematic drawings prepared from frontal section autoradiograms of rat brain, showing distribution of estrogen concentrating neurons (dots on left half of sections) in the preoptic-septal region, central hypothalamus and amygdala. Designations of structures are on right half of drawings. Reproduced from Stumpf, Sar and Keefer[8].

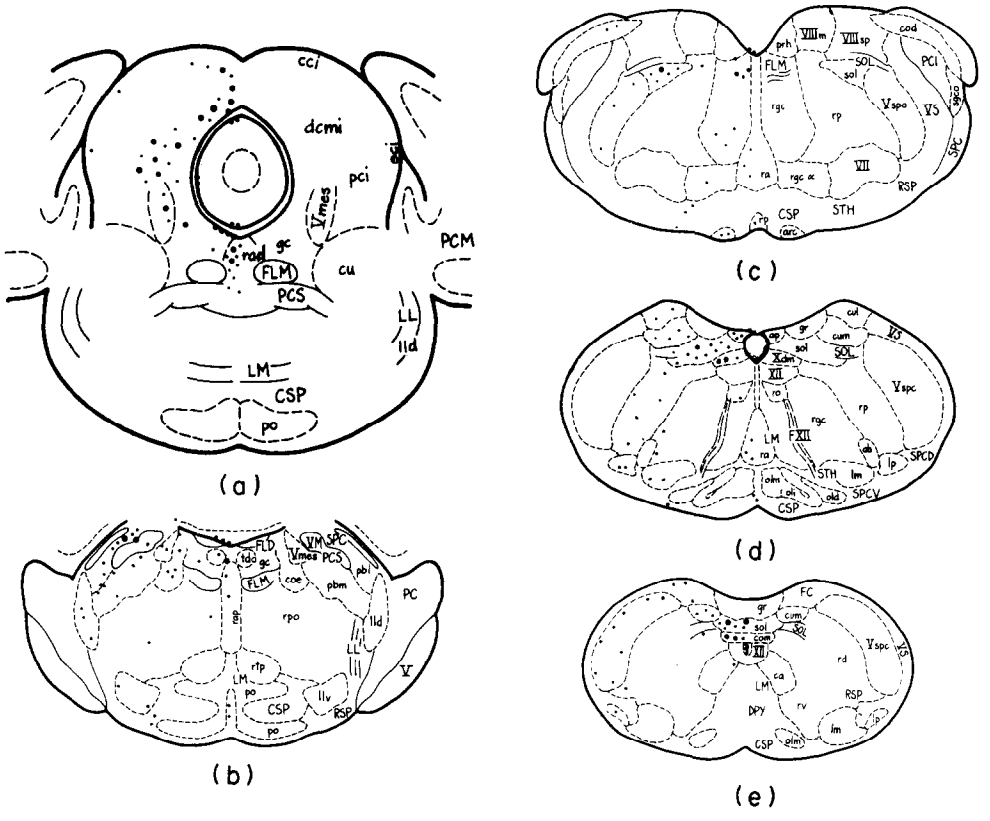


Fig. 8a–e. Schematic drawings prepared from frontal section autoradiograms of mouse midbrain, pons and medulla oblongata, showing distribution of estrogen concentrating cells (dots on left half of sections). Designations of structures are on the right half of drawings. Reproduced from Stumpf and Sar[9].

cerebelli are labeled.* Nuclear labeling with estrogen of ependymal cells is also observed along the ventral groove of the fourth ventricle and the adjacent central canal. In the spinal cord estrogen concentrating cells exist in laminae 1, 2 and 10 [8], while androgen concentrates mainly in motox neurons of the ventral horn (lamina IX) and in laminae IV, V, VI and X.

Corticosterone, different from the sex steroid hormones, is not concentrated and retained in neurons of the preoptic and central hypothalamus, but rather prevails in the hippocampus and dentate gyrus as well as in structures closely associated with the hippocampus, such as the dorsal and lateral septum and the entorhinal cortex (Figs. 10a–e). While estrogenic and androgenic sex steroids concentrate heavily in neurons of the nucleus medialis of the amygdala (Figs. 7c and 9c), the main portion of this nucleus does not retain [^{3}H]-corticosterone in the rat (Fig. 10d).

The autoradiographic results suggest that in the central nervous system selective cellular binding sites exist for estrogens, androgens, progestins, and glucocorticosteroids. Since there is considerable overlap in the topographical distribution of target cells among the sex steroids as well as between the sex steroids and the glucocorticosteroids it appears that the same cell contains binding affinities for multiple hormones. This can be further supported by the demonstrated mutually modulating effects of estrogen and progestin on their nuclear uptake. The wide distribution in the central nervous system of steroid hormone target neurons, including the lower brain stem, indicates neuroendocrine significance of extrahypothalamic structures and suggests reorientation and revision of current concepts.

We have postulated earlier that steroid hormone target neurons are oligopeptide messenger producing neurons. Further evidence is now forthcoming through immunocytochemistry that supports this concept [14]. Topographical relationships between

*Evidence for the existence of a specialized endocrine structure in the collicular recess of the fourth ventricle was provided earlier [2, 9, 10]. This includes high vascularization, sulcation of the ependyma, subependymal space and nuclear concentration of estrogen and androgen in neurons, ependymal cells, and subependymal cells, involving the griseum centrale of the colliculus caudalis and the lingula cerebelli, with a sleeve of substantia grisea covering the lingula. Steroid hormone labeled ependymal and subependymal cells are accumulated in the midline at the collicular recess dorsal to the lingula and rostral to the colliculo–cerebellar transition. Estrogen and androgen concentrating neurons are numerous in the vicinity of this region. The above structures are termed *collicular recess organ* (organum colliculare), analogous to other ventricular recess organs [2].

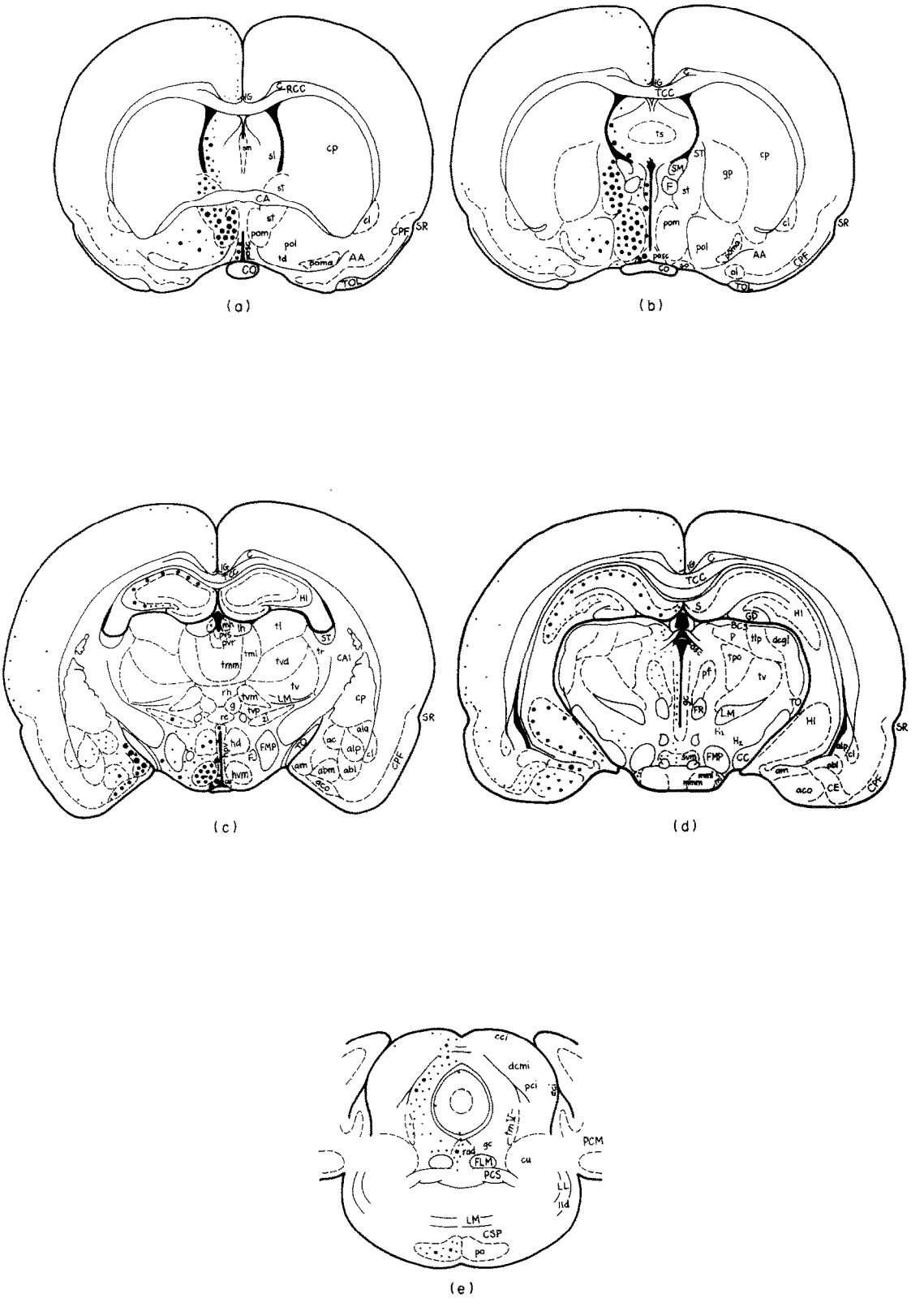


Fig. 9a–e. Schematic drawings prepared from frontal section autoradiograms of rat brain, showing distribution of androgen concentrating cells (dots on left half of sections) in preoptic-septal region, central hypothalamus, amygdala, hippocampus, piriform cortex, periallocortex, colliculus caudalis and pons. Designations of structures are on right half of drawings. Reproduced from Sar and Stumpf[10].

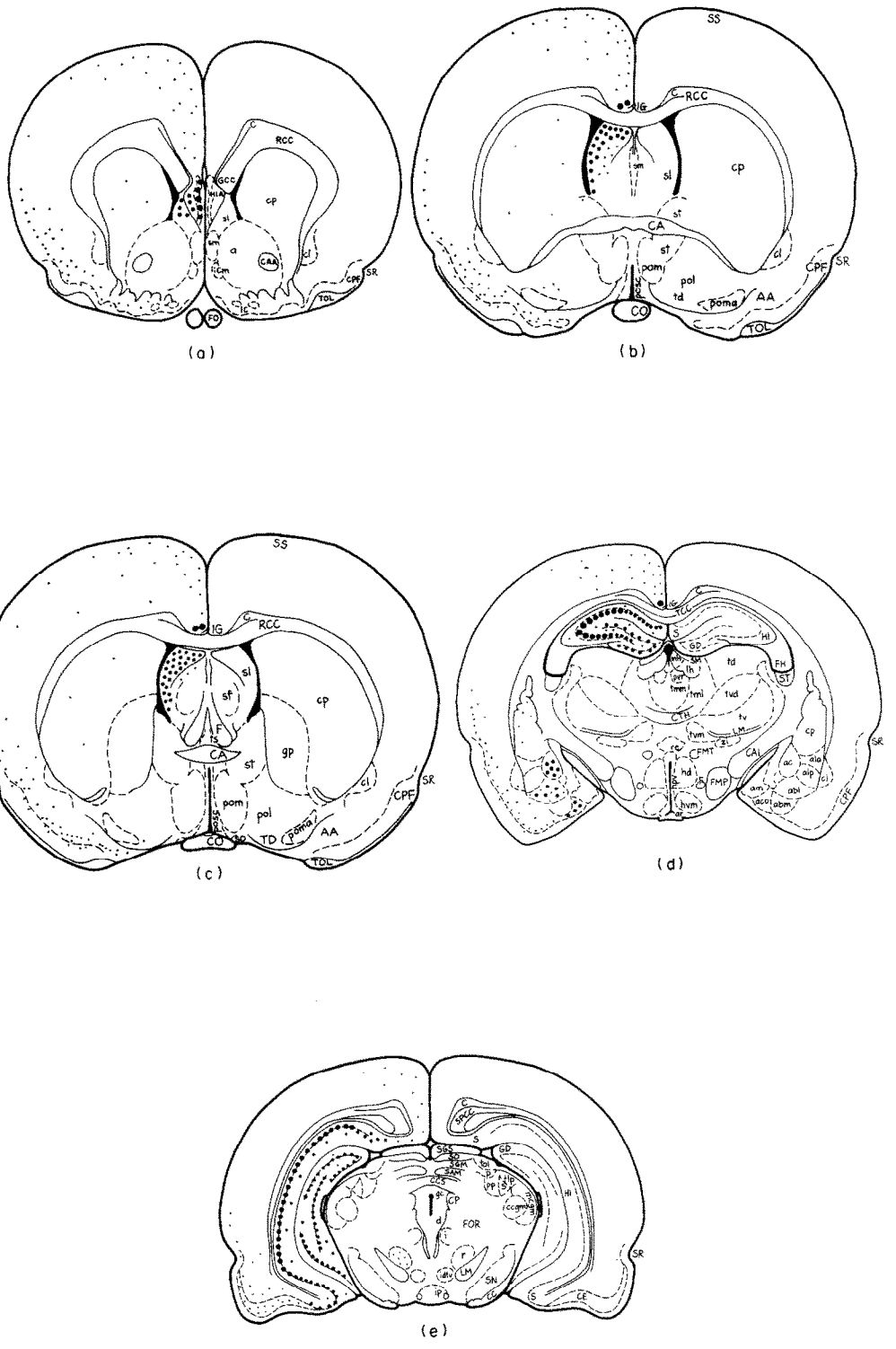


Fig. 10a–e. Schematic drawings prepared from frontal section autoradiograms of rat brain, showing distribution of corticosterone concentrating neurons (dots on left half of sections) in precommissural, supracommissural and subcallosal hippocampus, the dorsolateral septum, amygdala, piriform cortex, and periallocortex. Designations of structures are on right half of drawings. Reproduced from Stumpf and Sar[11]. Note the extrahypothalamic distribution of natural glucocorticosteroid target neurons, contrasting with the distribution of extrogen and androgen in Figs. 7 and 9. Also, contrasting with [^{3}H]-corticosterone is the distribution of [^{3}H]-dexamethasone. [^{3}H]-Dexamethasone is found in neurons, glial cells and ependymal cells throughout the brain [12].

estrogen target neurons and biogenic amine producing neurons have also been demonstrated [15, 16]. Thus, estrogen target neurons appear to include various amino acid-derived messenger-producing neurons.

REFERENCES

1. Stumpf W. E.: In *Methods in Cell Biology* (Edited by D. M. Prescott). Academic Press, New York, Vol. XIII (1976) pp. 171–193.
2. Stumpf W. E.: In *Anatomical Neuroendocrinology* (Edited by W. E. Stumpf and L. D. Grant). Karger, Basel (1975) pp. 2–8.
3. Stumpf W. E. and Grant L. D. (Editors): *Anatomical Neuroendocrinology*. Karger, Basel (1975).
4. Sheridan P. J., Sar M. and Stumpf W. E.: In *Anatomical Neuroendocrinology* (Edited by W. E. Stumpf and L. D. Grant). Karger, Basel (1975) pp. 134–141.
5. Martinez-Vargas M. C., Gibson D. B., Sar M. and Stumpf W. E.: *Science* **190** (1975) 1307–1308.
6. Stumpf W. E. and Sar M.: In *Receptors and Mechanism of Action of Steroid Hormones* (Edited by J. R. Pasqualini). Marcel Dekker, New York (1976), pp. 42–85.
7. Sar M. and Stumpf W. E. V. Intl. Cong. Endocrinology. Hamburg, Abstracts, p. 241 (1976).
8. Stumpf W. E., Sar M. and Keefer D. A.: In *Anatomical Neuroendocrinology* (Edited by W. E. Stumpf and L. D. Grant). Karger, Basel (1975) pp. 104–119.
9. Stumpf W. E. and Sar M.: In *Anatomical Neuroendocrinology* (Edited by W. E. Stumpf and L. D. Grant). Karger, Basel (1975) pp. 82–103.
10. Sar M. and Stumpf W. E.: In *Anatomical Neuroendocrinology* (Edited by W. E. Stumpf and L. D. Grant). Karger, Basel (1975) pp. 120–133.
11. Stumpf W. E. and Sar M.: In *Anatomical Neuroendocrinology* (Edited by W. E. Stumpf and L. D. Grant). Karger, Basel (1975) pp. 254–161.
12. Rees H. D., Stumpf W. E. and Sar, M.: In *Anatomical Neuroendocrinology* (Edited by W. E. Stumpf and L. D. Grant). Karger, Basel (1975) pp. 262–269.
13. Sar M. and Stumpf W. E.: *Science* **182** (1973) 1266–1268.
14. Stumpf W. E. and Sar M.: *Fedn. Proc.* (1976) in press.
15. Grant L. D. and Stumpf W. E.: In *Anatomical Neuroendocrinology* (Edited by W. E. Stumpf and L. D. Grant). Karger, Basel (1975) pp. 445–464.
16. Heritage A., Grant L. D. and Stumpf W. E.: *Neuroscience Abstracts*, Vol. II **2** (1976) 672.

DISCUSSION

Beyer. Do you have some data on estriol?

Stumpf. Unfortunately not; I think this type of study would be important. Also, I forgot to mention the 2-hydroxyestrogens: I was surprised that nobody brought this up in the discussion of the peripheral target tissues because Fishman thinks in the brain 40–50% of extractable estrogens may be this compound. In the peripheral tissues, 2-hydroxyestrogens may also play a role.

Crabbé. Did you have a chance to look at the caudal portion of the spine for gonadal hormone uptake?

Stumpf. We studied different regions of the spinal cord (Keefer, D. A., Stumpf, W. E. and Sar, M. *Proc. Soc. Exptl. Biol. Med.* **143** (1973) 414 and Stumpf, W. E. and Sar, *Excerpta Medica, Intl. Cong. Ser.* in press). Estrogen concentrates selectively in neurons of the substantia galatinosa and lamina X, while androgen preferentially concentrates in nuclei of motor neurons of the ventral horn, in the nucleus intermediomedialis and intermediolateralis, the nucleus dorsalis of Clark and lamina X.

Beyer. Do you have some data on possible uptake by neurons from raphe nuclei?

Stumpf. Yes, neurons of various raphe nuclei concentrate estrogen and androgen.

Terrenius. Have you any evidence that steroids are localized in anything else than in neurons, for instance in glial cells or in blood capillaries?

Stumpf. Yes. Autoradiography with [^{3}H]-estradiol revealed radioactive labeling of 'adenopituicytes' (Stumpf, W. E., Sar, M. and Keefer, D. A. in *Pituitary: Ultrastructure in Biological Systems* (Edited by A. Tixier-Vidal and M. Farquhar). Academic Press, New York (1975) pp. 63–82). These may be considered modified glial cells, similar to those found in other ventricular recess organs, such as, the subfornical organ, the pineal, the area postrema and the collicular recess organ. [^{3}H]-Dexamethasone appears to be a special case: in contrast to [^{3}H]-corticosterone and the tritiated sex steroids, it localizes in neurons and glial cells throughout the brain, however, depending on concentration and time, since this compound appears to enter the brain through the cerebrospinal fluid (Reese, H. D., Stumpf, W. E. and Sar, M. in *Anatomical Neuroendocrinology* (Edited by W. E. Stumpf and L. D. Grant). Karger, Basel (1975) pp. 262–269).