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# The A-B-C (Allocortex-Brainstem-Core) Circuitry of Endocrine-Autonomic Integration and Regulation:

## A Proposed Hypothesis on the Anatomical-Functional Relationships Between Estradiol Sites of Action and Peptidergic-Aminergic Neuronal Systems[1]

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STUMPF, W. E. AND L. JENNES. *The A-B-C (Allocortex-Brainstem-Core) circuitry of endocrine-autonomic integration and regulation: A proposed hypothesis on the anatomical-functional relationships between estradiol sites of action and peptidergic-aminergic neuronal systems.* PEPTIDES 5: Suppl 1, 221–226, 1984.—A sex steroid hormone sensitive brainstem-allocortex axis of neuronal cell groups and projections is recognized with convergent pathways of aminergic-peptidergic messenger systems, which subserves the adjustment for varying reproductive and environmental conditions and the coordination of endocrine-autonomic functions. Main stations in the A-B-C (Allocortex-Brainstem-Core) periventricular axis include the substantia gelatinosa, nucleus (n.) tractus solitarii-dorsal vagal nucleus-area postrema complex, locus ceruleus, n. parabrachialis, central gray and associated raphe nuclei, ventral tegmental area, lateral and periventricular hypothalamus, n. paraventricularis, bed nucleus of the stria terminalis, preoptic-septal nuclei and n. centralis amygdalae with associated amygdaloid nuclei, as well as the ventral and dorsal allocortex. All of these stations and their periventricular and medial forebrain bundle projections contain estradiol sites of action and represent elements of earlier defined periventricular estradiol-target neuron systems. Results from colocalization of $^3$H estradiol by thaw-mount autoradiography and aminergic and peptidergic messengers by immunohistochemistry or other histochemical techniques indicate direct nuclear effects of estradiol on certain noradrenalin, dopamine, gamma aminobutyric acid, somatostatin, and neurophysin neurons. Additional data about correspondence of estradiol-target neuron accumulations with neuronal sites of peptide messenger production suggest direct effects of estradiol on certain enkephalin, endorphin, corticotropin releasing hormone, adrenalin, serotonin, cholecystokinin, pancreatic polypeptide and gonadotropin releasing hormone neurons—and probably others. As documented for the pituitary, and as an approach to understand varying and dual effects, it is postulated that estradiol activation of brain messenger systems parallels the heterogeneous estradiol binding in the A-B-C system. This is expressed in the concept of differential Multiple Activation of Heterogenous Systems (MAHS). Different hormonal states probably result in differential alterations of estradiol receptor numbers in neuronal groups, and account for related changes in the differential stimulation of activational and inhibitory messenger systems, perhaps, providing one explanation for a transfer from a positive to a negative "feedback" and related effects. These effects, thus, include "indirect" actions of the steroid by involving not only one messenger system, but a number of such systems.

Allocortex-brainstem-cortex Endocrine-autonomic regulation Peptidergic-aminergic neuronal systems

THREE histochemical techniques have greatly influenced progress in our understanding of neuroendocrine regulation. These are the formaldehyde induced fluorescence for the localization of catecholamines and indolamines in the early and mid 1960's; dry-mount and thaw-mount autoradiography for the localization of steroid hormones in the mid and late 1960's; and immunohistochemistry for the localization of peptide hormones, synthesizing and degrading enzymes and other markers in the second half of the 1970s. The results obtained with these techniques all convey and support the idea of circuits with certain chemical and functional characteristics.

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## ESTROGEN-TARGET NEURON CIRCUITS

The pattern of nuclear localization of $^3$H estradiol in neurons of the diencephalon was reported in 1968 and recognized *not* to follow the concept of a "sex center" in the basal tuberal region of the hypothalamus and *not* to support the concept of the "hypophyseotrophic area." Rather, it was observed that the distribution of estrogen target neurons follows projections of the stria terminalis [34]. This lead in 1970 to the concept of "estrogen-neuron systems in the periventricular brain" [35]. Accordingly, estrogen target neurons were postulated to exist at extrahypothalamic sites and soon were found in the amygdala [38] and other projection sites [35]. The "stria terminalis as the sex circuit" was proposed [36]. Other sites in the brain were found to be involved, remote from the hypothalamus, including the nucleus of the solitary tract in 1970 [35] and the substantia gelatinosa, dorsal horn, and lamina X of the spinal cord in 1973 [16]. A comprehensive picture about the brain-distribution of cells which contain estradiol nuclear receptors had emerged [37]. The idea of a system or systems of target neurons was further established through additional phylogenetic and ontogenetic studies, but foremost through colocalization of steroid hormones with other messengers. The prerequisite technique of combined autoradiography for steroids and immunohistochemistry was developed in our laboratory in 1975 [17].

## ANTERIOR PITUITARY ESTRADIOL SITES OF ACTION: COLOCALIZATION OF $^3$H ESTRADIOL AND PITUITARY HORMONES

Autoradiographic studies with the pituitary revealed that most of the cells of the pars distalis concentrate $^3$H estradiol [33] and that, therefore, not only gonadotropes but also other cell types must be target cells for estradiol. Subsequently, this was verified through colocalization [17]. A quantitative autoradiographic assessment of $^3$H estradiol uptake in immunocytochemically characterized pituitary cells [15] indicated differential nuclear binding when different cell types were compared with each other in female castrated rats: gonadotropes > somatotropes > lactotropes > thyrotropes. This sequence was different in the castrated male: somatotropes=lactotropes > gonadotropes=corticotropes < thyrotropes [14]. Furthermore, changes in nuclear binding and modifications of the uptake hierarchy could be induced by long-term castration, or treatment with dihydrotestosterone or progesterone [13]. These results suggest steroid hormone induced changes in hormone receptor content and related modulation of cellular response to hormone stimulation at the pituitary level. Similar effects can be expected to occur in the brain.

## GnRH NEURONAL SYSTEMS: RELATIONSHIPS TO ESTRADIOL SITES OF ACTION

Are inhibitory and stimulatory effects of estradiol on GnRH secretion mediated via direct or indirect effects on GnRH secreting neurons? An answer to this question could be found through colocalization of $^3$H estradiol and antibodies to GnRH. Anatomical sites where cell bodies of GnRH neurons and estrogen target neurons are observed, overlap to some degree in the preoptic-septal region. But, upon close inspection, in the dorsal septum many of the GnRH-positive neuronal perikarya are located in the medial septal nucleus, an area devoid of estrogen target neurons in rodents. Attempts toward colocalization of $^3$H estradiol and GnRH antibodies showed negative results thus far—perhaps, due to technical difficulties—except for one report showing one neuron in the arcuate nucleus in the fetal guinea pig with silver grains over the cell nucleus and immunoreaction in the perikaryon [42]. Further studies are required to clarify for the rat and other species whether or not estradiol directly affects GnRH neurons, and if so, which population of GnRH cells. More successful has been the immunohistochemical demonstration of connections, or suggestive connections, between aminergic-peptidergic systems and GnRH neurons and the relationship of $^3$H estradiol to perikarya of such "modulatory" systems. Contacts with or close apposition to GnRH neurons have been demonstrated for serotoninergic, noradrenergic, and GnRHergic [9] as well as for dopaminergic and GABAergic fibers [10] by immunohistochemical double staining procedures.

In combined steroid autoradiography-immunohistochemistry studies, a nuclear concentration of $^3$H estradiol has been demonstrated in neurons with perikarya characterized by positive reactions for norepinephrine, dopamine [3,5] and GABA [30]. In addition, because of close congruity between anatomical sites of estrogen target neurons and immunoreactive perikarya, a direct (genomic) effect of estradiol can be expected to exist on certain serotoninergic, adrenalinergic, enkephalinergic and cholinergic neurons, all reported to be associated with the regulation or modulation of gonadotropin secretion. Further, nuclear localization of $^3$H estradiol has been shown to exist in neurophysin and somatostatin containing neurons as well [29]. Possible effects of these and other messengers on GnRH neurons must also be considered.

There exists a considerable body of evidence for most of the listed messenger systems about effects on GnRH secretion. The observation that estradiol directly addresses these systems is of interest in the understanding of the effects of estradiol on gonadal feedback regulation and on other brain functions.

## CATECHOLAMINE NEURONAL SYSTEMS: ESTRADIOL SITES OF ACTION

Effects of catecholamines on ovulation and effects of estradiol on brain catecholamine turnover are well documented. There is prevailing evidence that norepinephrine plays an important if not decisive role as a trigger for GnRH release prior to ovulation. Evidence for direct effects of sex steroids on catecholamine neurons was provided through combined autoradiography-formaldehyde induced fluorescence with $^3$H estradiol [5] and $^3$H dihydrotestosterone [6] and combined autoradiography-immunohistochemistry with $^3$H estradiol and antibodies to dopamine-beta-hydroxylase [29]. From these studies, it appears that 50–80% of the catecholaminergic cells in areas A1, A2, A6 and A7 and 30–40% in area A6 (locus ceruleus) concentrate $^3$H estradiol in their nuclei. Considering the likelihood of false negatives due to weak fluorescence or fluorescence fading and limited immunohistochemical detectability, it can be inferred that a large population of the catecholamine neurons in these areas contain receptors for estradiol and are thus activated according to blood levels of estradiol. Since the n. of the solitary tract (A2) and the n. reticularis lateralis (A1) are both interconnected with the rostral and central hypothalamic regions [2,27], a preovulatory increased turnover of catecholamines is observed, and a disruption of the ventral adrenergic bundle interferes with ovulation [4,20], the stimulus for ovulatory GnRH release is likely to involve estrogen-stimulated

catecholamine release from medulla oblongata originating projections.

Nuclear localization of $^3$H estradiol in dopaminergic neurons in the periventricular hypothalamus has also been observed [3], which agrees with the reported activation of dopamine turnover by estradiol.

### GABAERGIC NEURONAL SYSTEM: ESTRADIOL SITES OF ACTION

Colocalization of $^3$H estradiol and gamma-aminobutyric acid (GABA) decarboxylase antibodies [30] has been demonstrated in neurons in the periventricular region of the preoptic hypothalamus. The studies are incomplete and further congruent sites can be expected to be documented. Modulatory effects of GABA on LH release have been shown to exist by Wuttke and coworkers and have been found to be mediated by estradiol stimulation through increased GABA turnover [25,43].

### OTHER AMINERGIC-PEPTIDERGIC NEURONAL SYSTEMS AND ESTRADIOL SITES OF ACTION

Close anatomical correspondence of estrogen target cell accumulations with certain immunohistochemically characterized neurons and their projections exist for neurotensin, opioid peptides, serotonin, corticotropin-releasing hormone, pancreatic polypeptide, neurophysin, as well as somatostatin and substance P.

#### *Neurotensin*

Estrogen-addressed neurotensinergic neurons [8] can be expected to exist in the lateral septum, the bed nucleus of the stria terminalis, the medial and central nucleus of the amygdala, the lateral hypothalamus, the arcuate and periventricular hypothalamic nuclei, the raphe nuclei, the area postrema, the n. tractus solitarii, the n. ambiguus, and the substantia gelatinosa. Most likely, certain neurotensin neurons can be addressed by estradiol, whatever the undefined neurotensin effects will be.

#### *Opioid Peptides*

Since morphine has been known to inhibit ovulation [1], morphine related peptides can be expected to affect GnRH secretion. This has been studied and reviewed by Kalra (1983), who proposed that endogenous opioid peptides pose an axo-axonic link to catecholaminergic neurons, which are known to act upon GnRH neurons, and may, thus, exert an inhibitory effect on GnRH release prior to ovulation. The question remained open: how is the opioid system, which has been shown to mostly act inhibitorily, activated. A comparison of the localization of enkephalin neurons with estrogen concentrating neurons shows topographic correspondence in several regions, including the bed nucleus of the stria terminalis, paraventricular nucleus, n. perifornicalis, lateral hypothalamus, central nucleus of the amygdala, n. of the solitary tract and substantia grisea of the spinal trigeminal nucleus [7]. It is likely that in some of these regions, if not in all of them, estradiol is acting directly on enkephalinergic neurons. Although the presently available evidence strongly suggests effects of opiates and related peptides on GnRH secretion, it remains to be established whether or not such effects occur during the normal female cycle or only under certain stress conditions. The system probably is sensitized by estradiol.

#### *Serotonin*

Serotonin has long been known to affect ovulation, and is now recognized as being not only inhibitory, but also stimulatory under certain conditions [19]. Turnover of serotonin is enhanced by estradiol, suggesting an influence of gonadal steroids on serotonin secretion.

$^3$H estradiol localization has been reported to exist in the rodent brain in almost all raphe nuclei [37], which include, the n. raphe dorsalis, n. raphe pontis, n. raphe magnus, n. raphe obscurus and n. raphe pallidus. Especially in the n. raphe dorsalis, the percent index of $^3$H estradiol nuclear labeling is high, which suggests a direct action of estradiol on serotoninergic neurons. Since coexistence of serotonin, substance P and thyrotropin releasing hormone (TRH) in certain of these neurons has been demonstrated [11], estradiol effects on substance P and TRH secretion must be considered.

Effects of serotoninergic projections on certain estradiol target neurons can also be expected from the anatomical correspondence of certain projection sites [32] with the presence of estrogen target cells, for instance, in the ventral hypothalamus, medial amygdala, olfactory tubercle, periventricular thalamus, central grey, n. tractus solitarii and substantia gelatinosa.

#### *Corticotropin-Releasing Hormone*

The strongest accumulation of corticotropin-releasing hormone (CRH) immunoreactive cells is found in the paraventricular nucleus [21, 22, 26, 40] and corresponds with the presence of estradiol target cells in its parvo and magnocellular components. In addition, anatomical correspondence is conspicuous in the n. centralis amygdalae, n. interstitialis striae terminalis, n. preopticus medianus and periventricularis, n. parabrachialis, locus ceruleus, n. tractus solitarii, n. motorius nervi vagi, and substantia gelatinosa and lamina X of the spinal cord. Scattered labeled neurons in the n. arcuatus hypothalami, in the hippocampus, as well as in the allocortex are also candidates for CRH positive estradiol target neurons. The CRH-stained pathways and areas interconnected by these pathways overlap with those reported for neurotensin, pancreatic polypeptide, and catecholamines. All of them use the periventricular system and the medial forebrain bundle. Included in the CRH pathways are the central nucleus of the amygdala, substantia innominata, bed nucleus of the stria terminalis, preoptic region, lateral hypothalamus, central gray, dorsalolateral tegmental nucleus, locus ceruleus, parabrachial nucleus, dorsal vagal complex and n. reticularis lateralis. In all of the listed stations, neurons which concentrate estradiol are found. The close anatomical relationships between CRH immunoreactive perikarya and projections with target sites for estradiol argue for close functional interactions at these sites between the gonadal and adrenocortical systems.

#### *Pancreatic Polypeptide*

Comparing the extensive distribution of bovine pancreatic polypeptide-like immunoreactive neurons in rat brain [24] with the location of estradiol concentrating neurons, overlap can be noted at many sites. These include, the n. olfactorius anterior, n. septi lateralis, n. interstitialis striae terminalis, n. tractus diagonalis, n. centralis amygdalae, n. arcuatus hypothalami, zona incerta, griseum centrale, locus ceruleus, n. tractus solitarii, n. reticularis lateralis, certain

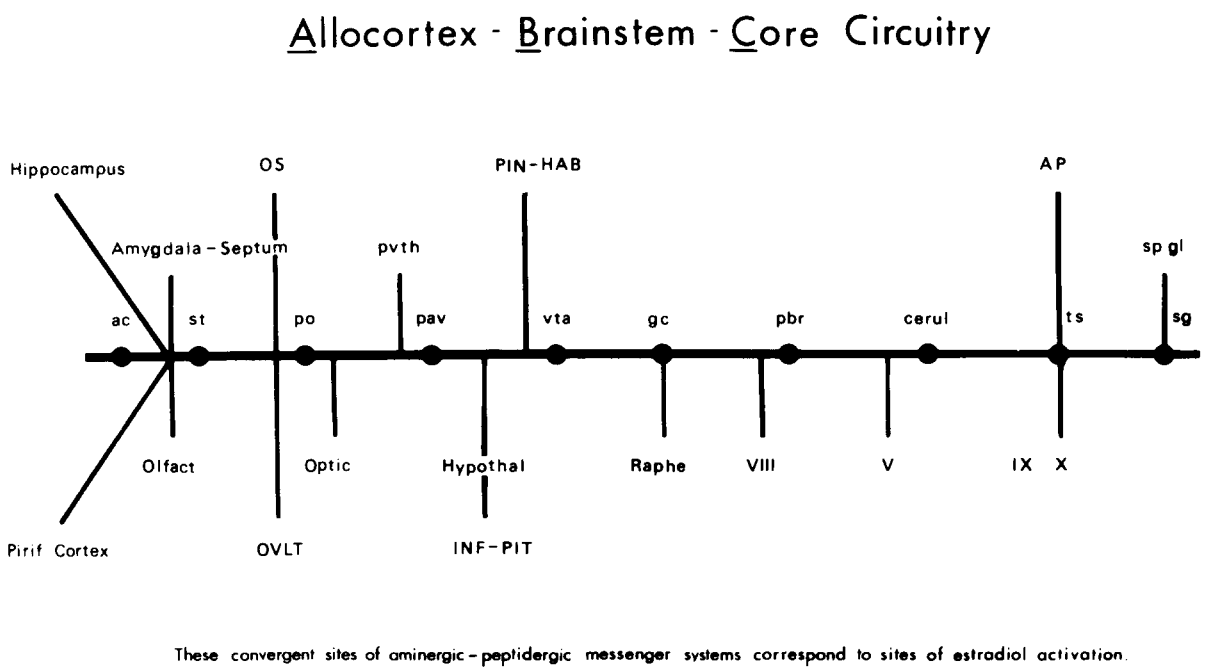


FIG. 1. The endocrine-autonomic A-B-C (Allocortex-Brainstem-Core) circuitry, depicted by the thick horizontal line, represents projections and coordinating nuclear groups of aminergic-peptidergic messenger systems and includes various periventricular brain stem pathways, the medial forebrain bundle, the stria terminalis and the fornix, as well as the stria medullaris and the fasciculus retroflexus. The various related nuclear groups and associated circumventricular organs all contain estradiol sites of action. The number of nuclear estradiol receptors in the differential neuronal groups varies. Therefore, the concept of *differential Multiple Activation of Heterogenous Systems (MAHS)* was proposed [39]. The A-B-C circuitry probably is both sensory and secretomotor. Piriform cortex and hippocampus as representatives of the limbic allo-cortex are characteristically linked with structures in the amygdala and septum: ac=nucleus (n.) centralis amygdalae, st=n. interstitialis striae terminalis, OS=organum subfornicale, OVLT=organum vasculosum laminae terminalis; the hypothalamus: po=preoptic region, pav=n. paraventricularis, INF-PIT=ventromedial hypothalamic nuclei and pituitary); the thalamus: pvth=n. periventricularis thalami and habenular-pineal complex; vta=ventral tegmental area; midbrain-pons with raphe nuclei, gc=griseum centrale, pbr=n. parabrachialis medialis and lateralis, cer=locus ceruleus; and medulla and spinal cord: AP=area postrema, ts=n. tractus solitarii, sg=substantia gelatinosa, sp gl=spinal ganglia. Endoceptive and exoceptive input from cranial nerves and spinal ganglia is indicated.

raphe nuclei, n. ambiguus, and substantia gelatinosa. Of interest is also the coexistence of pancreatic polypeptide within catecholamine neurons of groups A1, A2 and A6 [7,18] where colocalization with $^3$H estradiol has been reported. Since little is known about the role of pancreatic polypeptide in the brain, further information needs to be obtained.

### *Projections of Neurophysin Neurons*

Projections from neurophysin containing perikarya in the paraventricular nucleus [23,31] include nuclear groups, such as, the spinal cord substantia gelatinosa (and lamina X and n. intermediolateralis), n. tractus solitarii, locus ceruleus, n. parabrachialis, central gray, ventral tegmental area, epithalamus, septum and amygdala. Sites of origin as well as projection are known target areas for estradiol.

## CONCLUSION

Information about the distribution of estradiol sites of action and monoamine and peptide messenger sites of production and projection reveals a corresponding brainstem circuitry, common to the various types of messengers. A central or truncate circuitry emerges with common nuclear groups and pathways apparently subserving endocrine-autonomic regulation and coordination of reproduction, cardio-vascular function, respiration, temperature, feeding and drinking, as well as various forms of behavior, mood and emotion. Different messenger systems with varying and special emphasis to endoceptive and exoceptive sensory input are interlinked in this brainstem-allocortical-integrating and coordinating system. Its major stations are depicted in the figure and include the substantia gelatinosa (also lamina X and the n. intermediolateralis) of the spinal cord and medulla, the nucleus of the solitary tract, the locus ceruleus, the central gray, the ventral tegmentum, the paraventricular nucleus, the periventricular nucleus, and other hypothalamic nuclei, the amygdaloid central nucleus and other amygdaloid nuclei, as well as the bed nucleus of the stria terminalis and the septum. The latter structures are closely linked to the allocortex, also known as the limbic cortex. The hypothalamic paraventricular nucleus appears to play a pivotal role because of its complexity and presence of many different peptidergic neurons and coexistence of different peptides in the same neuron [42]. Other similarly complex neuronal groups exist in this circuitry, such as, the nucleus of the solitary tract, locus ceruleus, bed nucleus of the stria terminalis and central nucleus of the amygdala.

The Allocortex-Brainstem-Core (A-B-C) circuitry concept of endocrine-autonomic integration and regulation evolves from our earlier concept of the neuroendocrine periventricular brain [35]. Seemingly, it shows some resemblance to the concept of the "limbic system." But, the A-B-C concept differs: it encompasses components of the

whole brainstem and the spinal cord. The emphasis is on the brainstem origin of innervation, and the allocortex is perceived as an anatomical and functional outgrowth of the brainstem. It is, conceptually and semantically not a "limbic" system but rather a "central" or "core" brainstem system. Unlike the variably defined and forebrain (mesencephalon) restricted "limbic system," the dominant structures are not cortical, but reside in the circuits formed by medullary-pontine-mesencephalic-hypothalamic-septal nuclear groups. The limbic system concept, although useful, remained unsatisfactory. The present concept is in agreement with the notion that a characteristic feature of the limbic system is its non-"limbic" truncate nature of circuitry [35,36].

Estradiol nuclear concentration of neurons in the A-B-C system is *not* uniform and varies among individual neurons as well as certain nuclear groups. This suggests differential activation by estradiol, that is, different messenger systems are stimulated at a different functional level. As has been shown by Keefer [13] for the pituitary: treatment with steroid hormone or removal of steroid hormone secreting glands changes the number of estradiol binding sites differentially and characteristically for certain endocrine conditions and cell types. There are indications that similar mechanisms as demonstrated for the pituitary, and also for the uterus, exist in the brain. Such a steroid hormone receptor shift, together with a related differential Multiple Activation of Heterogenous Systems (MAHS) [39] is probably a cause for positive and negative "feedback" and other "dual" actions of steroid hormones.

The Allocortex-Brainstem-Core circuitry concept can be invoked as further support for our earlier arguments against the limited or erroneous concepts of a "sex center," and "the hypophyseotrophic area." The brainstem aminergic-peptidergic circuitry permits steroidal and environmental activation and tuning of various somatic, endocrine, autonomic and behavioral functions related to the needs of reproduction. All components of the endocrine-autonomic A-B-C systems seem to be involved to varying degrees: Reproduction is cardio-vascular, is respiratory, is sensory, is behavioral, and others. Estrogenic activation of GnRH release must be coupled to these functions, probably mediated by the simultaneous estrogenic sensitization and arousal of various messenger systems. Such diversity may account for the possibility of multiple elicitation, disturbance and adaptation of the GnRH system and other systems. The anatomical arrangement allows for a multiplicity of neural and humoral interactions, compensatory mechanisms, and variable and alternate routes of stimulatory and inhibitory activation.

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