MINIREVIEW

Estrogen Synthetase (Aromatase) Immunohistochemistry Reveals Concordance Between Avian and Rodent Limbic Systems and Hypothalami

FREDERICK NAFTOLIN,*[,1] TAMAS L. HORVATH,* and JACQUES BALTHAZART†

*Reproductive Neuroscience Unit, Department of Obstetrics and Gynecology and Center for Research in Reproductive Biology, Yale University, New Haven, Connecticut 06520; †Center for Cellular and Molecular Neurobiology, Research Group in Behavioral Neuroendocrinology, University of Liège, B-4020 Liège, Belgium

During amniote evolution, an early divergence occurred about 300 million years ago between the reptilian lines leading to the appearance of birds (anapsids) and mammals (synapsids). The different functional requirements of these vertebrate groups have involved divergent evolution of their brains. Even superficial examination reveals major anatomical differences between mammalian and avian brains, such as extensive development of the optic lobes and cerebellum in birds and a highly developed cortex in mammals. It has been nearly impossible to identify avian homologs of some mammalian brain regions by standard morphological criteria. This has long frustrated efforts at clarifying hypotheses regarding the anatomical location, field size, and regulation of brain functions shared between these two classes, despite the certainty that the principles of neurobiology apply equally at the cellular level in both groups. In an effort to remove this barrier, we have sought markers of common function that despite apparent anatomical dissimilarity, can allow recognition of homologous brain structures. We illustrate here how comparative analysis of the distribution of the steroid-metabolizing enzyme estrogen synthetase (aromatase) may help to understand the differences and similarities in the limbic system and hypothalamus of birds and mammals.

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[1] To whom requests for reprints should be addressed at Department Obstetrics and Gynecology, Yale University, School of Medicine, 333 Cedar Street, FMB 335, New Haven, CT 06520–8063. E-mail: Frederick.Naftolin@Yale.edu

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During the evolution of amniotes, an early divergence occurred about 300 million years ago between the reptilian lines that produced birds (anapsids) and mammals (synapsids) (1). The different functional requirements of successful adaptation for these two vertebrate classes involved a divergent evolution of their brains' anatomy. Even a superficial macroscopic examination reveals major differences such as the extensive development of the optic lobes and cerebellum in birds (presumably associated with the capacity to fly and the need to see small objects at great distances) and the presence of a developed and markedly expanded cerebral cortex in mammals (presumably related to the development of some aspects of higher brain functions).

Further, there were evolutionary disconnects between periods of positive adaptations so that flight and cortical development appear not to have had different evolutionary schedules. The resultant mix of neuronal and glial components from this evolutionary pushing and pulling on brain structures has differentially affected specific parts of the brain. It is, for example, nearly impossible to identify avian homologs of the mammalian hippocampus or amygdala by simple morphological criteria. In contrast, brain regions such as the preoptic area and hypothalamus, which regulate fundamental and usually more primitive physiological functions (i.e., reproduction or feeding), are more similar in

these two vertebrate classes, indicating both functional similarity and sturdiness in evolution.

To further complicate matters, brain nuclei and tracts were originally described on the basis of their gross or tinctorial appearance, leaving homologies between groups to be detected by size, shape, and relative location of these structures. As described above, this approach has been confusing when extensive adaptive changes have taken place, and forms similar to the common ancestor have been lost. These are problems frequently encountered when comparing birds and mammals. However, since all brain structures develop from the neural tube, homologies can be deduced from comparative study of ontogenetic development, the connections of the structures under examination, and functional responsibilities of cells in the studied regions. This article focuses on the latter approach.

Neurochemical Markers as Indicators of Brain Homology

The development of chemical neuroanatomy has provided a reliable and penetrating approach to comparative neuroanatomy. Immunohistochemical markers identifying neurotransmitters, neuropeptides, and their receptors are discretely distributed in the central nervous system. Since these biomarkers are associated with specific functions, it is usually assumed that cell groups containing such markers have remained relatively stable in the course of evolution. Therefore, the presence of the same neurochemicals in nuclei suspected to be homologous in different species, is often considered to be a key argument for defending nuclear homology. This approach has been used in studies analyzing the functional and phylogenetic relationships of different parts of the basal ganglia in amniote vertebrates (2). It must, however, be noted that there are contributions to the available aromatase made by migrating microglia (3), and there have been reports of the presence of aromatase in reactive glial cells (4, 5) that must be taken into account.

We illustrate below how the comparative analysis of the distribution of a specific and highly conserved steroid-metabolizing enzyme, estrogen synthetase (aromatase), may allow progress in understanding differences and similarities in the limbic system and in particular the preoptic area POA) hypothalamus of birds and mammals.

Earlier Studies of Brain Aromatase

Estrogen synthetase or aromatase (EC 1.14.14.1) is a key demethylase that irreversibly catalyzes the transformation of androgens into estrogens. This enzyme plays a crucial role in the control of many physiological and behavioral processes both during ontogeny and in adulthood (6–11). Its presence in the brain was first postulated based on enzyme activity measurements (12). Following the development of sensitive assays, aromatase activity was observed in the brain of representative species of all vertebrate classes with the possible exception of jawless fish (Agnatha) (13–15).

By combining the microdissection technique of brain samples with these sensitive assays (16–18), a relatively precise localization of aromatase was obtained in brains of a number of species including the rat (19), monkey (20), Japanese quail (21), and zebra finch (22). This biochemical approach revealed similarities between the distribution of aromatase activity in avian and mammalian brains, but did not permit analysis of specific nuclei. For this, aromatase localization had to reach the cellular level of resolution. It is only recently that reliable immunocytochemical (23) and *in situ* hybridization techniques could be established that allowed one to visualize this enzyme in the brain, first of birds (especially Japanese quail) (24, 25) and subsequently of mammals (see below).

Distribution of Aromatase-Containing Cells in the Quail Limbic System and Hypothalamus

For years, antisera raised against nonbrain aromatase failed to identify this enzyme in the vertebrate brain, either because of its low concentration or because of the absence of cross-reaction with brain enzyme isoforms. About 10 years ago, a polyclonal antiserum raised against purified human placental aromatase (23) was, for the first time, successfully used to visualize aromatase-immunoreactive cells in the quail brain (24, 25). More recently, this distribution was confirmed and extended using a more sensitive approach based on a homologous polyclonal antiserum directed against recombinant quail aromatase that had been expressed in *Escherichia coli* and subsequently affinity-purified (26). Subsequently, the partial sequence of quail aromatase that had been identified (27) was used to generate a specific RNA probe that also allowed the study by *in situ* hybridization of the neuroanatomical distribution of aromatase in the quail brain (28). Concordant information was obtained by these complementary experimental approaches. These studies identified, in coronal sections, four major groups of aromatase-immunoreactive (ARO-ir) cells in the quail brain: in the POA, one group is centered on the sexually dimorphic medial preoptic nucleus (POM) (29, 30) and defines its cytoarchitectonic boundaries; in a dorso-lateral position to this preoptic cell group, one other group extends from the most caudal part of the ventral paleostriatum to the caudal end on the anterior commissure; at this level, this dorsal group merges with the dorsal aspects of a V-shaped aromatase group that was originally described as overlapping the bed nucleus of the stria terminalis (BNST; see also below for a more accurate interpretation of this group); and finally, a less dense but more widespread group of ARO-ir cells runs in the tuberal region from the dorso-lateral edges of the ventromedial nucleus of the hypothalamus (VMN) to the level of the nucleus inferior hypothalami (see Figs. 1 and 2). Scattered weakly stained cells were also observed in the nucleus taeniae, the avian homologue of parts of the mammalian amygdala, and in a few other locations such as the mesencephalic central gray or the dorso-lateral edges of nucleus ovoidalis (26). A similar distribution of aromatase-immunoreactive cells has been identified by immunocyto-

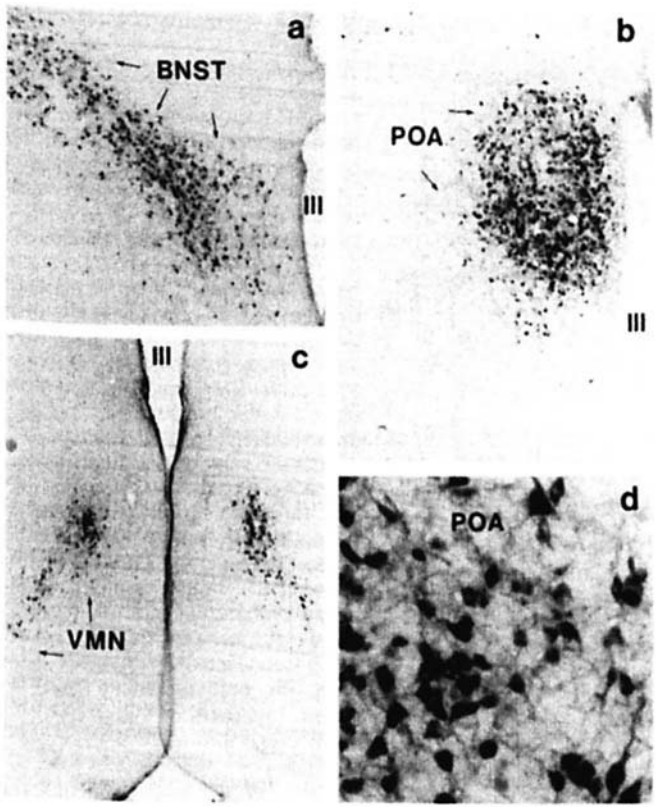

Figure 1. Photomicrographs illustrating the distribution of ARO-ir cells in the quail brain. (a) V-shaped group of cells centered on the BNST. (b) Cluster of ARO-ir cells in the medial POA outlining the sexually dimorphic POM. (c) ARO-ir cells in the medial hypothalamus at the level of the VMN. (d) High magnification of cells in the POA. III third ventricle.

chemistry with the same antibody in another avian species, the ring dove (*Streptopelia risoria*), as well as in one song bird species, the zebra finch (*Taeniopygia guttata*) (25, 31). In songbirds, large populations of aromatase-immunoreactive cells are additionally located in telencephalic regions, mainly in the medial and lateral part of the neostriatum and in the hippocampus (31).

Distribution of Aromatase Immunoreactivity in the Rodent Limbic System and Hypothalamus

Since the first studies of aromatase in mammalian brains were limited to positive findings of enzyme activity in relatively large blocks of brain (6, 19), the detailed discussion of apparent functional-anatomical homologies with avian species will be limited to description of immunocytochemical findings. Even so, major discrepancies were present in the first published reports describing immunocytochemical methods the distribution of ARO-ir cells in the rat brain (32–36). There were also differences between immunocytochemical data and biochemical assays quantifying aromatase activity in microdissected brain samples (19). Similarly, the first immunocytochemical study of the mouse brain using the antibody that had successfully localized aromatase in the quail (23–25) only identified one major population of ARO-ir cells in the dorso-lateral parts of the hypothalamus, in the area of the zona incerta (37). However, technical improvements to the immunocytochemical methods subsequently provided detailed anatomical results that match to a large extent the distribution of aromatase activity and molecular biological findings (see below).

Using the above methods in rats, the hypothalamic aromatase system was similar to what had been described in the mouse brain. It includes neurons scattered in the dorsolateral hypothalamic area, including the paraventricular, lateral, and dorsomedial nuclei, the subincertal nucleus, and the zona incerta (38). This system responded to gonadectomy by decreased immunostaining (38). In addition, the same authors identified a limbic system of ARO-ir cells that was composed of large populations of perikarya forming a continuous ring extending from the lateral septal area to the central amygdaloid nucleus, including the laterodorsal division of the BNST, the stria terminalis, and the substantia inominata-ventral pallidum-fundus striatal region (see Figs. 2 and 3). This group of cells did not change its immunohistochemical staining following gonadectomy. The implications of a gonad-sensitive and -insensitive system are broad, but need to be tested. The available biochemical data (39) are, however, consistent.

This study, however, still failed to detect immunoreactive cells in several brains regions such as the medial preoptic area, the medial amygdala, and the ventromedial nucleus of the hypothalamus, which were previously shown to contain high levels of aromatase activity (19, 40). A similar distribution of ARO-ir cells has also been reported in the female musk shrew (*Suncus murinus*) (41).

The immunocytochemical discrepancies have been partially resolved by amplifying the immunoreactivity in the brain for aromatase through the use of aromatase inhibitors. This is permissible, since these are generally issues of localization, not quantitation. Independent physiological studies have identified an increased immunoreactivity for aromatase induced by steroidal aromatase inhibitors such as Vorozole (42) or Fadrozole (42, 43). Although the basis of this amplification is not known, it has provided better visualization of aromatase. When applied to brains from mice that had been pretreated with Vorozole, the presence of dense populations of ARO-ir perikarya was confirmed in the septal region, the BNST, the central amygdala, and the dorso-lateral parts of the hypothalamus. In addition, weakly labeled, but clearly positive cells were observed in the medial preoptic nucleus, the ventromedial nucleus of the hypothalamus, and the medial amygdala (44). Therefore, a good neuroanatomical correspondence between aromatase activity and the presence of ARO-ir cells has been confirmed in rodent brains. Resolution of apparent differences such as the gonad-responsive hypothalamic system versus the nonresponsive limbic ring will require methods beyond the scope of this anatomical review.

The distribution of cells expressing aromatase has also been investigated by *in situ* hybridization in the rodent brain. This confirmed the presence of aromatase-expressing

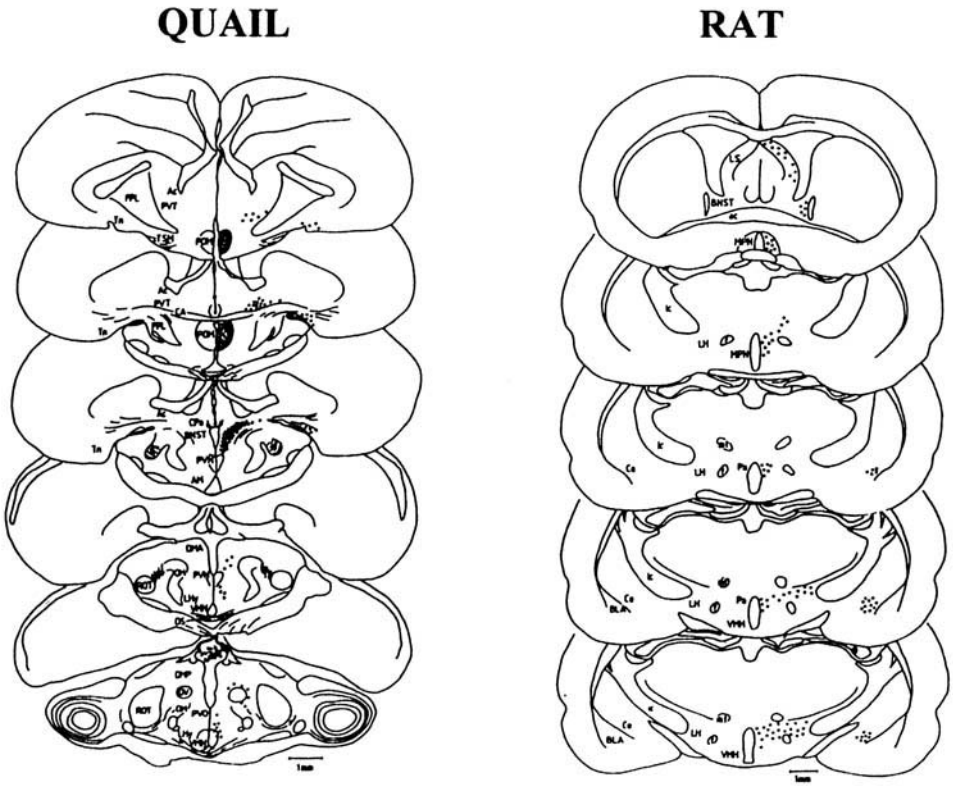

Figure 2. Schematic presentation of the distribution of aromatase in the quail (left) and rat (right) brain. Data illustrate the broader distribution of aromatase-positive cells that has been reported to this date with the use of either immunocytochemistry for visualization of the enzyme protein of *in situ* hybridization for its mRNA. Data derived from Refs. 24–26, 28, 37, 38, 44–47. Ac, nucleus accumbens; AM, nucleus anterior medialis hypothalami; BLA, basolateral amygdaloid nucleus, anterior part; CA, commissura anterior; Cpa, commissura pallii; DMA, nucleus dorsomedialis anterior thalami; DMP, nucleus dorsomedialis posterior thalami; DS, decussatio supraoptica; f, fornix; FPL, fasciculus prosencephali lateralis (lateral forebrain bundle); ic, internal capsule; LH, lateral hypothalamic area; Lhy, nucleus lateralis hypothalami; LS, lateral septum; MPN, medial preoptic nucleus; mt, mammillothalamic tract; OM, tractus occipitomesencephalicus; OV, nucleus ovoidalis; Pa, paraventricular hypothalamic nucleus; PVO, organum paraventriculare; PVT, paleotriatum ventrale; ROT, nucleus rotundus; Tn, nucleus taeniae; TSM, tractus septomesencephalicus.

cells in all brain regions that were shown to contain ARO-ir perikarya, including the medial preoptic nucleus, nucleus striae terminalis, and ventromedial nucleus of the hypothalamus (45–49). In some studies, however, the distribution of aromatase mRNA was suggested to be broader than the distribution of aromatase activity. This discrepancy presumably reflects the detection by *in situ* hybridization of noncoding transcripts or of transcripts coding for nonfunctional protein (for example, see Ref. 46).

Taken together, the immunocytochemical and *in situ* hybridization studies, therefore, identify a distribution of aromatase-expressing cells that matches fairly well the distribution of aromatase activity as identified by enzyme assays on microdissected brain regions (19, 50). Some quantitative discrepancies are, however, still present between immunocytochemical and biochemical studies, and, in particular, it appears that using presently available antisera, the number of ARO-ir cells identified in areas such as the POA and ventromedial nucleus of the hypothalamus remains at odds with the very high level of enzyme activity measured in these nuclei. Since these regions contain fairly large numbers of cells containing high densities of aromatase mRNA, perhaps some of these apparent disparities will be explained by the demonstration of aromatase-immunoreactivity in neural processes and axon terminals, far from the aromatase-positive somata (see below), or they may reflect post-transcriptional differences such as alternate splicing of the aromatase gene products (51–53).

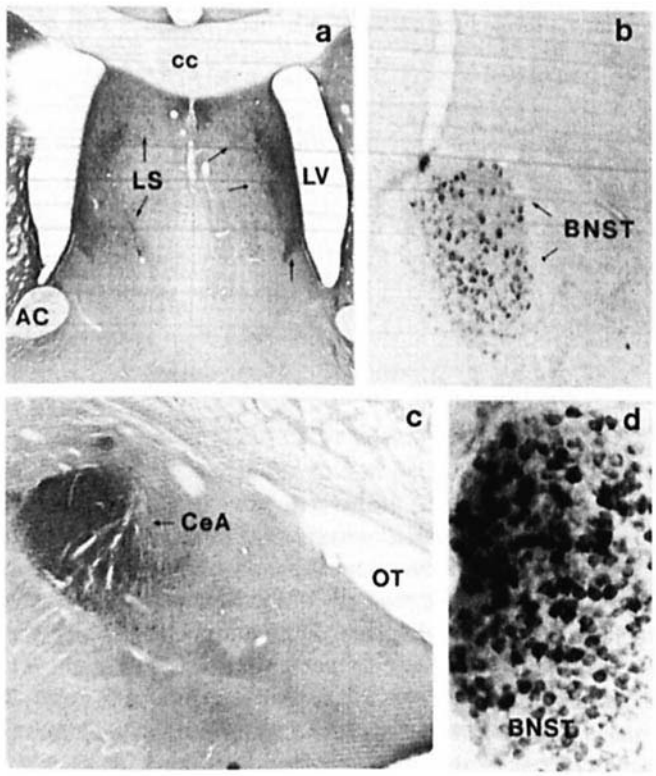

Figure 3. Light micrographs (a–d) of ARO-ir cells in the rat limbic system. (a–c) The distribution pattern of ARO-ir neurons in the lateral septum (LS), BNST, and central amygdala (CeA). (d) A high power magnification of ARO-ir cells in the BNST. AC, anterior comissure; cc, corpus callosum; OT, optic tract; LV, lateral ventricle.

Ontogeny Recapitulates Phylogeny

In rats, a peak in aromatase activity takes place during the critical period of sexual differentiation around birth, in the third week post-conception (6, 54). This high aromatase activity in young subjects is reminiscent of the high activity that is observed throughout the brain of lower vertebrates such as fish and amphibians. The evolution of vertebrates has indeed been accompanied by a progressive decrease in the total brain aromatase activity and in more specific localization of this enzymatic activity in limited brain areas (13–15). In tetrapods, aromatase activity appears to be lower than in fish and it becomes more concentrated in specific brain areas that bear direct relationship to functions such as reproduction.

The high aromatase activity in young rodents, however, provides additional opportunities to identify the localization of the enzyme by immunocytochemical methods. This biochemical anatomy can enrich our understanding of function and evolutionary changes in the brain of avian and mammalian species.

Three major groups of ARO-ir cells showing specific patterns of ontogenetic development were identified by immunocytochemistry (55) during this period in the anterior medial preoptic nucleus, the periventricular preoptic nucleus, the strial part of the preoptic area, and the rostral portion of the medial preoptic nucleus (during late embryonic/early postnatal phase); a medial preoptico-amygdaloid neuronal arc that extends from the medial preoptic nucleus through the principal nucleus of the BNST to the posterodorsal part of the medial amygdaloid nucleus (around birth); and in the lateral septal nucleus, the oval nucleus of the BNST, and the central amygdaloid nucleus (after postnatal Day 14, i.e., 5 weeks post-conception). *In situ* hybridization studies confirm this distribution and ontogeny of the aromatase system in the rat brain (45). Although the general localizations of ARO-ir cells described for the rodent correlate well with the finding in avian species, specific details of the distributions are better seen by molecular biology techniques.

Homologies between Avian and Mammalian Nuclei Suggested by Aromatase Neuroanatomy

Taken together, the above studies provide a consistent view of the distribution of the aromatase enzyme in both the avian and mammalian brains. This "functional map of the brain" allows correlation between functional-anatomical areas in avian and rodent species. Some examples follow that are diagrammed in Figure 2.

The POA. Previously, a sexually dimorphic nucleus (SDN) had been identified in the medial POA of rats (56, 57) and quail (the POM) (29, 30). However, the structures identified in rat and quail appeared to be dissimilar: the rat SDN is at least 10 times smaller than the quail POM and is not, or only marginally, affected by steroids in adulthood. In rats, the SDN differentiates during ontogeny under the influence of perinatal steroids (58). In contrast, the POM in adult quail is steroid-sensitive (its volume regresses after castration and is increased by a treatment with testosterone) (30, 59), but it is not affected in a permanent manner by embryonic treatments with estrogens that are known to irreversibly differentiate male-typical copulatory behavior (30, 60). The correspondence between sexually dimorphic structures described in the rat and quail POA's was, therefore, unclear until examined for concordance of aromatase staining.

Aromatase immunocytochemistry revealed that the quail POM is specifically labeled by the presence of a high density of ARO-ir cells. The cytoarchitectonic boundaries of the nucleus can be identified in sections stained by immunocytochemistry for aromatase (24, 25). On the other hand, no specific arrangement of ARO-ir cells has been observed in the rat SDN. This is of interest, since the determinant of final nuclear size in the rat SDN has been proven to be estrogen (61, 62), presumably formed *in situ* from androgens by aromatase (8). *Inter alia,* in respect to the homologies between the aromatase cells in anterior diencephalons, there is a preoptic population of aromatase cells identified in all studies within the medial preoptic nucleus (MPN) of the rat, allowing definition of its cytoarchitectonic boundaries, which suggests that this structure is homologous to the quail POM. Interestingly, physiological studies have shown that the volume of the rat MPN is also larger in males than in females and is affected by experimental manipulation of the circulating levels of testosterone in adulthood (63). The similarity of the endocrine controls of the rat MPN and quail POM, therefore, provides additional support for the homology of these structures.

Recent studies indicate that a specific part of the rat POA does contain aromatase neurons. In the neonatal rat, this neuronal population can be detected by immunocytochemistry (55) and by *in situ* hybridization of the aromatase mRNA (45, 64). Since the aromatase activity decreases during postnatal life, it becomes difficult to identify ARO-ir neurons in the POA of adult rats. However, a recent *in situ* hybridization study clearly identified ARO-ir neurons in the POA of adult males and females (46, 47), suggesting that immunocytochemistry may lack sensitivity or that different aromatase proteins are expressed during development.

When mice (and rats) are pretreated with a nonsteroidal aromatase inhibitor, a major increase in the density of the ARO-ir signal is observed. This has been interpreted as the reflection of an increased concentration of the enzymatic protein (65). For example the preoptic population of ARO-ir cells becomes visible (44). This implies that aromatase levels may change with age, even becoming undetectable without augmentation by this technique. Similar implications may be drawn from the finding that the highest levels of aromatase activity have been found in the developing rat and monkey (66). Furthermore, our recent *in vitro* studies

indicate that the neuroprotective effects of aromatase/estrogen on hypothalamic cells may be regulated by the type of estrogen receptor subtype expressed (67).

Mammalian and Avian Limbic Structures: The Extended Amygdala. The amygdala is an important component in the limbic system of mammals. It plays a pivotal role in a variety of behavioral and neuroendocrine functions. The extended amygdala includes brain nuclei that are connected to or continuous with the several subdivisions of amygdala and play a role in limbic function. To relate these structures with avian analogs, it is instructive to focus on parts of the extended amygdala: the central nucleus of the amygdala (CeA), the laterodorsal division of the BNST, and the substantia innominata-ventral pallidum-fundus striati region along the anterior commissure that connects the CeA and the BNST. In the rat, in addition to obvious anatomical landmarks, a continuous body of ARO-immunoreactive cells identifies this extended amygdala (38).

In quail, the homologous structures have previously been difficult to identify anatomically, but the nucleus taeniae is considered a part of the avian archistriatum that may be homologous to parts of the mammalian amygdaloid complex (68). Our immunocytochemical studies have demonstrated the presence of ARO-ir cells in this nucleus (26, 69), reinforcing the homology. In rats and mice, ARO-ir cells are heterogeneously distributed in the amygdala with higher densities of strongly labeled cells being present in the CeA (38, 44). While the density of ARO-ir cells is low in the quail nucleus taeniae compared with the CeA of rodents, it is the only nucleus in this area of the brain that contains ARO-ir cells. This could be taken as neurochemical evidence suggesting homology between the mammalian CeA and the avian nucleus taeniae. However, a variety of other criteria suggest that the nucleus taeniae is rather homologous to the medial amygdala of mammals (see Ref. 70 for additional discussion of this topic). The apparent conflict between species homologies based on aromatase distribution in the extended amygdala serves as a reminder that this form of anatomical rationalization has its limits. In these cases, additional techniques may be necessary to formally identify, if possible, the avian homologs of the different subdivision of the mammalian amygdala.

Finally, the anatomical identification of the avian homolog of the mammalian BNST in birds has been, until recently, elusive and a matter of discussion. A good candidate for this homology in quail is at the lateral and dorsal edges of the caudal part of the medial preoptic nucleus, a region with high neuronal density and a relative homogeneous cell typology in Nissl-stained sections (71). Rostrally, this area reaches the anterior commissure that is surrounded (dorsally and ventrally) by the more rostral portions of this cell cluster. At more caudal levels, the cell group assumes a V-shaped organization and is characterized by a high density of ARO-ir cells (24, 26, 69). Interestingly, both *in situ* hybridization (45) and immunocytochemistry (50) have identified a similar V-shaped structure in the same part of the brain in young rats. Because of these neurochemical-morphological correlates, we propose that the mammalian BNST and the V-shaped nucleus of the quail are homologous. This proposal is independently supported by the observation that this structure is characterized in both rodents and quail by the presence of a high density of vasopressin/vasotocin-immunoreactive fibers and cells (71). More specifically, it appears that the cell group characterized by a high density of ARO-ir cells specifically corresponds to the caudal part of the medial BNST. There is also no interruption between this V-shaped caudal group and the more rostral group located around the anterior commissure that must therefore be considered as the rostral part of the medial BSNT (see Ref. 71 for a more detailed discussion of these homologies). This conclusion should be further tested by tract-tracing studies, analyzing the connections of these nuclei in the two vertebrate classes.

Sub-Cellular Distribution of ARO-ir in Mammals and Birds

While a number of helpful anatomical-functional correlates may be made between brains in the two vertebrate classes, there are many areas of estrogen action and aromatase activity in birds and mammals where ARO-ir cell bodies have not been detected. Often, these areas also manifest sex steroid receptors.

Immunocytochemical studies appear to help resolve these apparent paradoxes in a way that maintains the applicability of such studies in uniting our understanding of anatomical homologs between mammals and birds. In both classes, immunocytochemical evidence of ARO-ir axons and axon terminals have been provided in several of these "blank spots." (69, 72, 73). Their distribution overlaps with the distribution of aromatase activity in the absence of ARO-ir cell bodies. We have commented further on the functional implications of distally placed aromatase-containing boutons in the central nervous system (72). It appears that the export of aromatase along neural fibers to the synaptic boutons may have similarities to the operation of classical neurotransmitter networks. In the sense of this review, these are supportive studies that underline and complete the correlative anatomical data described above.

Concluding Remarks

The evolutionary divergence of their antecedents has resulted in anatomical specialization that often makes avian and mammalian brain anatomy difficult to rationalize. The bird's sensory and motion systems occupy large functional and anatomical domains, while the mammal's cortical development has dominated. Despite the evolution of anatomical differences, ancient neurochemical systems were conserved and remain woven into the anatomic shaping of the respective species' brains. That this is a fortunate circumstance is well illustrated by the localization of the ancient estrogen synthesis system in the brain. The key en-

Table I. Aromatase Immunoreactivity of Four Limbic and Hypothalamic Regions Studied in Japanese Quail, Rat, Monkey and Human

	Japanese quail		Rat		African green monkey		Human	
	Cells	Axons	Cells	Axons	Cells	Axons	Cells	Axons
Amygdala/nucleus taeniae	++	+	+++	++	+++	++	++	+++
Bed nucleus of stria terminalis	++++	+++	++++	++	++++	++	+++	++
Lateral septum		+	+++	+++	+++	+++	+++	+++
Medial preoptic area anterior hypothalamus	+++	+++	+	+++	++	+++	+	+++

Note. The density of immunoreactive structures has been estimated on a semi-quantitative scale as follows: +, low cell or axon density; ++, moderate cell or axon density; +++, high cell or axon density; ++++, very high cell density.

zyme aromatase has had sufficient genetic conservation to furnish epitopes that span between classes. Using immunocytochemistry with antisera raised against these proteins and aromatase mRNA *in situ* hybridization, it has been possible to identify functiono-anatomical homologs between bird and mammal brains. Examples have been given of ARO-ir cells present in two such units in the mammalian and avian brain: The first of these examples consists in a preoptico-hypothalamic cell group that runs from the medial POA to the tuberal hypothalamus, including cells in the caudal preoptic area and anterior hypothalamus (rostral to the ventromedial nucleus). This structure is contiguous with the second grouping of limbic cells that encompasses the septal area, the BNST, the amygdala, or its avian homolog, the nucleus taeniae.

Besides these anatomical and neurochemical similarities, functions of the hypothalamic and limbic structures also appear to have remained similar in birds and mammals. In both taxa, for example, male copulatory behavior is largely controlled by the POA, whereas female receptivity is primarily under the control of neuronal activity in the ventromedial hypothalamus. The mammalian amygdala and its avian counterparts (parts of the archistriatum and nucleus taeniae) similarly appear to regulate a variety of instinctive behaviors. In addition, some specialization has occurred in the course of evolution as exemplified by the development of telencephalic song control pathways in oscine birds that are associated with the presence of telencephalic areas expressing aromatase (31, 74, 75). The detailed discussion of these functional similarities and differences is, however, beyond the scope of the present minireview.

From a purely anatomical point of view, the preoptic-hypothalamic group of aromatase cells appears to be more developed in birds, especially at its rostral pole. Numerous densely stained ARO-ir cells outline the medial preoptic nucleus of the quail, while these are hardly visible in the medial preoptic nucleus of the rat or mouse (they are nevertheless easily detected by immunocytochemistry or *in situ* hybridization in the neonate). In contrast, the limbic group appears more prominent in rodents, especially in its most lateral part. ARO-ir cells are indeed more numerous in the mammalian amygdala than in the avian nucleus taeniae.

Given these apparently minor differences, the overall pattern of distribution of this enzyme appears to have, however, remained relatively stable in the course of evolution and it can be used to assess interspecies homologies between components of the hypothalamic and limbic parts of the brain. One example of such studies is the recent work disclosing the presence in the areas of ARO-ir in axons and axon terminals. These studies have been able to explain the presence of aromatase activity in areas not known to contain ARO-ir cell bodies. It is anticipated that mapping these ARO-ir axons and boutons may allow more complete understanding of the homologies in brain structures between birds and mammals.

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