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# Cytoarchitectural analysis of the quail preoptic area. Evidence for a sex-related dimorphism in the medial preoptic nucleus

GIANCARLO PANZICA
CARLA VIGLIETTI-PANZICA
MARIA GRAZIA FIORI
MAURIZIO CALCAGNI
GIANCARLO ANSELMETTI
Dipartimento di Anatomia e Fisiologia umana, Sezione di Neuroscienze, Università di Torino, c.so M. D'Azeglio 52, I-10126 Torino (Italy)

JACQUES BALTHAZART
Laboratoire de Biochimie Générale et Comparée, Université de Liège, 17 Place Delcour, B-4020 Liège (Belgium)

ABSTRACT

Studies using the Nissl method on thick paraffin sections of the preoptic region of the Japanese quail hypothalamus reveal a complex cytoarchitecture with several differentiated nuclei. The most prominent nucleus is the medial preoptic nucleus whose staining and volume appear sexually dimorphic. A higher number of intensely stainable neurons are present in males than in females, probably reflecting different metabolic rates in the two sexes. The volume of the medial preoptic nucleus is also larger (40%) in males than in females. Present results represent the first description of a sex-related dimorphism in the hypothalamus of non-mammalian vertebrates.

KEY WORDS: Birds; Hypothalamus; Medial Preoptic Nucleus; Sexual dimorphism; Cytoarchitecture

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## INTRODUCTION

The analysis of the cytoarchitectonic pattern in the avian hypothalamus has been the aim of several studies dealing with different species of birds. Most valuable results on passerine species were reviewed by Oksche & Farner (1974), while Kuenzel & van Tienhoven (1982) have recently reviewed the nomenclature for the chicken hypothalamus. Starting from the pioneering works of Huber & Crosby (1929) and Kuhlenbeck (1937) the anatomical descriptions of avian rostral hypothalamus have been widely influenced by the presence of scattered large cells, successively identified as neurosecretory neurons (for an extensive review cf. Oksche & Farner, 1974). In the opinion of several authors the avian hypothalamus shows a cluster-like organization preventing any definite interpretation of the nuclear areas corresponding to those described in the mammalian brain. According to Kuenzel &. van Tienhoven (1982) however it is possible to recognize in the chicken hypothalamus several nuclei that have been named according to an international nomenclature. In an attempt to identify sexually dimorphic regions in the avian hypothalamus to be related to some biochemical dimorphism previously demonstrated in the quail (Schumacher & Balthazart, 1984; Balthazart & Schumacher, 1984; 1985), we conducted a study of the quail rostral hypothalamus with techniques similar to those that have led Gorski *et al.* (1980) in the rat and Commins & Yahr (1984) in the gerbil to identify a dimorphic region in the mammalian preoptic area.

## MATERIAL AND METHODS

*Animals*

In the present study we utilized hypothalami obtained from 16 males and 16 females of Japanese quail (*Coturnix coturnix japonica*). Birds, which ranged in ages between 6 and 16 weeks, were commercially obtained from a Belgian seller, and were photostimulated (16 h of light) for at least two weeks. All the males were calling and females were laying eggs.

*Histology*

Birds were anaesthetized with Hypnodil[R] (Janssen Pharmaceutica, Belgium) and them transcardially perfused with saline solution followed by Bouin's fluid without acetic acid. After a one-week in 50% ethanol, the dissected hypothalami were embedded in paraffin and serially sectioned. The 50 $\mu$m-thick sections were then processed according to the Nissl method (toluidine-blue), mounted and photographed at low magnification (objective 1.6×) by means of a Leitz Ortholux microscope. Micrographs (final enlargement 12×) from each hypothalamus were studied without knowledge of the sex of the animal and nuclei were drawn on transparent sheets.

*Morphometrical analysis*

The volume of selected nuclei (dorsolateral preoptic nucleus PD, suprachiasmatic nucleus SCN, medial preoptic nucleus POM, nucleus rotundus thalami nRT) was calculated by means of a BASIC program running on an Apple IIe[TM] computer provided with a graphic tablet. This program measures the area of the traced nucleus in each section by means of coordinates recorded by the graphic tablet. The volume, assuming that any change in cross-sectional area between the slides measured is linear, is calculated according to the formula of Uylings *et al.* (1984):

$$V = \frac{1}{M^2} \sum_{i=1}^{n-1} \frac{A_i + A_{i+1}}{2} d_i$$

in which $A_i$ = surface area in $i^{th}$ section, n = number of sections measured, $d_i$ = distance between surface area $A_i$ and $A_{i+1}$ and M is the linear magnification.

Due to difficulty in recognizing the boundaries of preoptic nuclei in Nissl's stained sections, the volumes were calculated separately from the drawings made independently by 3 observers for each specimen and nucleus. In cases of marked differences in the results of the calculations, the original pictures and sections were carefully re-observed to check for manual errors, i.e. loss of sections, or misinterpretation due to a stronger or weaker staining of the sections. For each animal both the right and left side of the hypothalamus have been measured in order to test the possibility of a lateralization of the various structures.

*Statistical test*

The mean values of the volumes measured by the 3 observers were used for the statistical analysis. Volumes of the nuclei in males and females and on the left and right side were compared by the bilateral Student t-test.

## RESULTS

*Cytoarchitecture of the preoptic region.*

In the preoptic region of the quail several magno- and parvocellular masses are easily recognizable in 50 $\mu$m thick Nissl-stained sections. Larger and more intensely stained neurons probably correspond to the neurophysin-immunoreactive system distributed in several cell clusters occupying a lateral or periventricular location, as described by Berk *et al.* (1982) in pigeon and by Viglietti-Panzica (1986) in quail, fowl and duck.

Parvocellular elements are on the contrary clustered in different, larger cell masses that can be more easily outlined only in thick sections (Fig. 1, a-h). In thinner ones (5-15 $\mu$m), it is in fact hard to distinguish the boundaries of the parvocellular nuclei. The most prominent nucleus present in our specimens is the medial preoptic nucleus (POM), located periventricularly and extending throughout the preoptic region (Fig. 1, b-g). Rostrally it extends upward to the dorsolateral preoptic nucleus that, in the quail, is indistinguishable from the nucleus of the diagonal band of Broca. At this rostral level the medial preoptic nucleus is continuous with the so called periventricular preoptic nucleus. P2 and P3 clusters of vasotocin-immunoreactive neurons (Viglietti-Panzica, 1986) are situated close to the ventricular wall in the region of the nucleus. In more caudal sections, the POM extends laterally and dorsally, reaching the region of the medial septal nucleus. At this level the nucleus is a round shape, elongated ventrally. The medial preoptic nucleus extends caudally to the level of the anterior commissure. After the anterior commissure it disappears quickly, while in periventricular position the P2 and P3 clusters also extend laterally to give rise to the so called paraventricular nucleus.

The dorsolateral preoptic nucleus (PD) is located ventrally to the septum-mesencephalic tract and has a roughly triangular shape (Fig. 1, a). Its elements are more intensely stainable than the surrounding nuclei, but they do not belong to the vasotocin-producing system. The other well recognizable cell group of the preoptic and rostral hypothalamus is the so called suprachiasmatic nucleus (Fig. 1, b-f), whose morphology and peptidergic content has been already described in quail and chicken (Panzica, 1985). Other cell masses are distributed in the preoptic area: the lateral preoptic area, whose rostral part extends laterally like the wing of a butterfly (Fig. 1, a-c), and the accessory nuclei of the anterior commissure (Fig. 1, f) earlier described by Yamauchi & Yasuda (1981).

*Sexual dimorphism of the preoptic nuclei*

The intensity of Nissl's staining of the neurons in the POM is higher in males than in females (Figs. 2, 3). Apparently, even if no morphometrical analysis was undertaken, the more intense staining is dependent on a higher number of neurons provided with a well developed Nissl's substance in the cytoplasm in males than in females, while no marked differences are found in cell density. This observation led us to consider, at first, this nucleus as a possible site for sexual dimorphism in the quail preoptic area. On this basis we started to measure the volume of 3 nuclei of the preoptic region of the quail (PD, SCN and POM), together with a thalamic nucleus (nRT) chosen for its easy identification, diencephalic location and clearly recognizable shape. The results of this analysis are reported in Tables I and II. No significant left-right assimmetry could be detected in the considered structures (Table I). The volume of the POM, measured according to the described methods, is significantly ($2p < 0.001$) larger (40%) in males than females. This difference is not statistically significant for the other nuclei considered (Table II).

TABLE I – *Mean volume of the medial preoptic nucleus on the left and right side of male and female Japanese quails. The volume is expressed as* $\mu m^3 \times 10^6$. *n = number of animals; s.d. = standard deviation; 2p = value of probability (bilateral Student's t-test; n.s. = not significant).*

| | LEFT | | RIGHT | | |
|---|---|---|---|---|---|
| | n | Mean volume ± s.d. | n | Mean volume ± s.d. | |
| Males | 16 | 140.32 ± 17.93 | 16 | 147.33 ± 20.70 | 2p > 0.1 n.s. |
| Females | 16 | 105.89 ± 14.74 | 16 | 113.03 ± 15.73 | 2p > 0.1 n.s. |
| | | 2p < 0.001 | | 2 < 0.001 | |

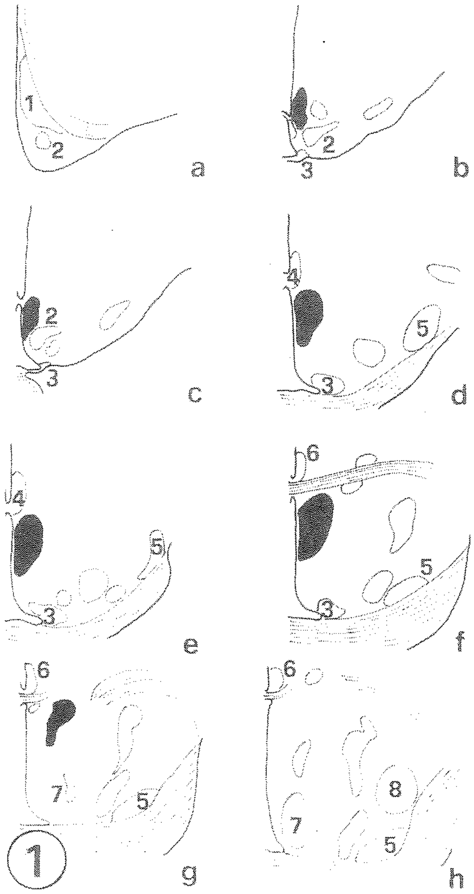


Fig. 1 - Schematic drawings showing the rosto-caudal (a-h) extent and topography of nuclei in the preoptic region of the male Japanese quail in transverse (coronal) sections. The medial preoptic nucleus is indicated by *black area.* 1 = nucleus preopticus dorsolateralis; 2 = n. preopticus lateralis; 3 = n.suprachiasmaticus; 4 = septum medialis; 5 = geniculatus lateralis ventralis; 6 = bed nucleus commissurae pallialis, 7 = n.anterior hypothalami; 8 = n.rotundus thalami.


Figs. 2,3 - Toluidine-blue stained paraffin sections of a male (2) and a female (3) Japanese quail medial preoptic nucleus (at level of sections 1d-e) showing different intensity of staining in the two sexes. × 125.

TABLE II – *Mean volumes ($\mu m^3 \times 10^6$) of diencephalic nuclei (only the left side) of the Japanese quail.* POM = *medial preoptic nucleus;* SCN = *suprachiasmatic nucleus;* PD = *dorsal preoptic nucleus;* nRT = *nucleus rotundus thalami. Other abbreviations as in Table I.*

| | MALES | | | FEMALES | | | |
|---|---|---|---|---|---|---|---|
| | n | Mean volume | s.d. | n | Mean volume | s.d. | |
| POM | 16 | 140.32 ± | 17.93 | 16 | 105.89 ± | 14.74 | 2p < 0.0001 |
| SCN | 8 | 15.538 ± | 3.48 | 14 | 13.89 ± | 3.169 | 2p > 0.1 n.s. |
| PD | 12 | 43.479 ± | 23.36 | 14 | 38.508 ± | 13.014 | 2p > 0.1 n.s. |
| nRT | 16 | 790.53 ± | 154.16 | 15 | 803.156 ± | 105.85 | 2p > 0.1 n.s. |

## DISCUSSION

The use of thick-paraffin sections in cytoarchitectural studies can improve the possibility of the identification of shapes and boundaries of clusters of parvocellular neurons. Former studies on the avian hypothalamus (Huber & Crosby, 1929; Kuhlenbeck, 1937; van Tienhoven & Juhasz, 1962; Karten & Hodos, 1967; Crosby & Showers, 1969; Baylé *et al.*, 1974) revealed a marked difficulty in describing preoptic nuclei. According to some authors (Oksche & Farner, 1974) this may be due to the peculiar arrangement of the parvo- and magnocellular neurons in a cluster-like entity. Recently Kuenzel & van Tienhoven (1982) revised the literature and proposed a new nomenclature for the preoptic hypothalamic nuclei in the chicken. In the present cytoarchitectural study, done on thick paraffin sections stained with toluidine-blue (Nissl's stain), we provide a clear picture of the arrangement of parvocellular neurons in the preoptic area of a galliform species, the Japanese quail. Our results are largely confirmatory of the nomenclature of Kuenzel & van Tienhoven (1982), except for the location and extension of POM that we identified according to the drawings of Berk & Butler (pigeon: 1981) and the description of Crosby & Showers (chicken and house sparrow: 1969). As discussed also by Kuenzel & van Tienhoven (1982) the naming of recognizable hypothalamic nuclei in classically stained preparations does not identify functional subunits. These latter may be well identified by means of different techniques such as immunocytochemistry or investigations of neuronal pathways. Nonetheless, the standardization of the naming of traditional nuclei could constitute a point of reference to those who wish to identify injection, lesion or implantation sites, as well as to perform biochemical measurements on small brain areas collected by the punch technique. The identification of hypothalamic nuclei in nonmammalian vertebrates could also improve our phylogenetic knowledge of the evolution of this ancient region of the brain. The present study shows that the avian preoptic region, at least in galliform birds, is more differentiated and organized than formerly supposed. Previous studies showing sexually dimorphic structures in the mammalian medial preoptic nucleus (Raisman & Field, 1973; Greenough *et al.*, 1977; Gorski *et al.*, 1980; Ayoub *et al.*, 1983; Commins & Yahr, 1984) and the identification of a well differentiated medial preoptic nucleus (POM) in the Japanese quail led us to investigate if this structure is sexually dimorphic also in birds. According to our results the POM is 40% larger in male than in female quails. The dimorphism in volume is specific to the POM: in fact, it does not affect the other preoptic nuclei which we considered (SCN, PD) nor the nucleus rotundus whose houndaries can be defined with high accuracy. The involvement of the preoptic region in the control of avian sexual behaviour has been demonstrated by several studies performed using different techniques (Ralph, 1959; Ralph & Fraps, 1959; Barfield, 1965; 1969; Meyer & Salzen, 1970; Meyer, 1973; Haynes & Glick, 1974; Hutchison, 1975). So far, the connections of the POM have been studied only in the pigeon (Berk & Butler, 1981). In this species, the POM is connected to the lateral septum, the dorsal thalamus and some mesencephalic regions. These neuroanatomical relationships suggest that this nucleus is a central structure which could be involved in the control of the LHRH neuronal system, distributed in birds mainly in the septal region (Sterling & Sharp, 1982). Considering functional studies and the connections of the POM with other brain areas, it appears likely that this nucleus is a target site for steroids and that it participates in the control of male sexual behaviour. Recently biochemical microanalysis of the quail preoptic area revealed sex differences in testosterone metabolism (Schumacher & Balthazart, 1984), in aromatase activity (Balthazart & Schumacher, 1984) and in catecholamine content (Ottinger *et al.*, 1986). Due to the fact that a denser staining of the Nissl substance probably reflects a higher rate of intracellular metabolism, we think that the POM could provide a morphologically defined region for the biochemical differences observed to date. Further studies are now in progress to determine whether hormonal signals could control the POM volume or the detail of its cytoarchitecture and to establish more precisely the role of POM in the integration of the reproductive processes in quail.

REFERENCES

Ayoub D. M., Greenough W. T., Juraska J. M., 1983 - Sex differences in dendritic structure ind the preoptic area of the juvenile macaque monkey brain. Science, *219*: 197-198.

Balthazart J., Schumacher M., 1984 - Organization and activation of behaviour in quail: role of testosterone metabolism, J. exp. Zool., *232*: 595-604.

Balthazart J., Schumacher M., 1985 - Role of testosterone metabolism in the activation of sexual behaviour in birds. In: R. Gilles & J. Balthazart (eds.), Comparative neurobiology. Springer Verlag, Berlin, pp. 121-140.

Barfield R. J., 1965 - Effects of preoptic lesions on the sexual behaviour of male domestic fowl. Am. J. Zool., *5*: 686-687.

Barfield R. J., 1969 - Activation of copulatory behavior by androgen implanted into the preoptic area of the male fowl. Horm. Behav., *1*: 37-52.

Baylé J.-D., Ramade F., Oliver J., 1974 - Stereotaxic topography of the brain of the quail (*Coturnix coturnix japonica*). J. Physiol., Paris, *68*: 219-241.

Berk M. L., Butler A. B., 1981 - Efferent projections of the medial preoptic nucleus and medial hypothalamus in the pigeon. J. comp. Neurol., *203*: 379-399.

Berk M. L., Reaves T. A., Hayward J. N., Finkelstein J. A., 1982 - The localization of vasotocin and neurophysin neurons in the diencephalon of the pigeon, *Columba livia*. J. comp. Neurol., *204*: 392-406.

Commins D., Yahr P., 1984 - Acetylcholinesterase activity in the sexually dimorphic area of the gerbil brain: sex differences and influences of adult gonadal steroids. J. comp. Neurol., *224*: 123-131.

Crosby E. C., Showers M. J., 1969 - Comparative anatomy of the preoptic and hypothalamic areas. In: W. Haymaker, E. Anderson, W. T. Nauta (eds), The hypothalamus. Charles C. Thomas, Springfield, pp. 61-135.

Gorski R. A., Harlan R. E., Jacobson C. D., Shryne J. E., Southam A. M., 1980 - Evidence for the existence of a sexually dimorphic nucleus in the preoptic area of the rat. J. comp. Neurol., *193*: 529-539.

Greenough W. T., Carter C. S., Steerman C., De Voogd T. J., 1977 - Sex differences in dendritic patterns in hamster preoptic area. Brain Res., *126*: 63-72.

Haynes R. L., Glick B., 1974 - Hypothalamic control of sexual behaviour in the chicken. Poultry Sci., *53*: 27-38.

Huber G. C., Crosby E. C., 1929 - The nuclei and fiber paths of the avian diencephalon, with consideration of the telencephalic and certain mesencephalic centres and connections. J. comp. Neurol., *48*: 1-225.

Hutchison J. B., 1975 - Target cells for gonadal steroids in the brain: studies on steroid-sensitive mechanisms of behavior. In: P. Wright, P. G. Caryl & D. M. Vowles (eds.), Neural and endocrine aspects of behaviour in birds. Elsevier, Amsterdam, pp. 123-137.

Karten H. J., Hodos W., 1967 - A stereotaxic atlas of the brain of the pigeon (*Columba livia*). The Johns Hopkins Press, Baltimore.

Kuenzel W. J., van Tienhoven A., 1982 - Nomenclature and location of avian hypothalamic nuclei and associated circumventricular organs. J. comp. Neurol., *206*: 293-313.

Kuhlenbeck H., 1937 - The ontogenic development of the diencephalic centers in a bird's brain (chicken) and comparison with the reptilian and mammalian diencephalon. J. comp. Neurol., *66*: 23-75.

Meyer C. C., 1973 - Testosterone concentration in the male chicken brain: an autoradiographic survey. Science, *180*: 1381-1383.

Meyer C. C., Salzen E. P., 1970 - Hypothalamic lesions and sexual behaviour in the domestic chick. J. comp. Physiol. Psychol., *73*: 365-376.

Oksche A., Farner D. S., 1974 - Neurohistological studies of the hypothalamo-hypophysial system of *Zonotrichia leucophrys gambelii* (Aves, Passeriformes). With special attention to its role in the control of reproduction. Adv. Anat. Embryol. Cell Biol., *48*: 1-136.

Ottinger M. A., Schumacher M., Clarke R. N., Duchala C. S., Balthazart J., 1986 - Comparison of monoamine concentration in the brains of adult male and female Japanese quail. Poultry Sci., *65*: 1413-1420.

Panzica G. C., 1985 - Vasotocin-immunoreactive elements and neuronal typology in the suprachiasmatic nucleus of the chicken and Japanese quail. Cell Tissue Res., *242*: 371-376.

Raisman G., Field P. M., 1973 - Sexual dimorphism in the neuropil of the preoptic area of the rat and its dependence on neonatal androgen. Brain Res., *54*: 1-29.

Ralph C. L., 1959 - Some effects of hypothalamic lesions on gonadotrophin release in the hen. Anat. Rec., *134*: 411-431.

Ralph C. L., Fraps R. M., 1959 - Long term effects of diencephalic lesions on the ovary of the hen. Am. J. Physiol., *197*: 1279-1283.

Schumacher M., Balthazart J., 1984 - Sexual dimorphism in the hypothalamic metabolism of testosterone in the Japanese quail (*Coturnix coturnix japonica*). Progr. Brain Res., *305*: 51-59.

Sterling R. J., Sharp P. J., 1982 - The localization of LH-RH neurones in the diencephalon of the domestic hen. Cell Tissue Res., *222*: 283-298.

Uylings H. B. M., van Eden C. G., Verwer R. W. H., 1984 - Morphometric methods in sexual dimorphism research on the central nervous system. Progr. Brain Res., *61*: 215-222.

Yamauchi K., Yasuda M., 1981 - Several cell masses around the commissura anterior in the chicken. J. Hirnforsch., *22*: 189-194.

van Tienhoven A., Juhasz L. P., 1962 - The chicken telencephalon, diencephalon and mesencephalon in stereotaxic coordinates. J. comp. Neurol., *118*: 185-198.

Viglietti-Panzica C., 1986 - Immunohistochemical study of the distribution of vasotocin reacting neurons in avian diencephalon. J. Hirnforsch, *5*: 559-566.