
Balthazart J (ed): Hormones, Brain and Behaviour in Vertebrates. 1. Sexual Differentiation, Neuroanatomical Aspects, Neurotransmitters and Neuropeptides. Comp Physiol. Basel, Karger, 1990, vol 8, pp 104–120

Anatomical and Biochemical Studies on the Sexually Dimorphic Preoptic Medial Nucleus of the Quail¹

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The Preoptic Region of Vertebrates and the Control of Reproductive Behaviour

Several studies have demonstrated during the last 15 years the presence of sexual dimorphisms in the vertebrate central nervous system (CNS). These dimorphisms involve both the structure of brain nuclei, and the biochemistry of the nervous tissue (metabolic pathways of some hormones, concentration of neurotransmitters and receptors). In a few cases it was possible to demonstrate that these dimorphisms can be directly correlated to some aspects of reproductive behaviour.

A large number of studies indicate that the anterior hypothalamus-preoptic area (POA) complex plays a major role in the control of physiological and behavioural aspects of vertebrate reproduction. The control namely concerns the male sexual behaviour and the gonadotrophin release. These functions are controlled by steroids, as shown by stereotaxic implantation studies [1]. The biochemical dimorphisms described so far in this region include differences in steroid action (differential concentrations or induci-

¹ This work was supported by grants from the CNR and MPI to G.C.P. and C.V. and by grants from NIH (HD 22064), the Belgian FNRS, the Medical School and the Fonds Spécial pour la Recherche of the University of Liège to J.B. The collaboration between the Italian and Belgian researchers was supported by grants from the ETP, NATO and EEC.

bility of receptors, differentiated metabolism of testosterone), and in the concentration or turnover of neurotransmitters, neuropeptides, receptors and enzymes.

Studies of sexual behaviour and of its control mechanisms require the use of animal models in which the behaviour can easily be induced in controlled conditions. It is also an advantage to have a model which shows a strong sexual dimorphism so that hopefully differences in behaviour can be related to differences in brain anatomy and/or physiology. Among birds, the Japanese quail (*Coturnix japonica*) fulfils these criteria and has been the subject of intense research in recent years.

During the last decade, many studies have been devoted to the problem of the sexual dimorphism in brain morphology in mammals and other vertebrates. Within the POA, sexual differences concerning the neuronal connectivity, the cell nucleus size and the volume of several groups of cells have been identified [for a list of references, cf. 2]. Assuming that this would also be the case in the Japanese quail we have concentrated our investigations on the anatomy and neurochemistry of this region.

Cytoarchitecture of the Preoptic Region in the Japanese Quail

In the preoptic region of the quail several magno- and parvocellular masses are easily recognizable in Nissl-stained sections. Larger and more intensely stained neurons probably correspond to the neurophysin-immunoreactive system distributed in several cell clusters located in a lateral or periventricular position. Parvocellular elements are on the contrary clustered in different, larger cell masses that can be more easily outlined [3]. The most prominent nucleus present in the region is the medial preoptic nucleus (POM), located periventricularly and extending throughout the preoptic region (fig. 1). Rostrally it begins right after the dorsolateral preoptic nucleus. Clusters of vasotocin-immunoreactive neurons are situated close to the ventricular wall in the region of the nucleus [4]. In more caudal sections, the POM extends laterally and dorsally to the ventral limit of the septal region. At this level the nucleus shows a round shape which is elongated ventrally. The POM extends caudally to the level of the anterior commissure. At this level, it quickly disappears. The dorsolateral preoptic nucleus (PD) is ventral to the septum-mesencephalic tract and shows a roughly triangular shape. Its elements are more intensely stainable than surrounding nuclei. Another well recognizable cell group of the preoptic and rostral hypothalamic region is the

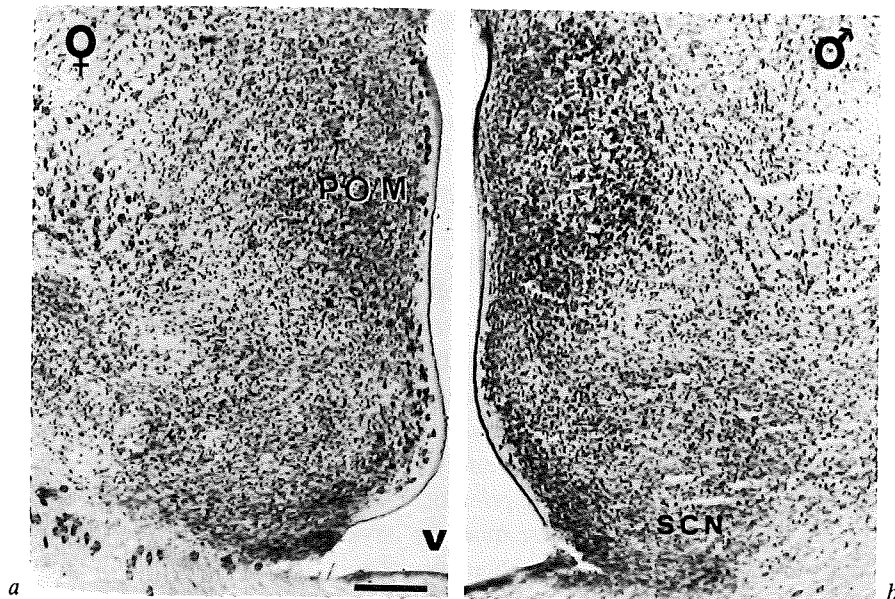


Fig. 1. Preoptic region of the adult quail at the level of the rostral POM. *a* Female; *b* male. v = Third ventricle; SCN = suprachiasmatic nucleus. Bar = 200 μ m.

so-called suprachiasmatic nucleus [5]. Two other cell masses have been described in the POA: the lateral POA and the accessory nuclei of the anterior commissure.

Sexual Dimorphism of the Quail POM

We measured by means of a semiautomatic analyser developed in our laboratory [6] the volume of some preoptic nuclei [7]. The results of this study are summarized in figure 2a. The volume of the POM was significantly ($2p < 0.001$) larger in males than in females. This difference was not statistically significant for the other nuclei considered (preopticus dorsalis, suprachiasmaticus). No significant left-right asymmetry could be detected in these structures. Some parts of the POM showed a denser staining than the rest of this nucleus and the volume of the dark staining region within the POM was measured. A strong sex-related difference also appeared in the dark volume of POM ($2p < 0.001$). These results demonstrated that one parvocel-

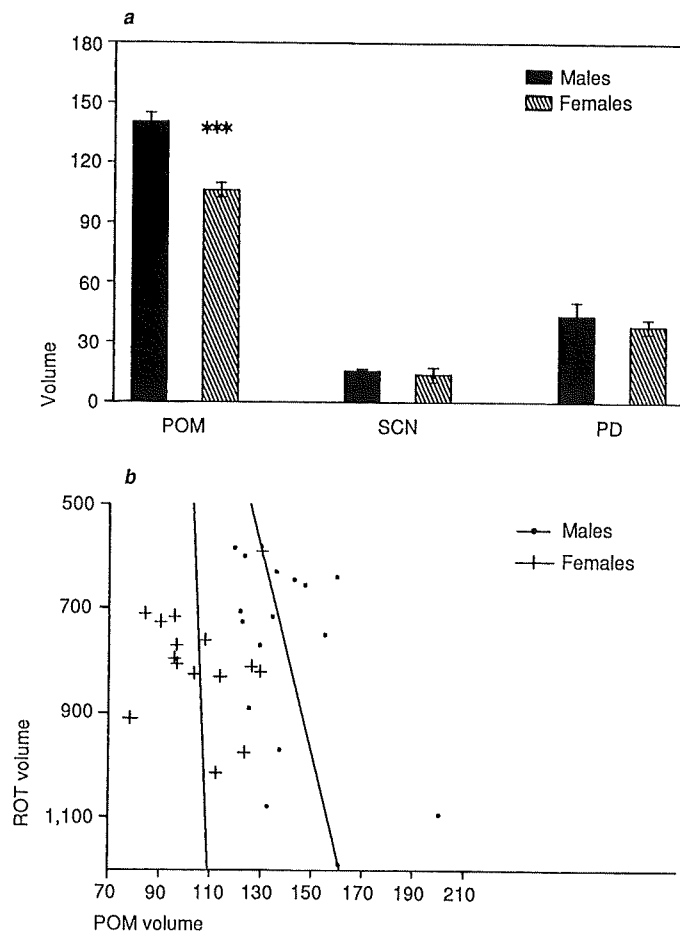


Fig. 2. *a* Volume (mean $\pm$ SE) expressed as $\mu\text{m}^3 \times 10^6$ of the left side of different preoptic nuclei (for abbreviations see the text). *** = $2p < 0.001$ [modified from 3, 7]. *b* Regression lines between POM and ROT volume to show the correlation between the size of ROT and the size of POM in both sexes. Male and female correlation lines are clearly separated.

lular group of neurons (POM) in the quail POA shows a strong sexual dimorphism. This dimorphism in volume is specific and does not simply result from a gross difference in brain size. In fact, it does not affect the other preoptic nuclei that were measured nor the thalamic nucleus rotundus (ROT) whose boundaries can be defined with high accuracy. This can be clearly demonstrated by plotting the POM volume against the ROT volume. The

results show that males and females belong to different populations characterized by different correlation lines (fig. 2b).

Steroid Effects on the POM Volume

Treatments which are known to affect quail sexual behaviour (adult castration and testosterone replacement; injection of estradiol benzoate in the egg) were applied to different groups of birds and their behavioural effects were verified. The brain of each animal was then collected, sectioned and stained in order to study the effects of the treatments on the POM volume [8].

Effect of Testosterone

In agreement with previously published papers, testosterone (T) treatment activated sexual behaviour in males but not in females despite the fact that the hormone implants produced similar levels of T in gonadectomized birds of both sexes [9]. The volume of the POM was significantly affected by the sex of the birds and their hormonal treatments. A decrease of about 25% was observed following castration and the nucleus size could be restored to the intact male level by treatment with T (fig. 4a). In the same experiment, no significant effect of sex, nor of treatment, nor of their interaction could be detected on the volume of the thalamic ROT.

The optical density of the POM (an index of the distribution of Nissl's substance, e.g. protein synthetic organelles) changed in the same way as the volume of the nucleus [10, 11] (fig. 3a-c). The POM volume seen in gonadectomized T-treated birds was however not sexually dimorphic which suggested that it is directly controlled by the circulating levels of T. The sex-related difference in the volume of this preoptic nucleus probably is not an irreversible sex dimorphism, i.e. it is presumably not differentiated by the perinatal hormones.

Effect of Photoperiod

Many wild birds are typical seasonal breeders which reproduce in spring or early summer. One of the major factors controlling this annual cycle is the photoperiod. In laboratory conditions, the sexual behaviour of the Japanese quail is also strongly controlled by the photoperiod. The behaviour can be activated by exposure to long days which stimulate the secretion of T by the testes [12-14]. The results of a recent study [15; Panzica et al., in preparation] demonstrated that the volume of the POM also changes in the male as a function of the pho-

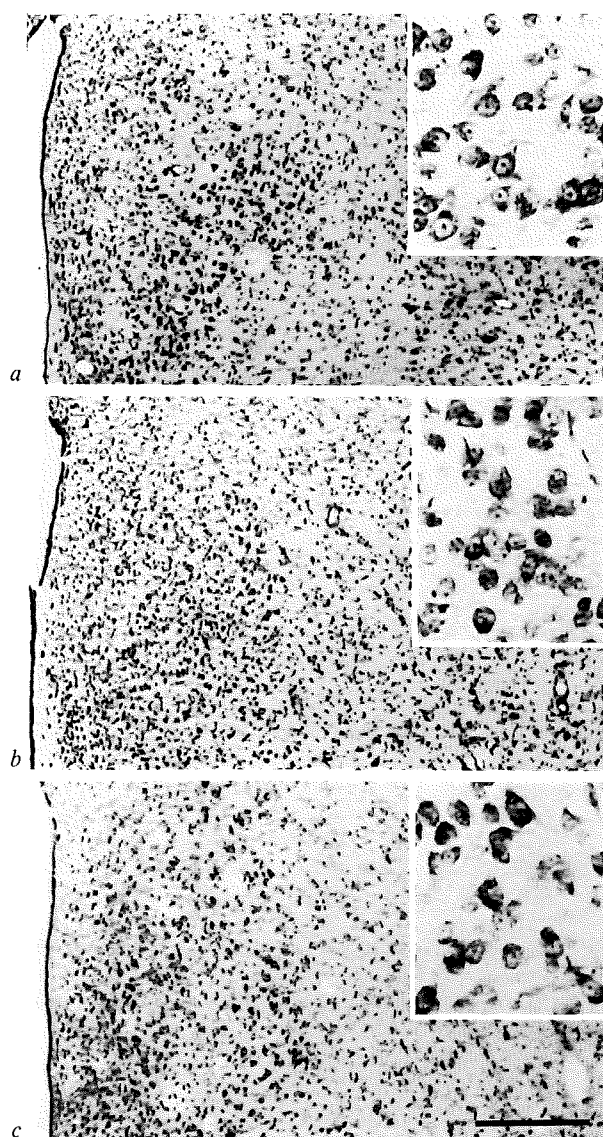


Fig. 3. Rostral part of the POM in a sham-operated male quail (*a*), in a castrated male (*b*), and in a castrated male implanted with T (*c*). The insets represent enlargements of the POM lateral part for each animal. Bar = 200 μ m.

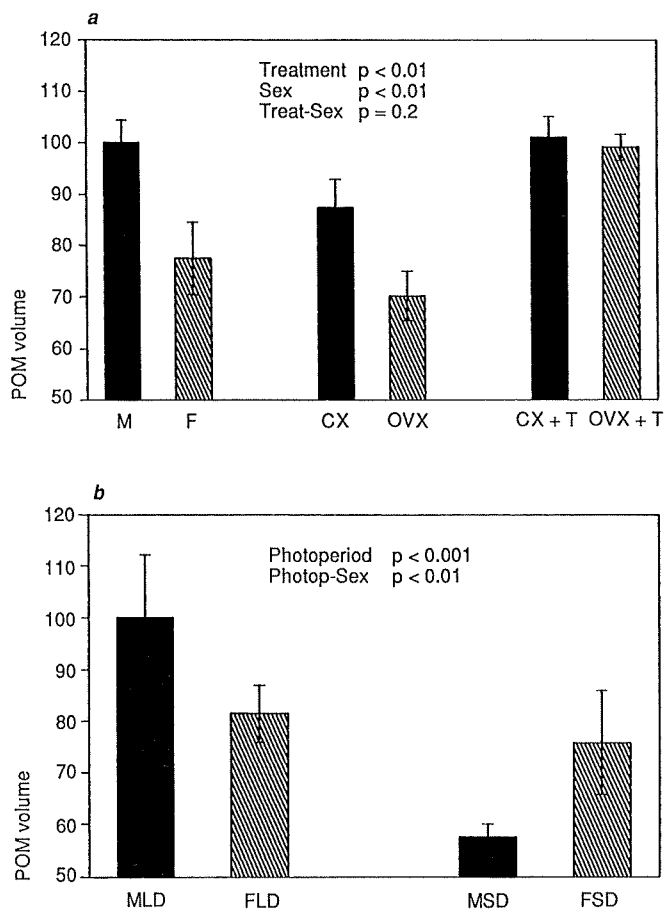


Fig. 4. Changes in the POM volume (mean $\pm$ SE) according to different experimental manipulations. The results of ANOVA analysis are also shown. *a* Effect of T upon POM volume (intact male mean = 100) [modified from 8]. *b* Effect of photoperiod (long-day male mean = 100) [modified from 15].

toperiod (increase in long days) whereas it remains substantially identical in the female (fig. 4b). These results further confirm the dependence of the POM volume in the adult quail on the levels of circulating T.

Oestradiol Benzoate Effect

Oestradiol benzoate (EB) treatment of embryonic quail is known to have irreversible organizational effects on the brain. This treatment suppresses the

sensitivity to the activating effect of T on male copulatory behaviour [16]. However, the injection of EB into 9-day-old quail embryos did not affect the volume of the POM in the adult birds which were grown from the injected eggs (studied both as intact and T-treated gonadectomized quails) [8]. This further supports the notion that the sex difference in POM volume is activational and not organizational in nature.

In conclusion, these experiments demonstrate that the volume of the quail POM is strongly affected by adult androgen levels. The nucleus can be considered as a good marker of androgen action in the POA. In addition, immunohistochemical [17] and autoradiographic [18] studies have shown that the quail POM contains a high density of estrogen receptors (more than 90% of cells were immunohistochemically labelled; fig. 5), and of androgen receptors. No sex differences in the number of estradiol-labelled cells were however found in the POM, nor in other hypothalamic nuclei.

POM and the Control of Copulatory Behaviour

In a recent study, Balthazart and Surlemont [19] demonstrated that the POM is a critical site of steroid action in the control of the copulatory behaviour. Stereotaxic implantation of needles filled with crystalline T restored the behaviour of castrated birds only when they were located within POM. Implants around the nucleus weakly activated the behaviour. Those that were distant by more than 200 μm were completely ineffective. Electrolytic lesions confirmed the role of the POM in the control of copulatory behaviour. Severe behavioural deficits were observed in male quail in which the POM had been partly destroyed by electrolytic lesions. In fact, the percentage of the POM that was lesioned was highly correlated with the behavioural deficit.

Cellular and Ultrastructural Organization of the POM

The change in POM volume observed following castration and T treatment could be due to an increase in the cell number of the cell size within the nucleus (true volume increase) or to an enhancement of the detectability of the nucleus at its edges as result of the increase of Nissl substance in the marginal neurons (apparent volume increase).

Recent observations [20] demonstrated that the POM of adult quail can be differentiated in a lateral part showing larger elements and in a medial one containing only small neurons. The difference in cell size between these two

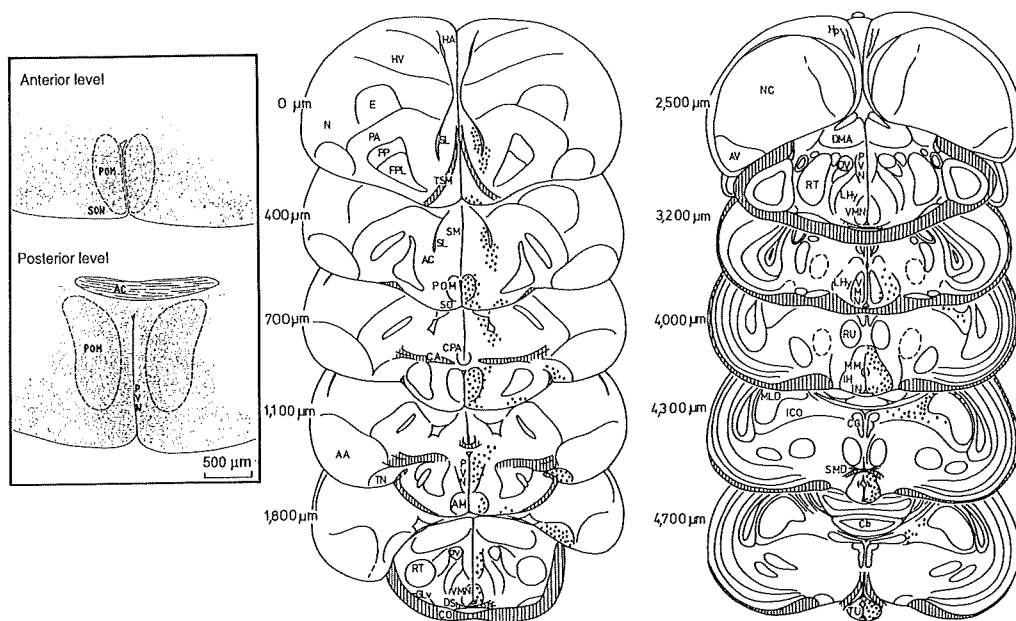


Fig. 5. Distribution of estrogen receptors in the quail brain as demonstrated by immunocytochemistry using a monoclonal antibody raised against estrogen receptors purified from a human breast tumour. Numbers at the left of each transversal section indicate the distance in micrometres from the level of the septomesencephalic tract. The inset shows the exact distribution of labelled neurons in a rostral and caudal section of the region of the medial preoptic nucleus. Each dot represents one labelled cell drawn with the help of a camera lucida. AA: Nucleus archistriatalis; AC: nucleus accumbens; AM: nucleus anterior medialis hypothalami; AV: archistriatum ventrale; CA: commissura anterior; Cb: cerebellum; CG: central gray; CO: chiasma opticum; CPA: commissura pallii; DMA: nucleus dorsomedialis anterior thalami; DS: decussatio supraoptica; E: ectostriatum; FPL: fasciculus prosencephali lateralis; GLv: nucleus geniculatus lateralis pars ventralis; HA: hyperstriatum accessorium; Hp: hippocampus; HV: hyperstriatum ventrale; ICO: nucleus intercollicularis; IH: nucleus inferior hypothalami; IN: nucleus infundibuli; LHY: nucleus lateralis hypothalami; MLD: nucleus mesencephalicus lateralis pars dorsalis; MM: nucleus mamillaris medialis; N: neostriatum; NC: neostriatum caudale; OV: nucleus ovoidalis; PA: paleostriatum augmentatum; POM: nucleus preopticus medialis; PP: paleostriatum primitivum; PVN: nucleus paraventricularis; RT: nucleus rotundus; RU: nucleus ruber; SL: nucleus septalis lateralis; SM: nucleus septalis medialis; SMD: supramamillaris decussatio; SO (SON): nucleus supraopticus; TN: nucleus taeniae; TSM: tractus septomesencephalicus; TU: nucleus tuberis; VMN: nucleus ventromedialis hypothalami.

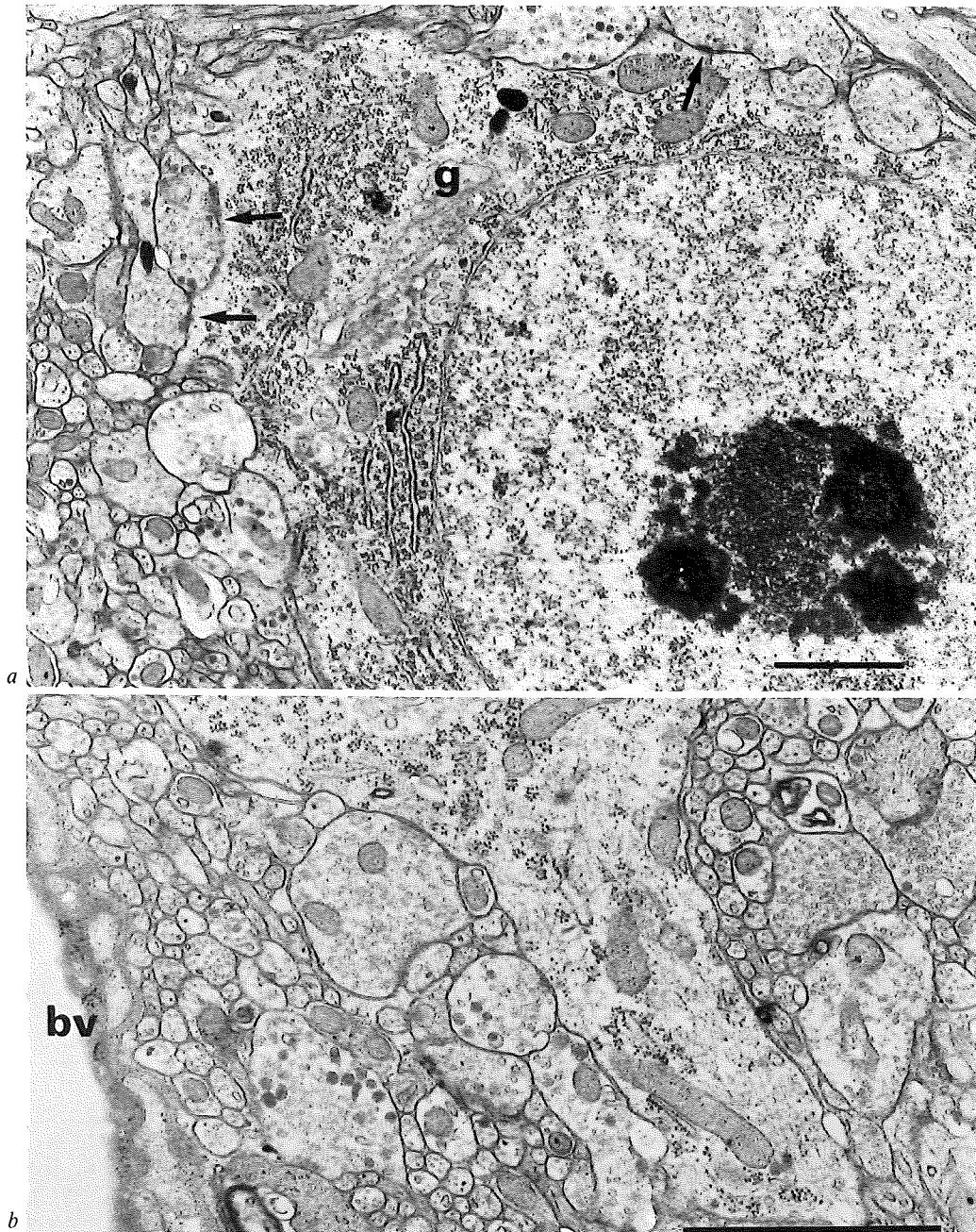
regions is statistically significant. In additional experiments [21], it was observed that the cell size in the lateral POM is controlled by the hormonal status of the bird, while in the medial part no change could be detected. Cell size in the lateral POM was significantly reduced in castrated males, as well as in short-day males. It returned to values typical of intact sexually mature males when castrated birds were treated with T (fig. 3a–c). This effect of T was sexually dimorphic, in fact no significant variations in cell size were observed in females subjected to the same treatment.

These results thus suggest that the increase in POM volume might be due to an increase of the cell size in the lateral portion of the nucleus (true volume increase). This neuronal population would represent the main target for steroid hormones within the POM.

Ultrastructural studies [22, 23] were also carried out to describe the neuronal and synaptic organization of the POM. The region is characterized by clusters of parvocellular neurons showing a rich supply of axosomatic synapses and a peculiar distribution of synthetic (rough endoplasmic reticulum) and secretory (Golgi complexes) structures (fig. 6a). The neuropil shows different types of synapses which contain clear and/or dense core vesicles. This suggests the presence of a rich aminergic and peptidergic innervation of the POM (fig. 6b).

Preoptic Aromatase

Schumacher and Balthazart [24] demonstrated that the quail POA contains an active aromatase, the enzyme which transforms T into estradiol (E_2). This transformation is a critical step in the production of the behavioural effects of T [25, 26]. The aromatase activity (AA) in the POA was, like the sexual behaviour, T-dependent: it was dramatically reduced by castration and restored by T treatment. T administration was less effective in ovariectomized female [27]. The induction of AA and of sexual behaviour by T were both dose-dependent [28]. The activation of sexual behaviour and of the AA were also correlated in time. The maximal increase in enzyme activity was seen after approximately 48 h and preceded by about 1 day the restoration of behaviour. When castrated males were treated with silastic implants filled with T and, in parallel, with implants containing ATD (androstatrienedione), a specific aromatase inhibitor, the increase of AA in the POA was suppressed and the activation of copulatory behaviour was delayed [11].



The application of the quail POA of the punch technique combined with a sensitive radioenzyme assay for the different enzymes involved in the T metabolism, demonstrated that the AA is specifically linked to the POM (fig. 7). Gonadectomy and replacement with T significantly affected the AA in the POM, and in other hypothalamic nuclei, but the magnitude of the changes and the absolute levels of enzyme activity were much larger in POM than in any other nucleus [29].

Preoptic Neurotransmitters, Neuropeptides and Receptors

Neuroanatomical studies on the distribution of neurotransmitters and neuropeptides in the avian POA are so far very scarce. The vasotocinergic system is the only one that has been studied in some detail [for a list of references, cf. 4]. Recently we started a systematic immunohistochemical study of the quail preoptic region. This demonstrated the presence of several peptidergic systems located in the POA but not exclusively in the POM [20]. So far, we have identified in this region immunoreactive fibers for substance P, vasotocin, corticotropin-releasing factor and neuropeptide Y. Substance P immunopositive cell bodies and a few LH-RH immunoreactive neurons were also observed in the quail POM. These neuropeptides are not exclusively located in the POM, but they are spread over the entire periventricular hypothalamus. However, the POM appears as one of the most important targets for these peptidergic systems. Former studies have also shown the presence of neurotensin-like immunoreactive cell bodies in the POM region of the Japanese quail [30].

Histochemical studies demonstrated the presence of catecholamines in the rostral hypothalamic and preoptic regions of the quail [31]. More recently, immunohistochemical studies [Cozzi et al., unpubl. results] demonstrated that serotonin is also present in high quantities in the quail POA and POM. Ottinger et al. [32] have shown by microdissection of the hypothalamic area that the noradrenaline content of the preoptic region is significantly

Fig. 6. Fine structure of the POM of intact adult male quail. *a* Low power enlargement of a POM neuron showing axosomatic synapses (arrows). The cytoplasm is filled with free polyribosomes, rough endoplasmic reticulum (r) and Golgi complexes (g). Bar = 1 μ m. *b* Higher enlargement of the neuropil surrounding a blood vessel (bv), showing different kinds of synapses provided of dense core and/or clear synaptic vesicles. Bar = 1 μ m.

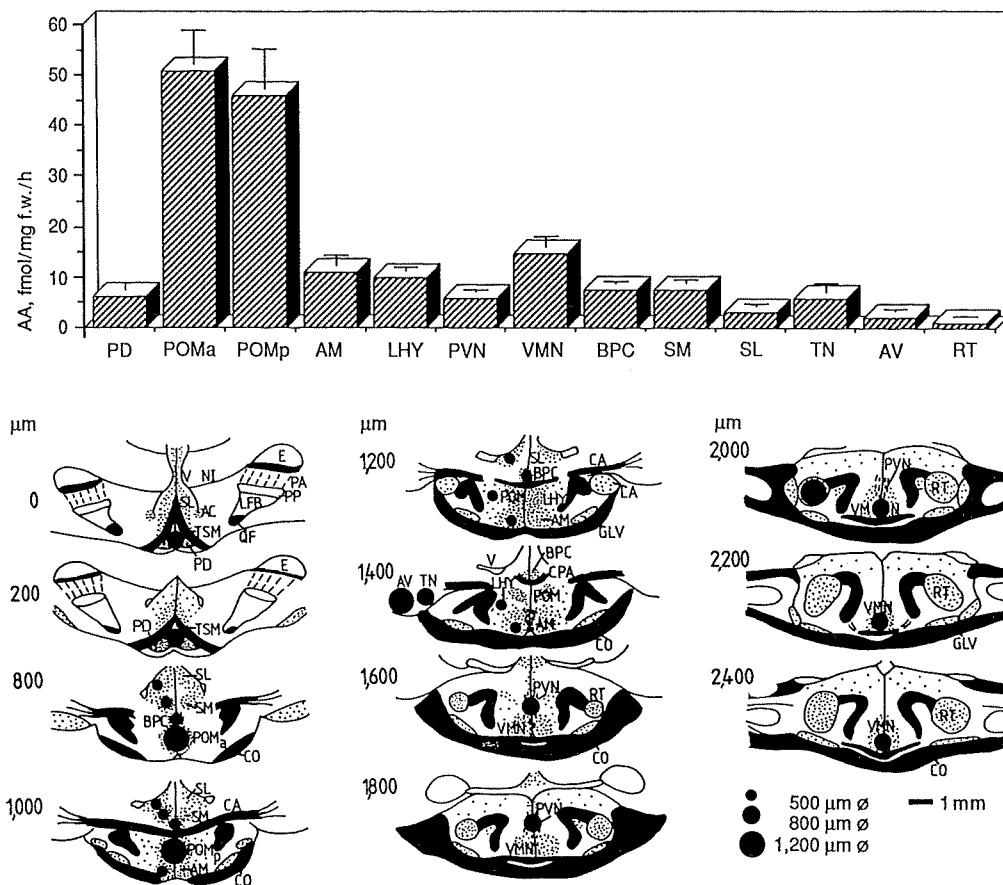


Fig. 7. Distribution of AA in different preoptic-hypothalamic nuclei of the male quail. The top part of the graph shows mean enzymatic activities determined by radioenzyme assays on nuclei dissected by the Palkovits punch technique. The bottom part shows the localization of the punched nuclei on a series of coronal sections. Numbers at the left of each transversal section indicate the distance in micrometres from the level of the septomesencephalic tract. PD: Nucleus preopticus dorsolateralis; POM: nucleus preopticus medialis (a = anterior level; p = posterior level); AM: nucleus anterior medialis hypothalami; LHY: area lateralis hypothalami; PVN: nucleus paraventricularis; VMN: nucleus ventromedialis hypothalami; BPC: bed nucleus of the pallial commissure; SM: nucleus septalis medialis; SL: nucleus septalis lateralis; TN: nucleus taeniae; AV: archistriatum ventrale; RT: nucleus rotundus. See also legend of figure 5 for additional abbreviations.

higher in female than in male Japanese quail. Additional experiments have demonstrated that the noradrenaline turnover is modulated by steroids in the male Japanese quail [33]. Recently the presence of high levels of α_2 -adrenergic receptors has been observed in the preoptic region. In particular, the POM was specifically labelled by an extremely high density of these receptors. They outlined the entire nucleus through its rostral to caudal axis [34]. This finding strongly suggests that the noradrenergic transmission plays a key role in the POM. The use of the specific noradrenergic toxin, DSP-4, actually demonstrated that the noradrenergic transmission modulates several aspects of the reproductive process including the copulatory behaviour, the secretion of LH, and the AA in the POA [35]. A treatment with DSP-4 potentiates the induction by T of the aromatase activity in the quail POA which suggests that, in intact animals, the noradrenergic transmission inhibits aromatase activity [35]. The lower AA in females compared to males [24, 27] could hence be a consequence of a higher inhibition in females due to a higher content of noradrenaline in the POA [32].

Conclusion

The present knowledge about the quail POM can be summarized as follows. The nucleus is particularly sensitive to the action of T. The steroid stimulates the dorsolateral part of the nucleus and determines an increase of the cell size which is reflected by an increase of the POM total volume. The sex dimorphism observed in adult animals reflects the different levels of T in male and female quail and is probably not organizational in nature. The cellular action of T is mediated by the presence of specific T and E_2 receptors in the POM region.

T increases the optical density of the POM in Nissl-stained sections. This strongly suggests that T increases the concentration of synthetic organelles in the POM neurons. This hypothesis is in agreement with the demonstration at the ultrastructural level of high densities of rough endoplasmic reticulum, free polyribosomes and other organelles related to secretion in the small cells of the POM.

One of the most important products of the POM synthetic activity is the enzyme aromatase, whose activity is T-dependent. The noradrenergic system is one important factor which might influence the activity of the aromatase. The sex difference in the adrenergic innervation of the POA might be responsible, at least in part, for the sexual dimorphism in aromatase activity.

Other peptidergic systems probably interact with catecholamines and serotonin, as suggested by immunohistochemical studies showing the coexistence of several peptidergic systems in the POM. Accordingly, the ultrastructural organization of the POM neuropil is very complex and differentiated, with an unusually high number of axosomatic (inhibitory?) synapses.

Finally, lesion and implant studies have demonstrated a direct involvement of the POM in the control of the male copulatory behaviour. Further studies are now needed to elucidate the embryonic and post-hatching development and differentiation of this structure, the afferences and efferences of the different portions of the POM, and its relations with some other important neuronal systems (e.g. the LH-RH septopreoptic system) which are also sexually dimorphic in quail [36].

In conclusion, data on the POM anatomy, biochemistry and physiology show that this nucleus represents an excellent and unique model to study the interactions between steroids, neurotransmitters and neuropeptides that regulate the activation of the behaviour.

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