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Effects of Testosterone on a Selected Neuronal Population Within the Preoptic Sexually Dimorphic Nucleus of the Japanese Quail

GIANCARLO PANZICA, CARLA VIGLIETTI-PANZICA, FERNANDO SANCHEZ, PHILIPPE SANTE, AND JACQUES BALTHAZART
Department of Human Anatomy and Physiology, Section of Neuroanatomy and Neuroembryology, I-10126 Torino, Italy (C.P., C.V.-P.), Department of Human Anatomy and Histology, Faculty of Medicine, Salamanca, Spain (F.S.), and Laboratory of General and Comparative Biochemistry, University of Liège, Liège, Belgium (P.S., J.B.)

ABSTRACT

The effects of testosterone on the volume and cytoarchitecture of the sexually dimorphic nucleus of the preoptic area (POM) were investigated in male and female Japanese quail. It was confirmed that castration decreases the POM volume in males and that, in gonadectomized birds of both sexes, testosterone increases this volume to values similar to those observed in intact sexually mature males. This suggests that the sex difference in POM volume results from a differential activation by T so that this brain morphological characteristic is not truly differentiated in the organizational sense. This conclusion was extended here by demonstrating that males exposed to a photoperiod simulating long days and that are known to have high plasma levels of testosterone have a larger POM than short-day males that have inactive testes. Detailed morphometric studies of POM neurons revealed a structural heterogeneity within the nucleus. A population of large neurons (cross-sectional area larger than 70–80 μm^2) was well represented in the dorsolateral but was almost absent in the medial part of POM. This lateral population of neurons was sensitive to variations of testosterone levels in males but not in females. The cross-sectional area, diameter, and perimeter of the dorsolateral neurons were significantly increased in males exposed to high testosterone levels (intact birds exposed to long days or castrated birds treated with the steroid). These changes were not observed in the medial part of the nucleus. Interestingly, the size of the dorsolateral neurons was not affected by testosterone treatments in females. These results suggest that the swelling of neurons in the lateral POM of males might be responsible for the increase in total volume of the nucleus, which is observed in physiological situations associated with a high testosteronemia. In addition, the sensitivity to testosterone of the dorsolateral neurons in the POM appears to be sexually differentiated. This differential response to testosterone might represent a truly dimorphic feature in the organizational sense and additional studies manipulating the early steroid environment should be performed to test this possibility.

Key words: photoperiod, sex dimorphism, medial preoptic nucleus, sexually dimorphic nucleus of the preoptic area, preoptic area

In mammals and birds, the preoptic area and anterior region of the hypothalamus (POA-AH) are involved in the control of several aspects of reproduction. Particularly the avian medial preoptic area plays a key role in the control of sexual behaviors (Barfield, '69, '71; Watson and Adkins-Regan, '89a), ovulation (Ralph and Fraps, '59a,b; Davies, '80; Follett, '84), and photoperiodically induced gonadal growth (Wada, '74, Davies and Follett, '80; Follett, '84). The preoptic area and the hypothalamus in birds also specifically accumulate steroid hormones such as ^{3}H-testosterone (e.g., Meyer '73, Arnold et al., '76, Barfield et al., '78, Watson and Adkins-Regan, '89b) and ^{3}H-estradiol

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Send reprint requests to G.C. Panzica, Dept. Human Anatomy and Physiology, Section of Neuroanatomy and Neuroembryology, c.so M. D'Azeglio 52, I-10126 Torino, Italy.

(Martinez-Vargas et al., '75, '76; Watson and Adkins-Regan, '89b).

Several studies have demonstrated the existence of sexually dimorphic structures in the preoptic region of different species of amphibia and mammals including the human (Raisman and Field '73; Greenough et al., '77; Gorski et al. '80; Ayoub et al. '83; Commins and Yahr '84; Takami and Urano '84; Hines et al. '85; Swaab and Fliers '85; Tobet et al. '86; Byne and Bleier '87; Allen et al., '89). In birds, extensive brain dimorphisms correlated with sex differences in behavior were reported in the telencephalic nuclei controlling song in passerine birds (Nottebohm and Arnold, '76). In addition, we demonstrated recently that one preoptic nucleus of the Japanese quail is sexually dimorphic. In adult quail, the volume of a nucleus identified as the nucleus preopticus medialis is larger in males than in females (Viglietti-Panzica et al., '86). Testosterone (T) directly controls the volume of this structure. Castration reduces it by approximately 25% and a 2-week T treatment restores the volume to values that are typical for intact sexually active males (Panzica et al., '87b). The volume of the nucleus is probably not truly differentiated in the organizational sense by perinatal steroids. It only depends on the adult circulating T levels because treatment of gonadectomized males and females with a same dose of T results in nuclei with similar volumes in both sexes (Panzica et al., '87b).

In a recent study confirming the presence of this dimorphism in the preoptic region of the Japanese quail, Adkins-Regan and Watson ('90) questioned the name of this nucleus. The nomenclature of the preoptic region seems rather confused in both birds (Huber and Crosby, '29; Crosby and Showers, '69; Oksche and Farner, '74; Kuenzel and Van Tienhoven, '82) and mammals (Allen et al., '89, Bloch and Gorsky, '88, Byne and Bleier, '87). The same clusters of cells are not always recognized by different authors, and even if they are, they often receive different names.

The aim of the present study was first to provide a more detailed description of the organization and nomenclature of the quail POA-AH. In addition, the study was designed to further test the hypothesis that adult T levels control the volume of the dimorphic nucleus. Birds were therefore exposed to different photoperiods simulating long and short days, and morphometric measures were then taken from these animals in an active or inactive reproductive state. The effects of these manipulations were compared to those observed following gonadectomy and T replacement therapy. We also performed detailed cytoarchitectonic studies on the dimorphic nucleus to investigate whether it is homogeneous or contains separate cell populations. As the study of intact adult birds clearly demonstrated the presence of different cell types, additional studies were performed to characterize their specific sensitivity to T.

MATERIALS AND METHODS

Subjects

A total of 52 adult Japanese quail (26 males and 26 females) were used. They were bought from a local breeder (Fr. Lefèvre, Boneffe, Belgium) at the age of 3 weeks. At their arrival in the laboratory, birds were randomly assigned to one of two experiments designed to study the effect of photoperiod (11 males, 12 females) and the effects of castration and T replacement therapy (15 males, 14 females). Before their arrival in the laboratory, birds had been kept in heterosexual groups and exposed to a long day photoperiod (16L:8D), but it has been shown that light has little or no stimulatory effect of the pituitary activity in quail before the age of 3 weeks (Siopes et al., '79). In the laboratory, the animals always received food and water ad libitum.

Experiment 1: Effect of the photoperiod

At their arrival in the laboratory, all birds assigned to this experiment were isolated in individual cages. Some of the birds (7 males, 6 females) were placed in an animal room in which lights were on for 6 hours a day from 9.00 A.M. to 3.00 P.M. (short day-SD-photoperiod; 6L:18D), whereas the other birds (4 males, 6 females) were exposed to 16 hours of light each day from 6.00 A.M. to 10.00 P.M. (long day-LD-photoperiod; 16L:8D). They were exposed to these conditions for 3 weeks. When 6 weeks old, they were tested once for sexual behavior in standard conditions (see below). On that occasion, their body weight and cloacal gland area (an androgen dependent structure, Sachs, '67) were recorded. The brains of these birds were perfused and dissected during the next week.

Experiment 2: Effect of castration and T replacement

Two days after their arrival in the laboratory, birds assigned to this experiment were gonadectomized or sham-operated under total anesthesia (Hypnodil, Janssens Pharmaceutica, Belgium; 15 mg/kg body weight) through a unilateral incision behind the ribs on the left side. Both testes were taken in males through this incision. In females, the left ovary only was removed as the right ovary is not developed and does not regenerate even after castration on the left side (Gibson et al., '75).

Nine days later, they were randomly assigned to one of six experimental groups defined as follows: (1) castrated males (n = 5): gonadectomized males with no hormone treatment (CX); (2) testosterone-treated males (n = 5): castrated males treated with 60 mm silastic implants filled with T (CX + T); (3) ovariectomized females (n = 5): gonadectomized females with no hormone treatment (OVX); (4) testosterone-treated females (n = 5): ovariectomized females treated with 60 mm silastic T implants (OVX + T); (5) intact males (n = 5): sham-operated males with no hormone treatment; (6) intact females (n = 4): sham-operated females with no hormone treatment.

The birds treated with T received 60 mm (3 × 20 mm) of silastic implants (Dow Corning tubing, number 602-252; i.d. 1.57 mm, o.d. 2.41 mm) filled with crystalline T (Sigma T-1500). These capsules were implanted subcutaneously in the neck region. The other four groups were implanted with empty silastic capsules of the same size. We have demonstrated previously that this type of T treatment restores in both males and females plasma levels of the steroid that are similar to those observed in intact sexually active males (Balthazart et al., '86; Schumacher and Balthazart, '86). Throughout the experiment, all birds were maintained under a photoperiod simulating long days (16L:8D).

Approximately 2 weeks after implantation when the birds were 6 weeks old, they were tested twice for sexual behavior and their body weight and cloacal gland were measured. They were killed (overdose of anesthetic and perfusion, see below) during the next week and the brains were collected for histology.

Behavioral tests

All birds were tested once or twice (see specific protocols) for sexual behavior in a test arena (60 × 40 × 50 cm) the floor of which was covered with sand. A stimulus egg-laying female was introduced in the arena and 30 seconds later the experimental subject was added. Its behavior was then observed for 3 minutes and the frequency of the following behavior patterns was recorded: neck grab (NG), mount attempt (MA), mount (M), cloacal contact movement (CCM), and strut. After the test, the area of the cloacal gland was measured with a caliper to the nearest millimeter (area = largest length × largest width). Body weight was recorded to the nearest gram.

Histological procedure

At the end of the experiment, birds received a 100-μl injection of heparin (30 mg/ml) into the wing vein and then were anesthetized with an overdose of Hypnodil (50 mg/kg). They were perfused with saline solution followed by Bouin fixative without acetic acid. Brains were dissected out of the skull and postfixed for 48 hours. The completeness of gonadectomy and presence of silastic implants were checked in all birds if appropriate. Birds that were not properly castrated or had lost their T implants were discarded, and all numbers mentioned in this study refer to the final number of specimen that had received adequate treatments.

The brains were dehydrated and embedded in Histosec (Merck). They were serially sectioned. Series of one 20-μm and eight 10-μm sections were collected in sequence and mounted on different slides. In this way, each section on a given slide was distant of 100 μm from the subsequent one. The 20-μm-thick sections, and one series of the 10-μm sections were then stained with toluidine-blue, coverslipped, and used for morphometry. Slides were then coded so that subsequent data analysis was conducted without knowledge of the sex or treatment condition of the animals.

Morphometrical analysis

The 20-μm-thick sections were used to measure the volume of the sexually dimorphic nucleus. It was calculated using a BASIC program running on an Apple//eTM computer connected with a graphics tablet and a SONY TV camera mounted on a Leitz Ortholux I microscope. This program was developed in our laboratory and is described elsewhere (Panzica et al., '87a). Drawings of the nucleus were performed on the tablet following the image obtained directly on the video screen using a 4× objective on the microscope. The volume was calculated from the surfaces in the serial sections according to the formula n.1 published by Uylings et al. ('84). This formula computes a volume for nuclei that is not affected by the thickness of the sections. It also calculates correct volumes even if a few sections are missing provided that the changes in area between successive sections are linear. The volumes of the left and right parts of the nucleus were always measured independently. As in previous experiments (Viglietti-Panzica et al., '86; Panzica et al., '87b,c), no asymmetry could be detected and the total volume (left + right) was therefore used in all analyses presented here.

Due to the difficulty in recognizing the POM boundaries in Nissl-stained sections, the volumes were calculated separately by 2 observers (GCP, CVP) for each specimen of experiment 1. As mentioned previously (Panzica et al., '87b), there is a good correlation between measurements taken in this way. As the castration and T replacement experiment was already a replication of previously published results from our laboratories (Panzica et al., '87b), the measures in this case were performed by only one person (G.C.P.).

For the measurements of cell size and other morphometric parameters, we used another BASIC program (MORPHO, release 2.1) running on the same hardware (see Panzica et al., '87a). For each bird, three 10-μm-thick sections corresponding to the medial part (rostrocaudal direction) of the dimorphic nucleus were selected. Within each section, quantitative data were collected in three fields (at 40× enlargements; 100 × 180 μm) in the left dorsolateral part of the nucleus, and three fields in its medial part. Measurements were done on all the neurons observed in these fields (see Fig. 3). Neurons were recognized based on the aspect of the nucleus (round and lightly colored) and the presence of Nissl substance in the cytoplasm. We only considered cells containing a clear nucleolus. Neurons that were largely superimposed to their neighbors or were not adequately preserved were not included in the data. In this way, 20–30 cells were usually measured in each field. The boundaries of these cells were drawn with the graphic tablet following the image obtained on the video screen through the camera. The computer program calculated the cross-sectional area of the cells (A), the diameter of the equivalent circle, the perimeter (P), and the shape factor ($4\pi A^2/P^2$). For each parameter, a statistical routine calculated the mean and standard deviation, and provided a distribution curve in percent with values distributed in 25 different classes.

Statistical tests

Mean values of POM volumes measured by the 2 observers were used for statistical analyses. Two-way analyses of variance (ANOVA) were used to evaluate all effects (POM volume and cell sizes). In the photoperiod experiment, they assessed the effect of the sex of the birds, the photoperiod, and their interaction. In the castration and T replacement experiment, the sex of the birds, the hormonal treatment, and their interaction were considered. Individual means were compared when appropriate by the Fisher protected least significant difference (Fisher PLSD) test. Differences were considered significant for $p < 0.05$. In both text and figures, data are represented by their mean and standard error of the mean. As the statistical analysis of the cells cross-sectional area, perimeter and diameter led to identical conclusions, only data concerning areas are reported here in detail.

RESULTS

Nuclear arrangement of the Japanese quail preoptic-anterior hypothalamic region

The results reported in this section are based partly on the experimental material collected in the present study and partly on 50-μm-thick Nissl-stained sections used in preceding studies to measure the volume of preoptic nuclei in adult sexually mature birds (Viglietti-Panzica et al., '86). Four different fiber systems are easily discernible and clearly circumscribe the preoptic-anterior hypothalamic region of the quail: (1) the tractus septomesencephalicus (TSM), whose fibers course dorsoventrally along the medial hemispheric wall (Fig. 1A,B); it defines the rostral and

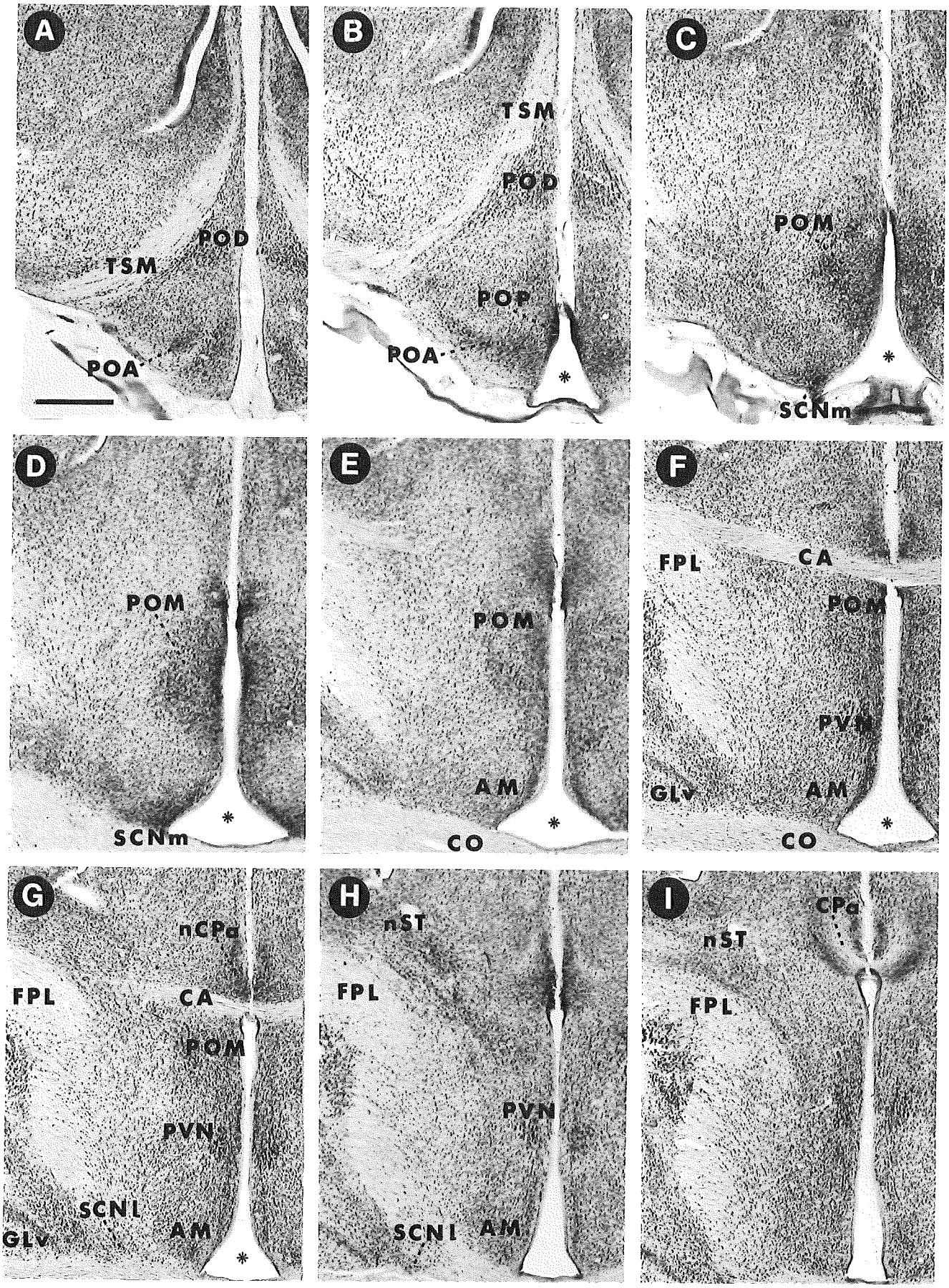
A
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Figure 1

dorsolateral boundaries of the preoptic region. (2) the commissura anterior (CA) marks the caudal and dorsal end of the region (Fig. 1F,G). (3) the chiasma opticum (CO) and the two optic tracts delineate the ventro-lateral limits of the region (Fig. 1D–I). (4) the fasciculus prosencephali lateralis (FPL) delimitating the caudolateral parts of the area (Fig. 1F–I).

Within the limits of these fiber systems, several magno- and parvicellular masses are recognizable. Large and intensely stained neurons probably correspond to the neurophysin-immunoreactive system. They are distributed in several cell clusters that occupy a lateral or periventricular location. In quail, this system has been described in several histo- and immunohistochemical studies (Oksche et al., '64; Bons, '80; Panzica, '85; Viglietti-Panzica '86).

Nuclear groups of parvicellular neurons have also been identified in the POA-AH of birds, e.g., in the domestic fowl (Van Tienhoven and Juhaz, '62; Kuenzel and VanTienhoven, '83; Kuenzel and Masson, '88), the pigeon (Karten and Hodos, '67), the ring dove (Vowles et al., '75), and the canary (Stokes et al., '74). Although the quail is a widely used bird in neuroendocrine research, very few studies have described the cytoarchitecture of the POA-AH in this species. The only available stereotaxic atlas does not distinguish the cell clusters of the preoptic region (Baylé et al., '74). For this reason, we present here a detailed photographic reconstruction of the region (see Fig. 1). In serial thick sections, a first well-recognizable nucleus is situated ventromedially under the tractus septomesencephalicus. This nucleus can be identified with the nucleus preopticus dorsolateralis (POD; Fig. 1A,B) described by in the chicken atlas (Kuenzel and van Tienhoven, '82). In the quail, this nucleus is indistinguishable from the nucleus of the diagonal band of Broca (Crosby and Showers, '69). Ventrally at a slightly more caudal level, an aggregation of parvicellular elements can be observed (Fig. 1A,B). At the level of the rostral pole of the third ventricle (Fig. 1B), this neuronal cluster is intensely stained and extends laterally like the wings of a butterfly. We identify this cellular group as the nucleus preopticus anterior (POA) according to the nomenclature of Karten and Hodos ('67), Stokes et al ('74), and Vowles et al. ('75).

According to Crosby and Showers ('69), one should recognize near the ventricle wall a parvicellular nucleus characterized by a columnar arrangement and called periventricular preoptic nucleus (POP). As stated by these authors, the extension of this nucleus is variable in different avian species. It seems that in the quail, as well in the chicken, the POP is not well developed and consists of only a few lines of neurons located rostrally (Fig. 1B). Lateral to this POP, there is a large nucleus that extends throughout most of the preoptic region in the rostro-caudal axis. At its most rostral level, this nucleus is contiguous with the POP. More caudally, it extends laterally and dorsally. Just rostral to the anterior commissure, it shows a characteristic oblong shape (Fig. 1C–G). The nucleus then extends dorsally until the level of the anterior commissure and after that, quickly disappears. In some specimens, however, a cluster of cells seems to extend more dorso-laterally and merges with the bed nucleus striae terminalis (nST, Fig. 1H–I). We used for this nucleus the name of nucleus preopticus medialis (POM) based on several photographic and schematic descriptions of the region in different bird species (Karten and Hodos, '67; Crosby and Showers, '69; Stokes et al., '74; Vowles et al., '75; Berk and Butler, '81; Yamauchi and Yasuda, '85). The lateral preoptic nucleus is not discernible in the quail preoptic area. It is probably composed of scattered small neurons that are lateral to the POM and interspersed within the lateral forebrain bundle fibers. Dorsal to the commissura anterior near the median line, there is a cluster of small intensely stained elements identified generally as the bed nucleus pallial commissure (nucleus commissurae pallii, nCPa; Fig. 1F–I). This group of neurons seems continuous with the more rostral similar elements located dorsal to the organum vasculosum laminae terminalis (Fig. 1B–E). Finally, near the lateral angle of the third ventricle there is an accumulation of cells classically identified (Crosby and Showers, '69, Kuenzel and van Tienhoven, '82) as the nucleus suprachiasmaticus (SCN). The nomenclature, function, and homology of this nucleus with the SCN of mammals is, however, a matter of controversy at present (Kuenzel and van Tienhoven, '82; Panzica, '85; Cassone and Moore, '87; Cassone, '88; Rivkees et al., '89; Norgren and Silver, '90; Sanchez et al., '90). According to Kuenzel and Masson ('88), two different nuclei should be respectively identified as nucleus suprachiasmaticus pars medialis (SCNm, Fig. 1C,D) and pars lateralis (SCN1, Fig. 1H).

Experimental manipulations of the POM volume

In order to limit to a minimum the number of birds that needed to be killed in these experiments, a small number of control birds only was included in each experiment. Considering that the intact birds exposed to a long photoperiod in experiment 1 and the intact sham-operated birds included in experiment 2 were exposed to very similar conditions (LD intact males), it was planned to pool these groups of birds and use them as control for both experiments. After the data were collected, we first determined that the small differences in procedures from one experiment to the other did not produce systematic differences between these two subgroups of birds. The body weight, cloacal gland area, and POM volume of the intact males and females were therefore analyzed by two-way ANOVA with the sex of the birds and the experiment in which they were originally included as factors. No significant effect of the experiment could be detected in this way (all $p > 0.15$). Therefore in all subsequent analyses, we have used the pooled data coming from these two subgroups of intact birds as control groups.

Fig. 1. Serial reconstruction of the preoptic region of the Japanese quail as observed in 50-μm-thick Nissl-stained sections. Sections from **A** to **I** run from rostral to caudal and are separated each by 100 μm. The asterisk (*) indicates the preoptic recess of the third ventricle. The bar in A represents 500 μm. AM: nucleus anterior medialis hypothalami; CA: commissura anterior; CPa: commissura pallii; CO: chiasma opticum; FPL: fasciculus prosencephali lateralis; GLv: nucleus geniculatus ventralis; nCPa: nucleus commissurae pallii; nST: nucleus striae terminalis; POA: nucleus preopticus anterior; POD: nucleus preopticus dorsolateralis; POM: nucleus preopticus medialis; POP: nucleus preopticus periventricularis; PVN: nucleus paraventricularis; SCNm: nucleus suprachiasmaticus, pars medialis; SCNl: nucleus suprachiasmaticus, pars lateralis (nucleus decussationis supraopticae); TSM: tractus septomesencephalicus.

Experiment 1: Effect of photoperiod. The body weight of the four groups of birds included in this experiment (LD and SD males and females) was analyzed by a two-way ANOVA that detected no effect of the sex of the birds, of the photoperiod they were exposed to, and of their interaction (all $p > 0.2$). The mean body weight of all birds was 227 ± 4 g (mean ± standard error). By contrast, the ANOVA of the cloacal gland areas revealed significant effects of the sex of the birds ($F_{1,29} = 13.51$, $p < 0.001$), of the photoperiod ($F_{1,29} = 80.24$, $p < 0.001$) and of their interaction ($F_{1,29} = 10.39$, $p = 0.003$). Gland size was larger in LD males than in LD females ($p < 0.05$ by Fisher PLSD test) and it was reduced to basal values in both sexes ($p < 0.05$ in each case by the Fisher PLSD test) following exposure to short day (LD males: 261.50 ± 25.06, LD females: 146.75 ± 7.21, SD males: 58.92 ± 3.68, and SD females: 51.41 ± 6.16 mm²). As expected based on previous experiments (Sachs, '69; Adkins-Regan, '83), all LD males showed sexual behavior (at least MA) during the standard tests, whereas all birds from the three other groups were sexually inactive.

The POM nucleus was clearly visible in all males and females exposed to long days. In short day animals, there was a general decrease in the staining intensity, similar to the decrease observed in gonadectomized birds (Panzica et al., '87b; this study). The boundaries of the nucleus were therefore more difficult to identify. There was nevertheless a good correlation between the volumes measured by the two independent observers ($r = 0.912$, $n = 26$, $p > 0.001$). The two-way ANOVA of the POM volumes revealed a photoperiod effect ($F_{1,22} = 18.99$, $p < 0.001$) and a strong interaction between sex and photoperiod ($F_{1,22} = 9.40$, $p = 0.005$). No overall sex difference was present ($F_{1,22} = 0.23$, $p = 0.63$). The significant interaction obviously resulted from the major decrease in volume observed in males exposed to short days, whereas females did not show this type of effect (see Fig 2A for the detail of statistical comparisons).

Experiment 2: effect of gonadectomy and testosterone therapy. Body weight was similar in all groups of birds included in this experiment, although females were as a mean about 20 g heavier than males (two-way ANOVA, effect of sex: $F_{1,34} = 3.59$, $p = 0.066$, effect of treatments: $F_{2,34} = 2.16$, $p = 0.130$, effect of interaction: $F_{2,34} = 0.46$, NS). Mean body weight in these birds was 235 ± 5 g. The measures of cloacal gland areas confirmed the success of the endocrine manipulations that had been made and the previously observed sex difference in this structure (Adkins, '75; Balthazart et al., '83; Panzica et al., '87b). The structure was larger in intact males than in intact females; it decreased after castration in males and it grew following exposure to T in both sexes (all $p < 0.05$ by the Fisher PLSD: Intact males: 261.50 ± 25.06, Intact females: 146.75 ± 7.21, CX males: 51.35 ± 4.16, OVX females: 87.7 ± 36.16, CX + T males: 263.15 ± 23.32, OVX + T females: 205.75 ± 13.75 mm²). This resulted in a significant effect of the sex of the birds ($F_{1,34} = 6.12$, $p = 0.0185$), their treatment ($F_{2,34} = 27.08$, $p < 0.001$) and the interaction of these two factors ($F_{2,34} = 6.33$, $p = 0.004$) in the ANOVA. The sexual behavior of the birds was also in full agreement with their endocrine status: all intact males and CX + T males showed at least MA during the tests, whereas birds from all other groups were completely inactive.

The analysis of POM volumes in the present experiment was actually a replication of a former study (Panzica et al., '87b). The results, presented in Figure 2B, are qualitatively similar to those reported previously. Absolute values are, however, different due to the different fixative used in this experiment. Bouin (used instead of formalin) produced more shrinkage of the tissue but resulted in a better preservation of the specimen. The two-way ANOVA identified as previously an overall effect of sex ($F_{1,29} = 6.36$, $p = 0.017$) and of treatments ($F_{2,29} = 11.70$, $p < 0.001$) but not of their interaction ($F_{2,29} = 2.16$, $p = 0.132$). Castration reduced the POM volume in males, whereas testosterone treatment of both males and females produced a volume increase resulting in values similar to those observed in intact males. POM volume was also smaller in intact males than in intact females (all $p < 0.05$ by the Fisher PLSD test).

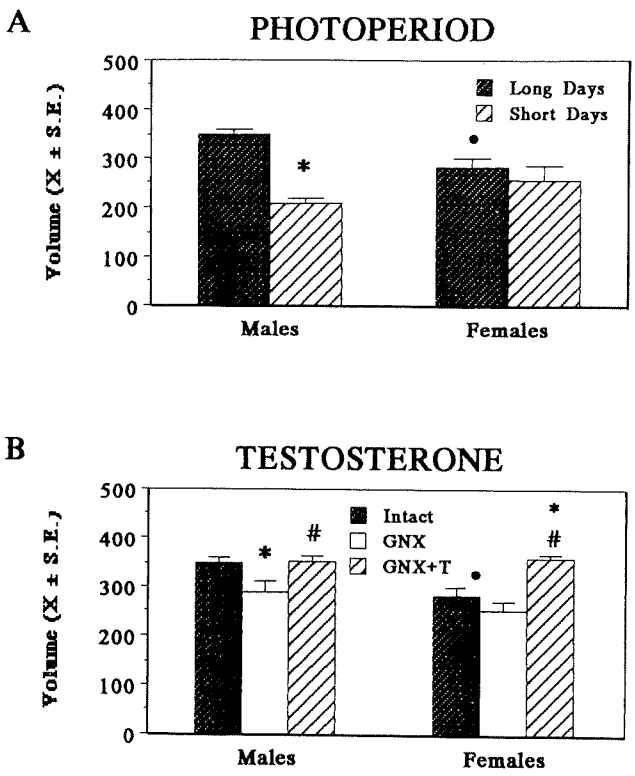

Fig. 2. Total volume of the nucleus preopticus medialis (POM) in male and female quail exposed to short or long days (**A**) and in quail exposed to long days and gonadectomized (GNX) or gonadectomized and submitted to a replacement therapy with T (GNX + T) or sham-operated (Intact) (**B**). The volumes are expressed in μm³ × 1,000. Data were analyzed by two-way ANOVA (see Results) followed by posthoc comparison of means using the Fisher PLSD test. These results are indicated in the graphs at the top of the bars as follows: * = $p < 0.05$ compared with LD or intact birds of the same sex, # = $p < 0.05$ compared to gonadectomized birds of the same sex, ● = $p < 0.05$ compared to males submitted to the same experimental treatment.

Effects of photoperiod, gonadectomy, and testosterone therapy on the POM cytoarchitecture

A qualitative examination of preoptic area sections in the region of the POM immediately revealed that there was a clear heterogeneity in the distribution of cells in the medial and dorsolateral parts of the nucleus. This was especially noticeable in thin section (10–20 μm). The dorsolateral

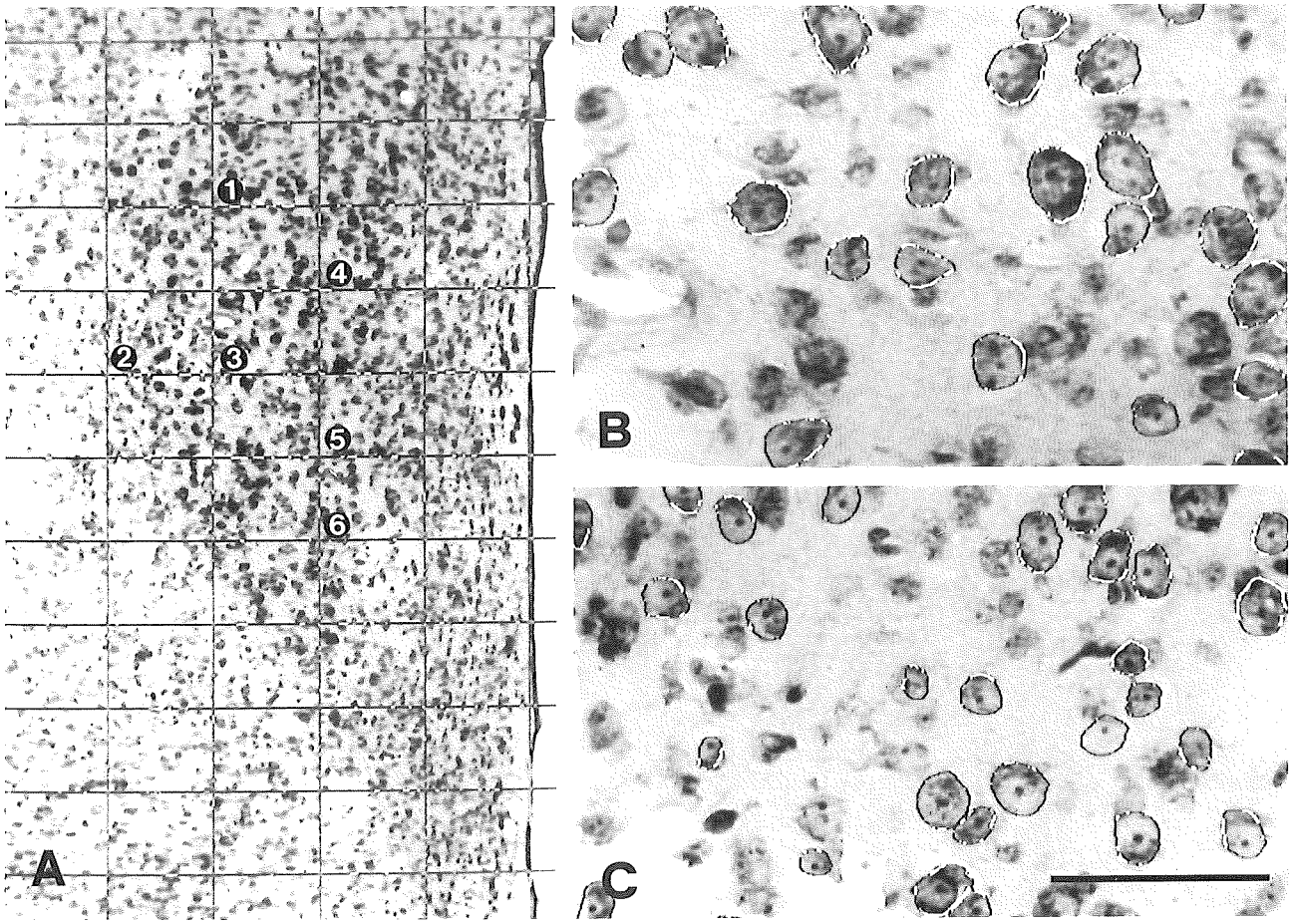

Fig. 3. **A.** Medium power enlargement of the POM illustrating the difference in cell typology between the dorsolateral and the medial part of the nucleus. The rectangular grid superimposed on the figure was used to randomly select the three microscopic fields of each subregions (1–3 dorsolateral, 4–6 medial) in which the cells were measured. **B,C.** Enlargement of one microscopic field in the dorsolateral (B) or medial (C) part of the POM showing the cell contours as they were drawn with the help of the image analyzer. The bar represents 50 μm.

elements seemed generally larger than those located more medially. To verify this impression, we decided to quantify cells sizes in the different parts of the nucleus of birds exposed to different hormonal conditions that were already known to influence the total volume of the POM. Figure 3 contains microphotographs that illustrate the medial and dorsolateral subdivisions of the POM and the methods that were used to collect these quantitative data.

Figure 4 presents the frequency distribution curves of cell areas for both the medial and the dorsolateral neuronal populations in LD males and LD females. In both sexes, the curves were similar and it was possible to observe important differences between the frequency distribution of the medial and lateral populations. In both males and females, there were fewer large cells in the medial part of the POM. Figure 4, however, clearly shows that only one cell population was present in the lateral and in the medial part of the nucleus (no suggestion of bimodal distribution in any of these curves).

Figure 5 summarizes the morphometric data collected separately for the two subregions of the POM in the two experiments: the mean cell areas in the different experimental groups are presented for the dorsolateral and medial parts of the dimorphic nucleus. In both experiments, the shape factor of the cells was not affected by the sex of the birds, the experimental treatments and the interaction of these two factors (all $p > 0.10$ by two-way ANOVA). Therefore, the cell areas essentially provided the same information as the cell perimeters and the diameters of equivalent circles. These two parameters were also subjected to statistical analysis and this always led to the same conclusions as the analysis of the cell areas. The latter results only are discussed here.

In the medial part of the nucleus, the cell area was always smaller than in the dorsolateral part. The cell size in the medial part was not affected by the sex of the birds, the photoperiod they were exposed to, and the hormonal manipulations (castration and T replacement therapy). By contrast, in males the size of the dorsolateral neurons markedly changed as a function of the hormonal status of the birds. Both the transfer from short days to long days and the treatment with T significantly increased the cell area (see Fig. 5 for the detail of statistics). These effects of photoperiod and of T treatment were not observed in females in which all morphometrical parameters were constant at values intermediate between those of sexually mature males and those of castrated or short day males. In the two-way ANOVA, this resulted in very significant

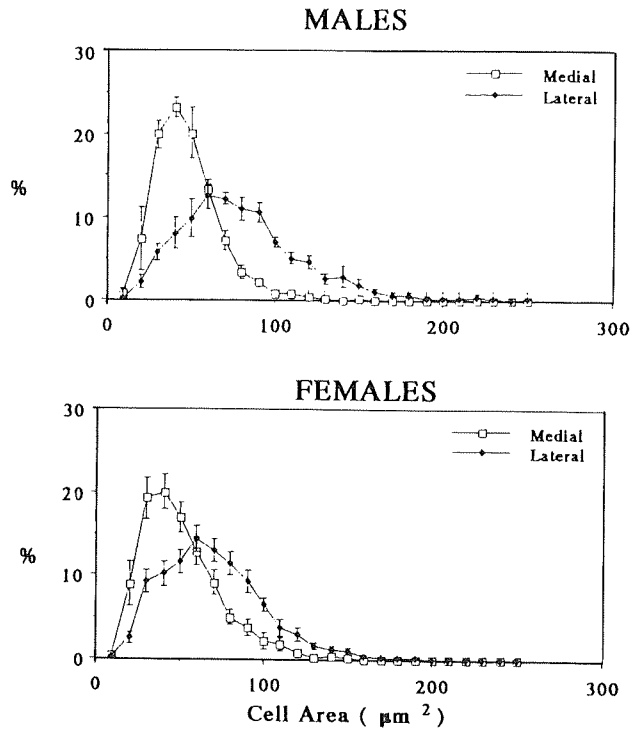

Fig. 4. Frequency distribution curves of the cell areas measured in the medial and in the dorsolateral part of the POM in male and female quail that were exposed to long days. The distribution was constructed with classes of 10 μm^2. Each curve is based on the data collected in 4–9 birds (Males medial: n = 4, Males lateral: n = 7, Females medial and lateral: n = 9). In each bird, at least 150–200 cells were measured in both the lateral and medial part of the nucleus (3 independent fields in 3 sections containing 20–30 cells each).

interactions between the effects of sex and of photoperiod in the experiment 1 and between the effects of sex and of hormonal treatments in experiment 2. The shape factor of the cells was not significantly affected by any of the factors that were considered in the present experiments (sex, endocrine condition, location within the POM).

These data therefore demonstrate that neurons located in the dorsolateral part of the male POM are sensitive to T action. Figure 6 presents of few microphotographs that illustrate the morphological changes observed in this cell population of males following an increase in the photoperiod or treatment of castrated birds with T. The changes in mean cell size are quite visible. In addition, the higher content in Nissl substance in LD and in T-treated birds by comparison with the SD and castrated birds can be observed.

It is also important to note that the increase in mean cell area observed following exposure to high levels of T (due to implantation of silastic capsules or exposure to long days) did not result from the differentiation of an additional subpopulation of large cells: the distribution curves of cell sizes was unimodal in birds exposed to both high and low levels of T presumably indicating a volume increase in the entire population (see Fig. 7).

DISCUSSION

The preoptic area-anterior hypothalamic region of birds is of crucial importance in the control of different aspects of reproduction (copulation, oviposition, secretion of gonadotropin-releasing factors). Many experimental studies using different avian species have analyzed the neuroanatomical aspects of these regulations (see introductory section). Surprisingly however, the detailed morphology of this region in birds has been poorly studied. Two studies only (Kuenzel and van Tienhoven, '82; Yamauchi and Yasuda, '85) provide precise descriptions of the cytoarchitecture and nomenclature of the chicken hypothalamus. For this reason, we described in detail the preoptic-anterior hypothalamic region of the Japanese quail, a widely used laboratory bird. The description is based on Nissl-stained sections as was also the case in most previously published studies. Generally the nomenclature adopted by Kuenzel and van Tienhoven ('82) and by Kuenzel and Masson ('88) for the chicken can easily be adapted to the quail hypothalamus. Unfortunately these authors did not describe or name in the chicken a very large cluster of cells present in the medial part of the quail preoptic region, which is sexually dimorphic as shown by earlier publications of our laboratories (Viglietti et al. '86; Panzica et al., '87b). This probably is a consequence of the facts that they used relatively thin sections to establish their atlas and that, in addition, they studied birds that were not sexually mature. As the staining intensity of the dimorphic nucleus is T dependent, it is possible that this structure was not clearly visible in their material.

Adkins-Regan and Watson ('90) in a recent morphometric study of the quail POA-AH experienced difficulties in naming the different cell clusters that they measured. They also identified in the medial part of the POA-AH a sexually dimorphic cell cluster. This dimorphic nucleus in their study is without a doubt identical to the nucleus that we previously identified as the nucleus preopticus medialis or POM. Based on the facts that in the chicken atlas, the name POM had been used for another more lateral and anterior structure and that the sexually dimorphic nucleus was not described in the chicken, they suggested to introduce the name nucleus preopticus medianus for the sexually dimorphic structure.

Based on a careful analysis of the available descriptions of the preoptic region in several species of birds and mammals and on the previous use of the terminology, we believe that the name nucleus preopticus medialis (POM) should be maintained for the dimorphic nucleus. In almost every publication on the avian preoptic area, POM has been used to name a large, caudal structure of the preoptic area located close to the third ventricle and extending to the level of the anterior commissure. This is true for studies on the chicken (Crosby and Showers, '69; van Tienhoven, '80; Yamauchi and Yasuda, '85), the house sparrow (Crosby and Showers, '69; Oksche et al., '74), the pigeon (Karten and Hodos, '67; Berk and Butler, '81), the ring dove (Vowles et al., '75, Martinez-Vargas et al., '76; Fechner and Buntin '89), and the quail (Yamada and Mikami, '81), to name only a few. Kuenzel and van Tienhoven ('82) and more recently Kuenzel and Masson ('88) are therefore the only authors who have so far associated in the chicken the name POM to a more lateral structure located in the rostral preoptic area and named POA in our present description (see Results). Even in the chicken, Yamauchi and Yasuda ('85) described the medial preoptic nucleus as a structure immediately lateral to the stratum cellulare internum (identified as the POP of our nomenclature) and extending in the dorsal and caudal direction until the level of the anterior commissure

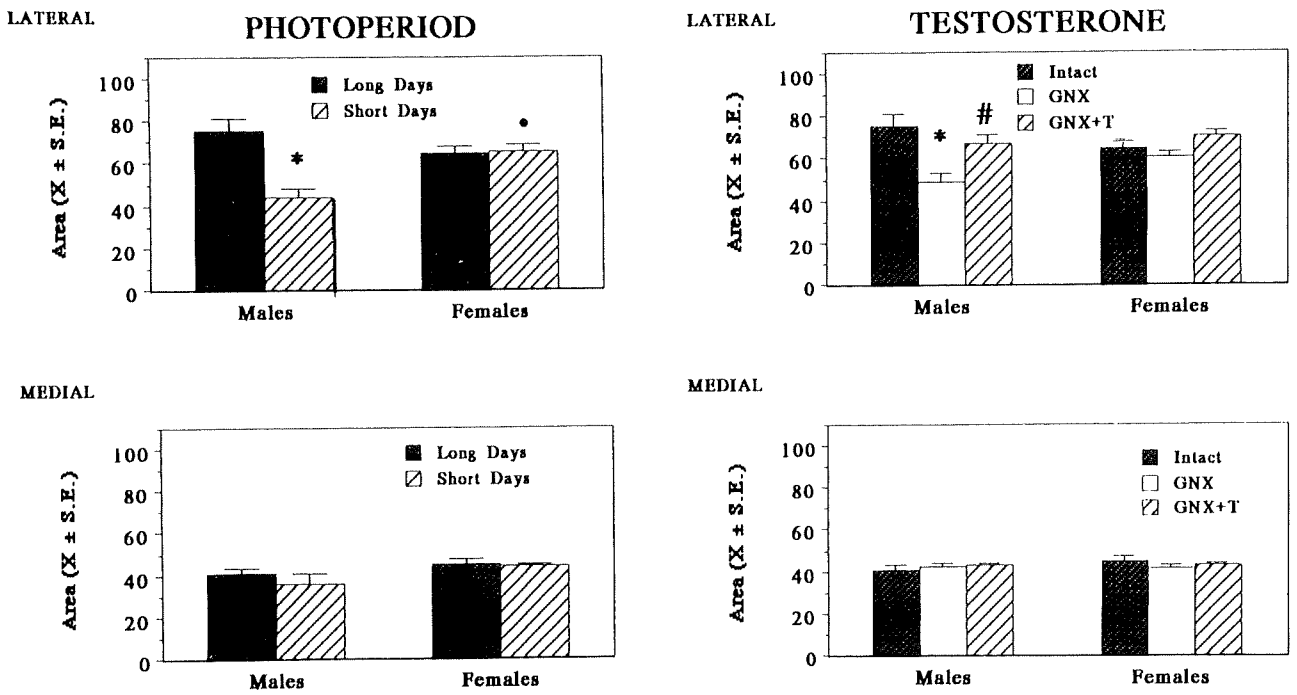

Fig. 5. Mean cell areas (in μm^2) measured in the two subdivisions of the POM (dorsolateral on top and medial at the bottom) during experiment 1 (photoperiod, left) and experiment 2 (testosterone, right). Within each experiment, data were analyzed by two-way ANOVA with the sex of the birds, the experimental treatments and their interaction as factors. No significant effect was observed in the medial part of the POM. During experiment 1 (photoperiod), it was shown that the cell area is influenced by the experimental treatments (photoperiod; $F_{1,22} = 10.29$, $p = 0.004$) and by the interaction sex treatment ($F_{1,22} = 11.44$, $p = 0.002$) but there was no overall effect of the sex ($F_{1,22} = 1.39$, ns). Similar results were obtained in experiment 2 (effect of sex: $F_{1,30} = 0.17$, ns; effect of treatments: $F_{2,30} = 7.05$, $p = 0.003$; effect of interaction: $F_{2,30} = 3.40$, $p = 0.046$). When appropriate, ANOVA were followed by posthoc comparison of means using the Fisher PLSD test. These results are indicated in the graphs at the top of the bars as follows: * = $p < 0.05$ compared with LD or intact birds of the same sex, # = $p < 0.05$ compared to gonadectomized birds of the same sex, ● = $p < 0.05$ compared to males submitted to the same experimental treatment.

(Figs. 2A, 3A in that study). They described, as we did, that the caudal part of the nucleus merges with the nucleus interstitialis of the dorsal olfactory projection (analogous of the bed nucleus striae terminalis of the Karten and Hodos pigeon atlas). Their description in the chicken therefore closely resembles our description of the POM in the quail. Based on this common use of the nomenclature, we propose to maintain the name POM for the medial sexually dimorphic nucleus and to use POA (nucleus preopticus anterior) for the more lateral and rostral cell cluster identified as POM by Kuenzel and van Tienhoven ('82). This use of POA is also consistent with many previous studies (Karten and Hodos, '67; Baylé et al., '74; Stokes et al., '74; Vowles et al., '75).

The nomenclature that we propose to keep has additional advantages when trying to compare avian and mammalian studies and also from the functional point of view. In mammals, the nucleus located in medial region of the preoptic area close to the third ventricle is classically referred to as POM (Christ, '69; Bleier et al., '82; Paxinos and Watson, '86; Bloch and Gorski, '88). This nucleus also contains subregions that were shown to be sexually dimorphic (e.g., Gorski et al., '78; Bloch and Gorski, '88). The medial preoptic region of mammals is characterized by the presence of specific receptors for androgens and estrogens (for review: Kelley and Pfaff, '78; Blaustein and Olster, '89) as is also the case for the nucleus that we call POM in the quail (Balthazart et al., '89; Watson and Adkins-Regan, '89b). Finally, the name nucleus preopticus medianus, which is proposed by Adkins-Regan and Watson ('90) for the dimorphic nucleus of the quail, has already been used in mammals to identify a different median structure located in the caudal part of the preoptic area above and below the anterior commissure (see Paxinos and Watson, '86). For all these reasons, we believe that the name nucleus preopticus medialis should be kept to identify the sexually dimorphic, T-sensitive group of neurons in the quail preoptic area.

In previous studies, we demonstrated that the quail POM is larger in males than in females (Viglietti-Panzica et al., '86), and this finding was recently confirmed in another laboratory (Adkins-Regan and Watson, '90). Based on the facts that castration decreases and T treatment increases the volume of this nucleus, whereas embryonic treatment with estradiol benzoate does not affect it, we concluded that the sexual dimorphism results from a different activation by T in adult birds and does not represent a truly differentiated feature in the organizational sense (Panzica et al., '87b). This conclusion was confirmed in the present study. We were first able to replicate the effects of castration and T replacement on the nucleus volume. Once again, no difference in POM volume was observed between males and females when they were exposed to the same hormonal conditions (T-treated gonadectomized birds). Spontaneous variations in plasma T are associated in quail with a

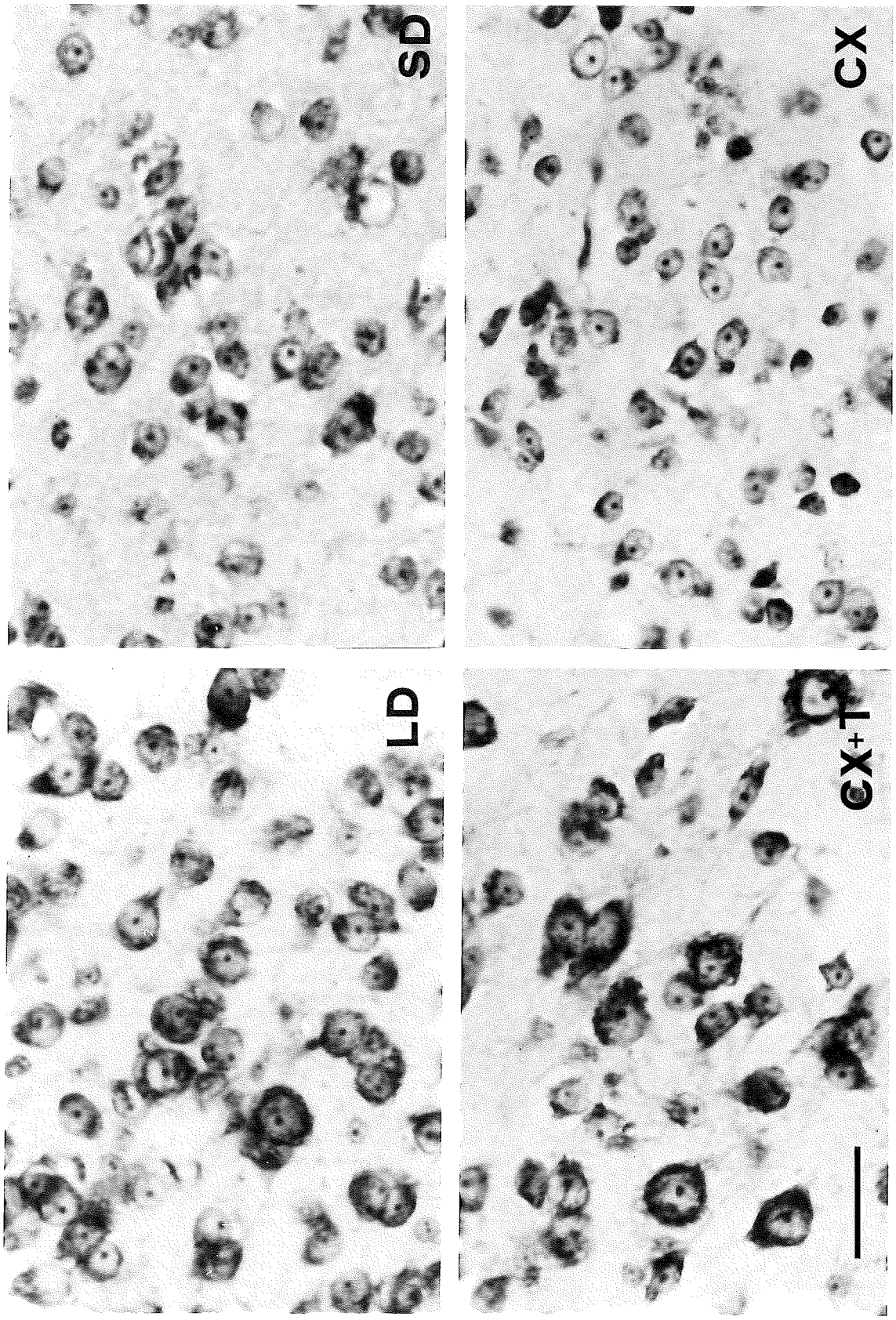

Fig. 6. Microphotographs of the dorsolateral region of the POM in male quail exposed to long days (LD) or short days (SD) and in LD males castrated (CX) or castrated and submitted to a replacement therapy with T (CX + T). The bar represents 25 μm.

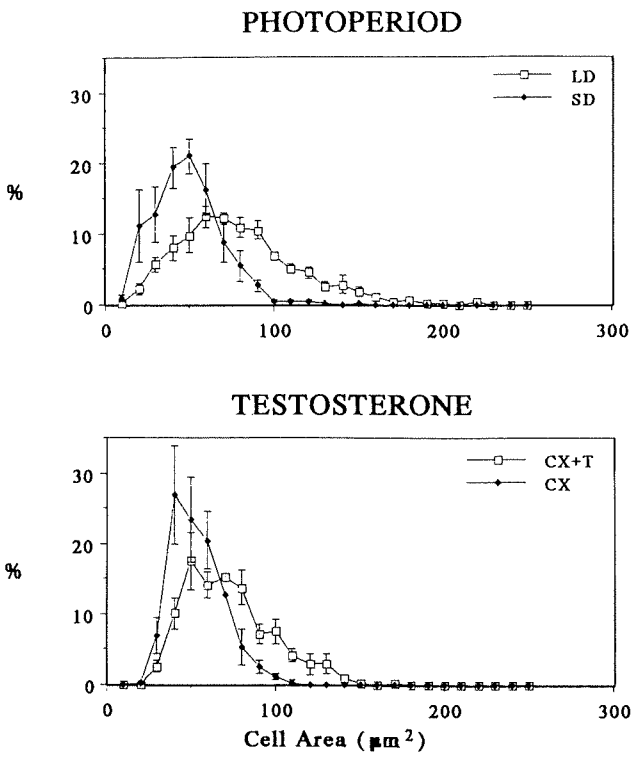

Fig. 7. Frequency distribution curves of the cell areas measured in the dorsolateral part of the POM of male quail that were exposed to long days (LD) or short days (SD) and in LD males castrated (CX) or castrated and submitted to a replacement therapy with T (CX + T). The distribution was constructed with classes of 10 μm^2. Each curve is based on the data collected in 5–7 birds (males LD: n = 7, all other curves: n = 5). In each bird, at least 150–200 cells were measured (3 independent fields in 3 sections containing 20–30 cells each).

modification of the photoperiod. Birds exposed to long days have much higher T levels than those exposed to short days (Balthazart et al., '79; Follett, '84). These changes were indirectly confirmed in the present experiment by the variations in cloacal gland area and in sexual behavior. They were also associated in the males with major modifications in POM volume. The volume of this nucleus in females was kept constant at a level intermediate between that of LD and SD males. Seasonal variations in the volume of nuclei related to the control of reproductive activities were also demonstrated in several preoptic and telencephalic nuclei of the Japanese toad (parts of the preoptic area and of the amygdala: Takami and Urano, '84) and of the male canary (song control nuclei: Nottebohm, '81; Nottebohm et al., '86). These are also presumably driven by the changes in plasma T levels. The data collected here in experiments 1 and 2 are therefore totally consistent with the idea that the sex difference in the total volume of the POM in quail is activational in nature.

The main goal of the present studies was to provide a more detailed cytoarchitectonic description of this dimorphic nucleus. This was motivated by three independent reasons. First it was recognized that the POM was a very large structure of the preoptic area. In all probability it was heterogeneous and this aspect should be described. In addition, we wanted to approach the mechanisms underlying the changes in total volume of the nucleus and this could only be done through morphometric analyses of its constituents. Finally, recent studies on the rat medial preoptic area demonstrated that subregions could be defined that were differentialy affected by hormonal treatments (Bloch and Gorski, '88), and it was important to research whether a similar situation occurred in quail.

Distribution curves of cell sizes in the dorsolateral and medial parts of the POM indeed demonstrated major differences between these two subregions. The lateral part of the nucleus contains a population of relatively large neurons that are not present in the medial part. This regional differentiation is present in both intact males and females. Experimental manipulations of the endocrine condition of the subjects revealed that these two separate cell populations show a differential sensitivity to T. The morphometric characteristics (cross-sectional area, diameter, perimeter, and shape factor) of the neurons in the medial part of the POM were not affected by the photoperiod the birds were exposed to nor by the castration and T replacement therapy. This was true in both males and females. By contrast, these same treatments had profound effects on the dorsolateral population of neurons in males. All parameters reflecting the size of the cells (area, diameter, and perimeter) were markedly increased in the physiological and experimental conditions associated with high plasma T levels (LD and CX + T males). The shape factor was, however, not modified. None of these changes was observed in females.

Further studies will have to establish whether the two subpopulations of neurons can also be differentiated on the basis of other morphological or neurochemical criteria. At present, these results have important consequences for our understanding of the sexual differentiation of the preoptic area and behavior. They identify a new sexually dimorphic characteristic: cell size is sensitive to T in males but not in females. During experiment 1, the change in photoperiod affected the cell size only in males. Considering that this effect was possibly mediated by the changes in plasma T, it was not surprising that a similar modification was not seen in females. It is indeed clear that no major change in plasma T takes place when female quail are transferred from short to long days. The cloacal gland that is extremely sensitive to changes in androgen levels does not grow in LD females. A small increase in area is detected, but it only reflects the enlargement of the cloacal diameter associated with the beginning of egg laying. The gland of LD females is not swollen and reddish like the gland of a T-treated female.

In the second experiment, the T treatment did not significantly increase the cell size in gonadectomized females, whereas it was fully effective in males. Gonadectomized T-treated males and females are known to be exposed to similar hormonal milieu and have similar levels of testosterone, androstenedione, 5α-dihydrotestosterone, estradiol, and progesterone as measured by radioimmunoassay (Balthazart et al., '83, '86; Schumacher and Balthazart, '86). Therefore this suggests that the sensitivity to T of this neuronal population is sexually differentiated in the organizational sense, and future experiments including manipulations the embryonic and neonatal hormonal environments should be conducted to test this hypothesis. These should also explain why the volume of the POM in SD females and the size of the lateral neurons in all females are intermediate between the levels seen in castrated and in T-treated males, whereas a more parsimonious hypothesis would have predicted female data similar to those of castrated

males. Long-term effects of the neonatal exposure to estrogen might therefore be contemplated.

We confirmed here that the volume of the POM is significantly affected following castration and T replacement therapy. These changes could be due to an increase in the cell number, in the cell size, or in the cell spacing within the nucleus (true volume increase) or to an enhancement of the detectability of the nucleus at its edges as a result of the increase of Nissl substance in the marginal neurons (apparent volume increase). The present results suggest that the T-induced increase in POM volume might be due, at least in part, to the swelling of the dorsolateral neurons following exposure to the hormone. We showed indeed that the mean cell diameter in this region of the nucleus is 1.16 times larger (area is 1.36 times larger) in CX + T than in CX males. This means that the mean cell volume is 1.58 times higher (1.16^3) following treatment with T (58% increase). As neuronal size only increase in the lateral part of the POM, this increase in mean cell volume might therefore be responsible for the changes in POM volume (22.6% increase) observed following exposure of castrated males to T. To confirm this conclusion, it would, however, be necessary to demonstrate that the spacing between these enlarged cells is also increased in conditions associated with high T levels. The present data nevertheless suggest that the volume changes of this nucleus are real and do not only reflect variations of the detectability at the edges. If this interpretation is true, it then remains to explain why similar treatment of females with T also increases the total volume of the POM without having any major effect on the mean cell size in the nucleus. Answers to this question can only be speculative at present and will require additional studies of the cell typology and cell spacing within the nucleus.

Finally and most importantly, this study demonstrates that in males the action of T on the POM is limited to the dorsolateral part of the nucleus at least as far as morphological changes are concerned. This might have important implications for the functional analysis of the system. It is quite possible that the behavioral effects of T on copulation, which are known to take place specifically in the POM (Balthazart et al., '88; Balthazart and Surlemont, '90), are in fact limited to the dorsolateral part of this structure. Detailed stereotaxic studies involving electrolytic lesions and implantation of small quantities of T should investigate this possibility. It is important to mention in this context that in a recent immunocytochemical study we could demonstrate that the aromatase-immunoreactive cells are preferentially located in this part of the nucleus (Balthazart et al., '90). Considering the key role played by the aromatization of T in the activation of copulatory behavior in quail (Balthazart, '89), this observation strongly supports the idea that the dorsolateral POM is the specific site of T action on behavior. It has been demonstrated that aromatase activity in the quail preoptic area and more specifically in the POM is T-dependent (Schumacher and Balthazart, '86; Balthazart et al., '88). Recently we could show that the aromatase immunoreactivity in the POM is also controlled by T suggesting that new enzyme molecules are synthesized in response to the steroid (Balthazart et al., '90). Again, this induction of aromatase immunoreactivity was preferentially located in the lateral parts of the POM (Balthazart et al., '90). It is therefore conceivable that the increase in cell volume that is seen in this region in T-treated birds actually reflects the induction of protein synthesis and in particular of aromatase by the steroid. The morphological changes would consequently represent a direct marker of the cellular mechanisms mediating the activation of sexual behavior by androgens.

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