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EFFECTS OF LESIONS OF THE MEDIAL PREOPTIC NUCLEUS ON THE TESTOSTERONE-INDUCED METABOLIC CHANGES IN SPECIFIC BRAIN AREAS IN MALE QUAIL

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Abstract—The effects of bilateral lesions of the medial preoptic nucleus in association with testosterone on the metabolic activity in discrete brain regions was studied quantitatively by the *in vivo* autoradiographic 2-deoxyglucose method. Adult male quail were castrated and then left without hormone replacement therapy or treated with testosterone or treated with testosterone and submitted to a bilateral lesion of the medial preoptic nucleus, a brain region that plays a key role in the activation of male copulatory behavior by testosterone. Treatment for about 10 days with testosterone activated the expression of the full range of male sexual behaviors and these behaviors were completely suppressed by the medial preoptic nucleus lesions. Mapping of 2-deoxyglucose uptake revealed both increases and decreases of metabolic activity in discrete brain regions associated with the systemic treatment with testosterone as well as with the lesion of the medial preoptic nucleus. Testosterone affected the oxidative metabolism in brain areas that are known to contain sex steroid receptors (such as the nucleus taeniae and the paraventricular and ventromedial nuclei of the hypothalamus) but also in nuclei that are believed to be devoid of such receptors. Effects of testosterone in these nuclei may be indirect or reflect changes in terminals of axons originating in steroid-sensitive areas. Bilateral medial preoptic nucleus lesions affected 2-deoxyglucose uptake in a variety of brain regions. Some of these regions are known to be mono-synaptically connected to the medial preoptic nucleus. Metabolic depression in these areas may reflect retrograde changes in the neurons projecting to the damaged field.

The metabolic changes identified in the present study confirm the prominent role of the preoptic area in the control of sexual behavior, show that changes in the physiology of the visual system represent one of the ways through which testosterone influences the occurrence of this behavior and demonstrate that the medial preoptic nucleus has marked effects on the metabolic activity in a variety of limbic and telencephalic structures. This study also indicates that the medial preoptic nucleus affects the activity of the area ventralis of Tsai, a dopaminergic area known to send projections to a variety of hypothalamic, thalamic and mesencephalic nuclei that are implicated in the control of male sexual behavior. These data therefore support the notion that the control of the dopaminergic activity in the area ventralis of Tsai by the medial preoptic nucleus represents one of the ways through which the medial preoptic area regulates male reproductive behavior.

Key words: sexual behavior, 2-deoxyglucose, dopamine, visual system, brain metabolic activity.

In most vertebrate species, male-typical copulatory behavior is activated in adult subjects by testosterone originating from the testes (Meisel and Sachs, 1994). A host of studies has demonstrated that the medial preoptic area (POA) is an essential site where testosterone acts in order to activate copulation. Lesions of this nucleus disrupt the expression of this behavior and stereotaxic implants of testosterone are sufficient to activate the behavior in castrated males. The medial POA is characterized in many species such as the rat, gerbil or ferret by the presence of sexually dimorphic cell clusters or nuclei (the so-called sexually dimorphic nuclei) that differentiate sexually during ontogeny in parallel with copulatory behavior under the influence of sex steroids (Arnold and Gorski, 1984; Tobet and Hanna, 1997).

In Japanese quail (*Coturnix japonica*), the expression of male-typical copulatory behavior is also sexually differentiated. Females never exhibit male-typical behaviors such as mounts and cloacal contact movements even after ovariectomy and treatment with exogenous testosterone at doses that would be more than sufficient to activate these behaviors in males (Adkins and Adler, 1972; Adkins, 1975; Balthazart et al., 1983). A sexually dimorphic nucleus has also been discovered in the POA of this species: the medial preoptic nucleus (POM) is larger in males than in females (Viglietti-Panzica et al., 1986). The POM is also testosterone-sensitive: its volume

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Abbreviations: ANOVA, analysis of variance; AR, androgen receptor; CCM, cloacal contact movements; CX, castrates; CX+T, castrates treated with testosterone; CX+T+L, castrates treated with testosterone and bearing a POM lesion; 2-DG, 2-^{14}C deoxyglucose; ER, estrogen receptor; L, left; M, mount; MA, mount attempt; NG, neck-grab; POM, nucleus preopticus medialis; R, right; see Table 1 for abbreviations of anatomical structures.

is decreased by castration and restored to normal values by a 2-week treatment by testosterone (Panzica et al., 1987; Panzica et al., 1991). In contrast to what has been described for several mammalian sexually dimorphic nuclei, the volume of the POM in female quail increases to male-typical values after a treatment with exogenous testosterone. The quail POM is therefore sexually dimorphic because adult males and females are exposed to different hormonal milieus (higher plasma testosterone in males than in females) and its size is a signature of testosterone action in the POA (see Panzica et al., 1996 for discussion).

The POM is a specific key site for the action of testosterone on male copulatory behavior in male quail in the presence of adequate external stimuli (e.g. a receptive female; Balthazart and Surlemont, 1990a,b; Balthazart et al., 1992b). This nucleus can be identified by the presence of a dense cluster of cells that express aromatase, the enzyme that catalyzes the transformation of testosterone into estradiol (Balthazart et al., 1990a,b). This enzymatic conversion within the POM plays a key limiting role in the activation of both the appetitive and consummatory components of male sexual behavior (Balthazart and Foidart, 1993, 1997b). The control of the expression of this enzymatic protein and of its activity has therefore been the focus of a lot of experimental work (see Balthazart and Ball, 1998b).

Although the POM appears as a necessary site of testosterone action for the activation of male copulatory behavior, this nucleus is obviously included in a complex circuitry that is required for the normal expression of copulation. Parts of this circuitry have been identified by a variety of experiments using techniques such as tract-tracing (Balthazart et al., 1994; Balthazart and Absil, 1997), immunocytochemical visualization of immediate early genes such as *c-fos* and *zenk* (Meddle et al., 1997, 1999; Tlemçani et al., 2000; Ball et al., 1997) or autoradiographic localization of the 2-^{14}C deoxyglucose (2-DG) uptake (Dermon et al., 1999).

In a recent study, we measured the 2-DG uptake in a large number of brain areas of male quail that had just been exposed to a female and had a chance to express either the appetitive part of male sexual behavior (watching a sexually mature female through a 'window' without having the possibility to physically interact with her) or the consummatory component of this behavior (actual interactions including mounts and cloacal contact movements). We identified in this way many brain areas in which local metabolism is significantly modified (increased or decreased) after expression of these behaviors by comparison with control birds that had not interacted with the females (Dermon et al., 1999). A number of the areas that were identified by this study are known to contain steroid (androgen or estrogen) receptors (Watson and Adkins-Regan, 1989; Balthazart et al., 1989, 1992a, 1998b) and/or have been previously shown to be implicated in the control of sexual behavior (Panzica et al., 1996; Balthazart and Ball, 1998a) and/or are known to be connected mono-synaptically to the POM (Balthazart et al., 1994; Balthazart and Absil, 1997). For other brain areas, the change in local glucose metabolism had however not been anticipated and its association with sexual behavior could not receive an immediate explanation. However, in general, this study indicated that the 2-DG uptake technique is a useful tool with adequate sensitivity to identify localized changes in brain activity that are related to the repeated performance of a specific set of behaviors during a specified time period. In the present study, we used the same method to further investigate the brain mechanisms that mediate the expression of male sexual behavior. The experiments described here had two related goals in mind.

Testosterone action in the brain is known to be required for the expression of male sexual behavior in the presence of sexually mature females. It is usually assumed that testosterone-induced changes in behavioral reactivity are caused by local changes in neural activity in specific brain areas that either process the stimuli originating from the female or organize the pre-motor and motor outputs that will ultimately result in the performance of the copulatory acts (Hull, 1995; Hull et al., 1999). The POM should be one of the main areas where testosterone exerts this type of action but many other brain regions however also contain androgen and estrogen receptors that could be activated by testosterone or estrogens derived from its aromatization. The first specific goal of the present research was therefore to identify all the areas of the male quail brain where oxidative metabolism is modified by a systemic treatment with exogenous testosterone. It was anticipated that many of these regions would contain either androgen or estrogen receptors that could be held responsible for the direct action of the steroids on this neural activation (or inhibition) but indirect effects in regions that are devoid of sex steroid receptors were also expected.

Because the action of testosterone in the POM only is unlikely to be sufficient to organize the execution of the entire copulatory sequence, we postulated that metabolic activation/inhibition should also be seen in a number of brain regions that have direct mono-synaptic or poly-synaptic connections with the POM, irrespective of whether they do or do not contain sex steroid receptors. To test this idea we therefore compared, as a second goal of the present study, the 2-DG incorporation in the brain of testosterone-treated castrates that had or had not received bilateral electrolytic lesions of the POM.

EXPERIMENTAL PROCEDURES

Subjects and neuroendocrine procedures

Experiments were carried out on 17 male Japanese quail (*Coturnix japonica*) that were purchased at the age of 3 weeks from a local breeder in Belgium (Elevage Paulus, B-4520 Vinalmont-Wanze, Belgium). Three days after their arrival in the laboratory, all birds were castrated under total anesthesia (Hypnodil, Janssen Pharmaceutica, Beerse Belgium, 15 mg/kg) through a unilateral incision behind the last rib on the left side using procedures that were previously described (Balthazart and Schumacher, 1984). Two weeks later (day 0 of the experiment), all subjects received two 20-mm-long silastic implants (Dow Corning no. 602-252; 1.57 mm inner diameter; 2.41 mm outer

diameter) filled with crystalline testosterone ($n = 11$) or left empty as control ($n = 6$). They were left undisturbed for 9 days and then were submitted on days 9 and 10 to two behavior tests lasting 5 min each in order to quantify their copulatory behavior when introduced to a sexually mature female (see details below).

Three days later (day 13), five males in the testosterone-treated group that had shown active copulatory behavior during the tests were submitted to bilateral electrolytic lesions of the POM while all other birds were submitted to a sham surgery (see details of procedure below). During the next two days (days 14 and 15), all birds were submitted to two additional behavior tests of 5 min each, in the same conditions as before to evaluate the behavioral consequences of the POM lesions.

The cloacal gland of each subject was measured with calipers (greatest length × greatest width = cloacal gland area) at regular intervals during the experiment in order to confirm the effectiveness of the testosterone treatment. This gland is an androgen-dependent structure (Sachs, 1967) and its size is highly correlated with plasma testosterone levels (Delville et al., 1984). Throughout their life at the breeding colony and in the laboratory, birds were maintained under a photoperiod simulating summer long days (16 h light:8 h dark). During the behavioral experiment, all birds were isolated in individual cages. All experimental procedures were in agreement with the Belgian laws on 'Protection and Welfare of Animals' and on the 'Protection of Experimental Animals' and the International Guiding Principles for Biomedical Research involving animals published by the Council for International Organizations of Medical Sciences. The protocols were approved by the Ethical Committee for the Use of Animals of the University of Liège.

Behavioral tests

Nine and 10 days after the start of the treatment with testosterone and during the first two days after stereotaxic surgery, all subjects were tested for the occurrence of male-typical copulatory behavior during two presentations to a sexually mature female. Presentations lasted 5 min each and took place in a small arena (60 × 40 cm) following a standard procedure that has been previously described (Balthazart and Schumacher, 1984; Balthazart et al., 1998a). In brief, the stimulus female was introduced in the arena containing the experimental subject for 5 min and the frequencies and latencies for the following behavior patterns were recorded: strut, neck-grab (NG), mount attempt (MA; counted only when a bird showing an NG raised one leg and put it over the back of the test female), mount (M) and cloacal contact movements (CCM) (see Adkins and Adler, 1972; Hutchison, 1978 for a detailed description). Because the different sexual behaviors that are directly related to copulation (NG-MA-M-CCM) occur in sequence, their frequencies are usually highly correlated. The following detailed analyses will be limited to the behaviors MA and CCM. The analysis of NG and M leads to similar results and their presentation would be redundant.

Electrolytic lesions

Birds were anesthetized with Hypnodil (15 mg/kg) and placed in a stereotaxic apparatus (Trent Wells, with pigeon head holder) with the beak holder placed 45° below the horizontal axis of the stereotaxic assembly. Bilateral lesions were produced using electrodes that were made of No. 00 steel insect pins insulated with Eukitt (O. Kindler, Freiburg, Germany) except at the tip. Before use, the insulation of the electrodes was tested by passing current while the electrodes were immersed in egg albumin and the presence or absence of coagulation could be checked. Current was produced by a Grass S48 stimulator and passed simultaneously in both electrodes (1.5 mA for 10 s). A metal clamp was fixed to the skin of the head and served as the indifferent electrode. The same manipulations, including the lowering of the electrodes to the desired brain target were performed in control birds (either treated or not treated with testosterone) but no current was passed through the electrodes in this case. Electrodes were subsequently removed, the opening in the skull was closed with dental cement, and the skin was sutured. Birds were then allowed to recover from the anesthesia in a warm environment and returned to their cage where they usually started to drink and eat within an hour.

Electrode coordinates were 5.0 mm anterior (x) and 2.3 mm above the zero reference point (center of the inter-aural axis, z) and 0.25 mm lateral to the sagittal midline (identified on each bird by the suture of the frontal bones, y). These coordinates had been originally obtained from the quail brain stereotaxic atlas (Baylé et al., 1974) and adjusted by trial and error for the heavier body weight of our birds (Balthazart and Surlemont, 1990a,b; Balthazart et al., 1998a).

The combination of the endocrine treatment and lesions determined three experimental groups: castrated birds with no electrolytic lesions (CX, $n = 6$), castrates treated with testosterone but with no electrolytic lesions (CX+T, $n = 6$) and castrates treated with testosterone and bearing an electrolytic lesion aimed at the POM (CX+T+L, $n = 5$).

2-Deoxyglucose experiments

Based on results of the behavioral tests, four subjects in each of the three experimental groups were selected for the 2-DG uptake study. They were selected to be fully representative of the expected neuroendocrine conditions of their experimental group. We selected subjects in the CX and CX+T+L group that had shown no copulatory behavior during the two post-operative tests and subjects in the CX+T group that had been sexually active during these tests.

Two days after the last behavioral test (day 17), each subject received an i.p. injection of 2-DG (New England Nuclear NEC-495) at a dose of 100 µCi/kg in 200 µl sterile saline, as previously described Dermon et al., 1999). The same batch of deoxyglucose was used for this entire experiment so that the specific radioactivity of the injected compound was identical in all birds (50.75 mCi/mmol, i.e. approximately 1.86 GBq/mmol). Food and water had been removed 7–8 h before the i.p. injection of 2-DG. Immediately after the 2-DG injection, quail were returned to their home cage. They were killed by decapitation 45 min later. Their brain was immediately dissected out of the skull, frozen on dry ice, and stored at −75°C until sectioned for autoradiographic experiments.

Autoradiography and data analysis

All brains were cut in the coronal plane with a Leica cryostat at −20°C in 20-µm-thick sections. Throughout the brain, one set of two adjacent sections every 200 µm was collected, thaw-mounted on coverslips, rapidly dried on a hot plate (60°C) and exposed to Amersham ^{14}C-sensitive autoradiographic Hyperfilm β max, along with a set of plastic autoradiographic standards (^{14}C, Amersham), as previously described (Dermon et al., 1999). Adjacent sections were placed on microscope slides and counterstained with Cresyl Violet for cytoarchitectonic identification. At the level of POA, every second section was collected to permit the detailed reconstruction of the location and size of the electrolytic lesion.

After 3 weeks of exposure, the films were developed and analyzed with a Macintosh-based image analysis system (NIH Image, v. 1.61, Wayne Rasband, NIH, Bethesda, MD, USA). Local metabolic activity (2-DG incorporation) was measured in 77 discrete brain structures separately in the left and right side of the brain. Each structure was outlined and measured in four to six consecutive sections depending on its antero-posterior extent, using as reference the corresponding Nissl-stained sections. Mean metabolic activity (as calibrated with standards, expressed in nCi/g tissue) in each structure of each subject, was normalized by a factor F, to minimize overall individual subject differences (see Dermon et al., 1999). The whole brain mean 2-DG incorporation was estimated in each subject by measuring uptake in all sections in this animal and dividing

the total by the number of sections. The *F* factor for each subject was determined as the ratio of the average 2-DG incorporation in the whole brain of all animals analyzed ($n = 12$) divided by the individual animal's whole brain mean 2-DG incorporation.

For each brain structure considered, data were originally analyzed by a two-way mixed design analysis of variance (ANOVA) with the three experimental groups (CX, CX+T and CX+T+L) representing the independent factor and the two brain sides (left and right) representing the repeated factor. Because these analyses indicated that brain metabolism is not or only very slightly lateralized (see Results), left and right values for each subject and brain area were then averaged and the resulting data were re-analyzed by one-way ANOVAs with the three experimental groups as an independent factor. When appropriate, post hoc testing was carried out to compare the three groups using the Tukey–Kramer test, that provides an optimal compromise between the risks of type I and type II errors. All tests were carried out with the software SuperANOVA (Abacus Concepts, CA, USA). Nomenclature used in this paper is based on the atlas of the chicken or quail brain (Kuenzel and Masson, 1988; Baylé et al., 1974) with minor modifications for the POA and anterior hypothalamus as described in Panzica et al. (1991) and Aste et al. (1998).

RESULTS

Behavioral and morphological data

During the first behavior tests that were performed approximately 10 days after the beginning of the treatment with exogenous testosterone (but before the stereotaxic surgery), the behavior of all experimental subjects conformed to what could be expected based on previous studies. The six castrated males (CX group) were sexually inactive when presented to a female and the 11 castrates that had been treated with testosterone showed an active copulatory behavior in the same circumstances. Five testosterone-treated birds were then submitted to a bilateral electrolytic lesion aimed at the POM (CX+T+L group) and during the behavioral tests performed two days later, four of them remained completely inactive during the entire duration of the presentations (two times 5 min) to sexually mature females. The fifth individual in this group was still sexually active presum-

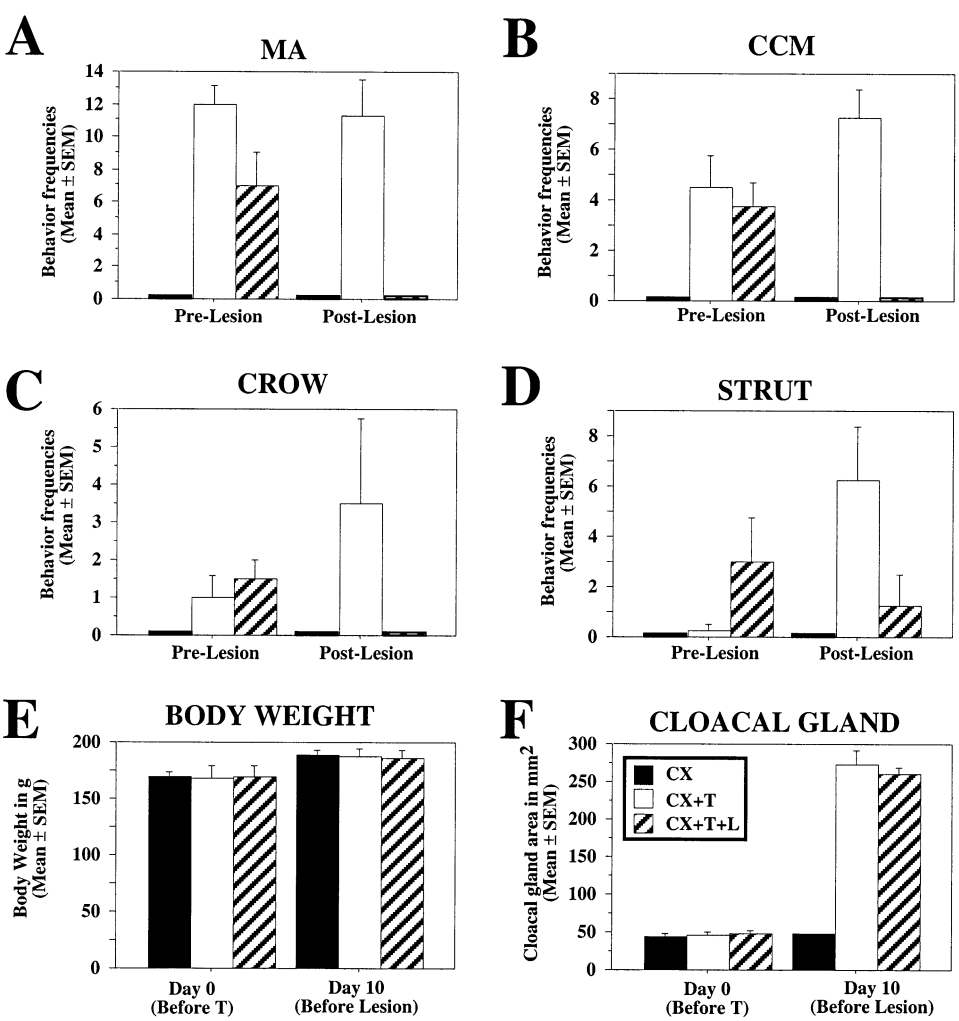

Fig. 1. Bar graphs summarizing the effects of testosterone associated or not with bilateral lesions of the medial preoptic nucleus on the expression of male sexual behavior (A–D), the body weight (E) and the size of the cloacal gland (F) in castrated male quail. Behavioral data collected during tests performed before and after the POM lesion are shown separately. Before lesions, birds in the CX+T and CX+T+L groups had already been treated with testosterone for 9–10 days. Morphological data shown in E and F were collected at the start of the experiment before the initiation of the treatment with testosterone and just before the electrolytic lesions.

ably because the lesion had missed its target and he was excluded from the experiment. The sham surgery performed on all other birds did not affect their behavior during the post-operative tests: the six CX males remained inactive and the six CX+T birds continued to display an intense copulatory behavior toward females. Four birds in each of these two groups were then randomly selected for the 2-DG uptake study. The behavior displayed during the pre- and post-surgery tests by all subjects that were finally included in the 2-DG uptake analysis (four males in each of the CX, CX+T and CX+T+L groups) is summarized in Fig. 1A–D.

Behavioral occurrence frequencies throughout the experiments were analyzed by two-way ANOVA with a mixed design in which the different groups were considered as an independent factor (three levels: CX, CX+T and CX+T+L) and the successive observations as a two-level repeated factor (before and after lesion). MA frequencies were significantly affected by the two factors (groups: $F_{2,9} = 34.901$, $P < 0.0001$; lesion: $F_{1,9} = 6.752$, $P = 0.0288$) as well as by their interaction ($F_{2,9} = 4.981$, $P = 0.0350$). These effects obviously resulted from the complete disappearance of the behavior after the lesions in the CX+T+L group. Similar effects were obtained in the analysis of CCM frequencies except that the overall effect of the lesion factor (before vs. after) was not statistically significant (groups: $F_{2,9} = 50.853$, $P < 0.0001$; lesion: $F_{1,9} = 0.189$, $P = 0.6740$; interaction: $F_{2,9} = 6.035$, $P = 0.0218$).

Although crowing occurrence frequencies were similarly depressed in the CX+T+L group after the lesion and this behavior was completely absent in the CX group, no significant change in this behavior was

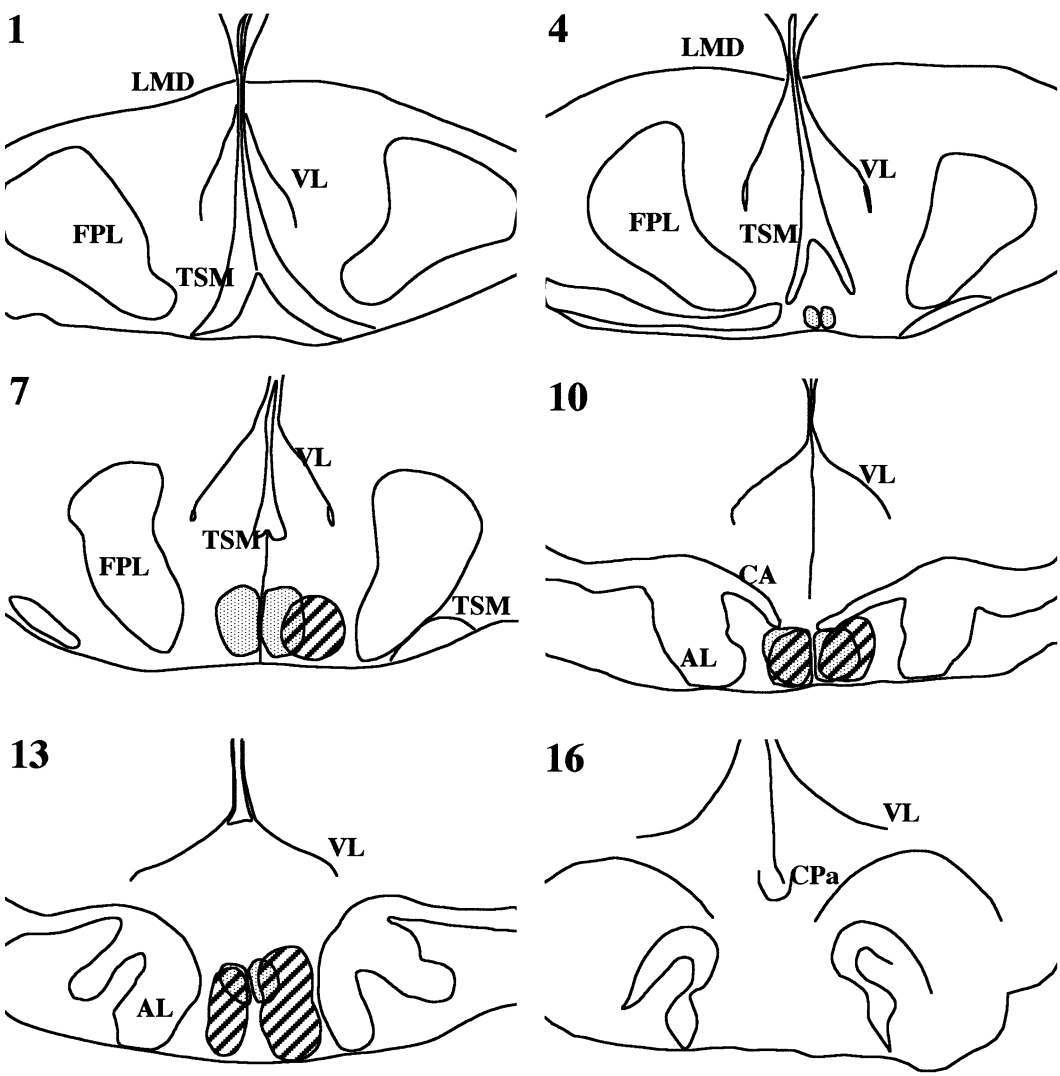

Fig. 2. Schematic drawings representing the location and size of the bilateral POM lesion (hatched area) in the subject (male number 20) that had a lesion size closest to the average size observed in the entire group (1.86 mm^3 for the total of left and right lesions). The POM itself is shown by the dotted areas. As can be seen this lesion was also slightly asymmetrical. The different panels illustrate successive coronal sections in a rostral to caudal order. Sections used for the reconstruction were 160 μm apart but every third section only is shown in the figure (numbers in the top left corner). AL, ansa lenticularis; CA, comissura anterior; CPa, commissura pallii; FPL, fasciculus prosencephali lateralis; LMD, lamina medullaris dorsalis; TSM, tractus septomesencephalicus; VL, ventriculus lateralis.

Table 1. Effects of testosterone treatment associated or not with a bilateral lesion of the medial preoptic nucleus on 2-DG incorporation in the Japanese quail brain

Brain region	CX (mean ± S.E.M.)	CX+T (mean ± S.E.M.)	CX+T+L (mean ± S.E.M.)	*F* value	*P* value
Dorsal ventricular ridge (DVR)					
Hyperstriatum accessorium (HA)	515 ± 7	512 ± 16	506 ± 3	0.100	0.904
Hyperstriatum ventrale rostral (HVr)	473 ± 11	470 ± 6	509 ± 10	2.330	0.153
Hyperstriatum dorsale (HD)	497 ± 9	480 ± 9	474 ± 14	0.520	0.613
Hyperstriatum intercalatum supremum (HIS)	634 ± 4	652 ± 9	578 ± 14[b]	6.890	**0.015**
Neostriatum frontale (Nf)	627 ± 5	537 ± 34	613 ± 6	2.580	0.130
Neostriatum intermedium (NI)	626 ± 12	614 ± 14	630 ± 8	0.220	0.808
Neostriatum (N)	650 ± 13	531 ± 8[a]	637 ± 18[b]	10.150	**0.005**
Neostriatum caudolaterale (Ndc)	536 ± 6	485 ± 13	560 ± 21	2.990	0.101
Field L (FL)	906 ± 19	851 ± 37	912 ± 17	0.730	0.510
Ectostriatum (E)	1279 ± 40	1130 ± 42	1169 ± 34	1.780	0.224
Nucleus basalis (Bas)	565 ± 15	512 ± 22	552 ± 12	1.210	0.343
Fasciculus prosencephali lateralis (FPLr)	644 ± 10	662 ± 46	615 ± 8	0.330	0.730
Paleostriatal complex					
Paleostriatum augmentatum (PA)	796 ± 17	738 ± 52	738 ± 23	0.420	0.667
Paleostriatum primitivum (PP)	594 ± 11	533 ± 23	582 ± 12	1.770	0.225
Lobus parolfactorius (LPO)	550 ± 14	561 ± 7	509 ± 11	2.760	0.117
Archistriatal complex					
Nucleus taeniae (Tn)	473 ± 3	547 ± 7[a]	519 ± 11[a]	10.460	**0.005**
Archistriatum anterior (AA)	480 ± 13	469 ± 9	458 ± 8	0.490	0.627
Archistriatum intermediate pars dorsalis (Ad)	493 ± 6	485 ± 15	493 ± 4	0.110	0.897
Archistriatum intermediate pars ventralis (Av)	439 ± 2	423 ± 18	448 ± 8	0.510	0.617
Tuberculum olfactorium (TO)	407 ± 11	361 ± 11	335 ± 14[a]	9.5	**0.001**
Limbic pallium					
Area parahippocampalis (APH)	390 ± 22	382 ± 7	381 ± 13	0.050	0.953
Hippocampus (Hp)	444 ± 10	456 ± 19	446 ± 8	0.110	0.896
Hippocampus, medial part (Hp-DM)	510 ± 24	480 ± 9	521 ± 8	0.830	0.465
Area corticoidea lateralis (CDL)	416 ± 11	379 ± 21	437 ± 8	1.880	0.208
Cortex piriformis (Cpi)	380 ± 9	355 ± 5	347 ± 8	2.460	0.141
Nucleus septalis lateralis (SL)	341 ± 3	349 ± 5	321 ± 3[b]	6.760	**0.016**
Nucleus septalis medialis (SM)	373 ± 9	376 ± 5	307 ± 7[a,b]	12.370	**0.003**
Area temporo-parietal-occipitalis (TPO)	490 ± 16	388 ± 9[a]	468 ± 10[b]	9.290	**0.007**
Preoptic region					
Preoptic area (POA)	323 ± 8	343 ± 10	287 ± 8[b]	4.53	**0.044**
Nucleus preopticus medialis (POM)	339 ± 6	311 ± 6	xxx	5.55	**0.065**
Thalamus					
Nucleus dorsolateralis anterior thalami (DLA)	496 ± 7	493 ± 14	487 ± 6	0.09	0.914
N. dorsomedialis anterior thalami (DMA)	489 ± 8	522 ± 15	438 ± 10[b]	6.52	**0.018**
N. dorsomedialis posterior thalami (DMP)	567 ± 8	564 ± 5	539 ± 10	1.77	0.225
Nucleus habenularis medialis (HM)	500 ± 9	505 ± 7	460 ± 7	4.55	**0.043**
N. geniculatus lateralis pars ventralis (GLv)	590 ± 9	598 ± 15	571 ± 6	0.80	0.479
Nucleus rotundus (ROT)	807 ± 11	788 ± 19	771 ± 3	0.85	0.458
Nucleus ovoidalis (Ov)	899 ± 23	829 ± 18	887 ± 12	1.86	0.210
Nucleus pretectalis (PT)	673 ± 17	629 ± 23	689 ± 8	1.47	0.281
Stratum cellulare externum (SCE)	485 ± 5	545 ± 11[a]	511 ± 5	7.07	**0.014**
Substantia grisea centralis (GCt)	453 ± 6	468 ± 8	458 ± 13	0.30	0.748
Nucleus subrotundus (SRt)	633 ± 9	610 ± 10	737 ± 12[a,b]	17.51	**0.001**
Hypothalamus					
Nucleus anterior medialis hypothalami (AM)	340 ± 9	323 ± 13	279 ± 17	2.68	0.129
N. ventromedialis hypothalami (VMN)	344 ± 6	300 ± 4[a]	326 ± 5	8.54	**0.001**
Regio lateralis hypothalami (LHyp)	386 ± 12	375 ± 9	354 ± 7	1.32	0.314
N. inferioris hypothalami (IH)	310 ± 6	337 ± 8	323 ± 9	1.69	0.244
N. mamillaris medialis (MM)	404 ± 10	463 ± 11	383 ± 15[b]	4.98	**0.035**
Nucleus suprachiasmaticus (SCN)	338 ± 8	336 ± 11	323 ± 1	0.37	0.703
N. paraventricularis magnocellularis (PVN)	413 ± 6	364 ± 6[a]	362 ± 10[a]	7.18	**0.014**
Mesencephalon					
Nucleus ruber (Ru)	640 ± 7	665 ± 4	628 ± 13	1.95	0.198
Nucleus mesencephalicus lateralis pars dorsalis (MLd)	989 ± 16	924 ± 25	1016 ± 11	2.99	0.101
Nucleus intercollicularis (ICo)	480 ± 4	461 ± 3	489 ± 8	2.93	0.105
Nucleus opticus basalis (nBOR)	501 ± 13	491 ± 11	525 ± 6	1.23	0.337
Nucleus nervi oculomotorii (OM)	573 ± 16	678 ± 19[a]	664 ± 13	5.42	**0.029**

Table 1 (*Continued*).

Brain region	CX (mean ± S.E.M.)	CX+T (mean ± S.E.M.)	CX+T+L (mean ± S.E.M.)	*F* value	*P* value
Nucleus tegmenti pedunculo-pontinus pars compacta (TPc)	629 ± 17	613 ± 14	620 ± 13	0.13	0.877
Nucleus interpeduncularis (IP)	367 ± 10	347 ± 9	360 ± 9	0.49	0.628
Nucleus isthmi pars magnocellularis (Imc)	485 ± 3	412 ± 16[a]	434 ± 7	5.92	**0.023**
Nucleus isthmi pars parvocellularis (Ipc)	586 ± 9	561 ± 19	575 ± 25	0.20	0.821
Nervus oculomotorius (NIII)	331 ± 12	278 ± 7	299 ± 11	3.18	0.091
Area ventralis tegmentalis of Tsai (AVT)	452 ± 4	451 ± 8	415 ± 6[a]	5.07	**0.034**
Nucleus of Edinger–Westphal (EW)	571 ± 7	617 ± 3[a]	571 ± 9[b]	6.42	**0.019**
Optic tectum (TeO)					
Stratum griseum et fibrosum superficiale (SGFS)	519 ± 6	499 ± 10	516 ± 12	0.55	0.597
Stratum griseum centrale (SGC)	534 ± 9	500 ± 5	516 ± 12	1.44	0.287
Stratum album centrale (SAC)	389 ± 5	330 ± 10[a]	351 ± 8	5.99	**0.022**
Tractus opticus (TrO)	301 ± 12	237 ± 8[a]	299 ± 5[b]	7.53	**0.012**
Rhombencephalon					
Nucleus nervi trochlearis (nIV)	546 ± 10	514 ± 5	526 ± 12	1.31	0.318
Nucleus isthmo-opticus (IO)	536 ± 11	513 ± 8	548 ± 7	1.75	0.228
Nucleus semilunaris (SLu)	510 ± 9	513 ± 20	559 ± 27	0.84	0.463
Locus ceruleus (LoC)	460 ± 8	433 ± 18	457 ± 15	0.48	0.632
Nucleus subceruleus (SC)	500 ± 11	486 ± 15	481 ± 11	0.28	0.760
Nucleus pontis lateralis (PL)	517 ± 40	460 ± 11	432 ± 9	1.39	0.298
Nucleus pontis medialis (PM)	501 ± 5	517 ± 11	459 ± 7[b]	6.45	**0.018**
Nucleus linearis caudalis (LC)	570 ± 16	544 ± 9	540 ± 13	0.68	0.530
N. lemnisci lateralis pars intermediate (LLi)	679 ± 14	643 ± 13	633 ± 20	1.01	0.402
Cerebellum (Cb)					
mol	475 ± 14	534 ± 16	496 ± 5	2.55	0.133
gr	622 ± 10	678 ± 25	658 ± 8	1.39	0.297
wh	414 ± 8	417 ± 23	432 ± 7	0.18	0.842
Nucleus cerebellaris internus (CbI)	739 ± 17	771 ± 15	764 ± 14	0.55	0.596

Values presented are means ± S.E.M. of 2-DG uptake expressed in nCi/g tissue. Data were analyzed by one-way ANOVA (*F* and *P* values shown in the last two columns) followed by Tukey–Kramer post hoc tests.
[a] $P < 0.05$ compared to the CX group.
[b] $P < 0.05$ compared to the CX+T group.

detected between groups or when comparing data before and after lesions (groups: $F_{2,9} = 3.375$, $P = 0.0806$; lesion: $F_{1,9} = 0.150$, $P = 0.7075$; interaction: $F_{2,9} = 1.837$, $P = 0.2142$). The high inter-individual variability in the expression of this behavior presumably prevented the differences from reaching statistical significance.

Overall, strutting frequencies were not statistically different between groups ($F_{2,9} = 2.767$, $P = 0.1157$) and did not significantly differ before and after the lesions ($F_{1,9} = 2.752$, $P = 0.1315$) but there was a significant interaction between these two factors ($F_{2,9} = 7.552$, $P = 0.0119$). This interaction also resulted for a large part from the behavior decline after lesion in the CX+T+L group.

Morphological measures collected during the course of this experiment indicated that the treatment with testosterone had no gross non-specific effects on the subjects' physical condition. The body weight of subjects was similar in the three experimental groups ($F_{2,9} = 0.022$, $P = 0.9779$) and did not change differentially after the treatment with the steroid of two of the three groups (interaction: $F_{2,9} = 0.155$, $P = 0.8584$). The normal increase in body weight that is normally observed in young quail at that age was also present in all three experimental groups which resulted in a statistically significant overall difference in weight between measures taken before and after testosterone therapy ($F_{1,9} = 77.481$, $P < 0.0001$).

As expected also, testosterone induced a major growth of the cloacal gland in the two groups of subjects treated with this steroid and this testosterone-induced growth of the cloacal gland was similar in the group that was later submitted to POM lesions and in its direct control (groups: $F_{2,9} = 99.971$, $P < 0.0001$; repeated measures: $F_{1,9} = 397.933$, $P < 0.0001$; interaction: $F_{2,9} = 94.792$, $P < 0.0001$).

Reconstruction of the electrolytic lesions

Histological analysis of the POA of males in the CX+T+L group confirmed the presence of significant lesions in all four subjects (average volume: 1.54 ± 0.45 mm^3, mean ± S.E.M., $n = 4$). Reconstruction of the lesions demonstrated that these lesions were bilateral and affected both sides of the POA to a similar extent (left lesions: 0.83 ± 0.28 mm^3; right lesions: 0.71 ± 2.22 mm^3; means ± S.E.M.). There was no overall significant difference in the size of the lesions on the left and right side although the lesion in some subjects affected one brain side slightly more than the other. An example of such a limited asymmetry is shown in Fig. 2. In all subjects, the lesions largely overlapped with the POM and

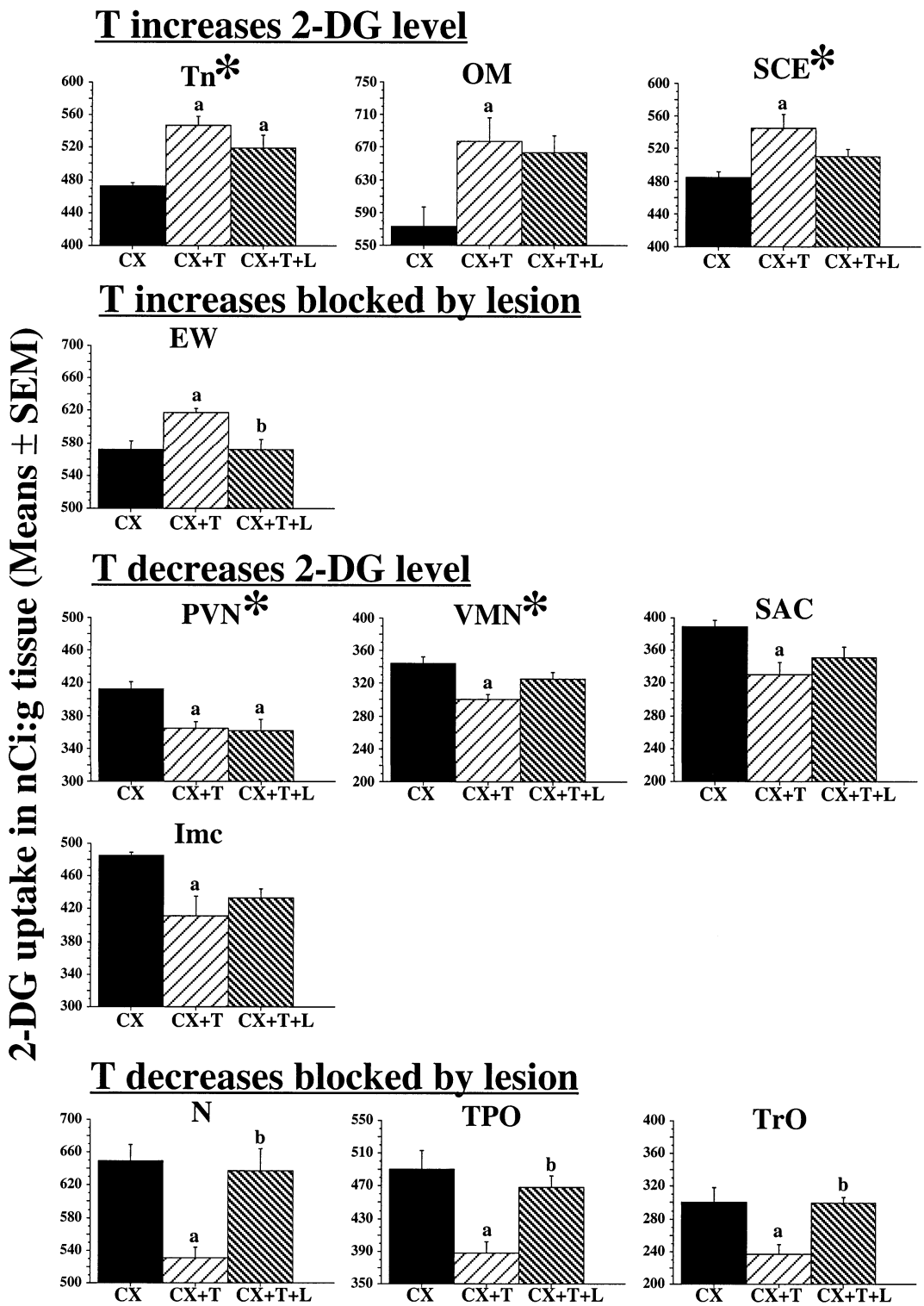

Fig. 3. Bar graphs illustrating the effects of testosterone (T) associated or not with bilateral lesions of the POM on the 2-DG uptake in brain nuclei where statistically significant effects of testosterone were observed. The scale used in each panel is identical to permit a direct comparison of the magnitude of effects but the basis of the vertical axis has been truncated differentially from one panel to the other to facilitate these comparisons. Steroid-sensitive nuclei (i.e. brain regions containing AR and/or ER) are indicated by an asterisk adjacent to the name of the nucleus. See Table 1 for abbreviations of the names of nuclei.

they destroyed a significant portion of this nucleus (see Fig. 2). Lesions were usually confined within the POA except in one subject in which the posterior part of the lesion extended in the rostral and medial hypothalamus to the level of the ventromedial nucleus (VMN). All metabolic values in this bird were however within two standard deviations from the mean of the experimental group so that data relative to this bird were kept in the final analyses.

Effect of treatments on overall brain activity and 2-deoxyglucose uptake

A number of studies previously identified effects of ovarian steroids on the ability of the female rat brain to use glucose as its primary source of energy. Estrogen treatment of ovariectomized rats was shown to increase glucose utilization in the brain by 20–39% (Namba and Sokoloff, 1984; Bishop and Simpkins, 1995). Because the testosterone systemically administered to castrated quail was likely to be aromatized into estrogens in substantial amounts, we first analyzed whether this steroid had any effect on the average 2-DG accumulation throughout the brain.

The mean 2-DG incorporation in the whole brain was estimated in each subject by measuring uptake in all sections in this animal and dividing the total by the number of sections. Comparison of these data for the three experimental groups by one-way ANOVA revealed no significant group differences. Although uptake was slightly higher in the two testosterone-treated groups by comparison with castrates (CX: 497.70 ± 81.58; CX+T: 559.29 ± 70.46; CX+T+L: 515.63 ± 33.33), this effect was far from significant ($F_{2,9} = 0.236$, $P = 0.7942$).

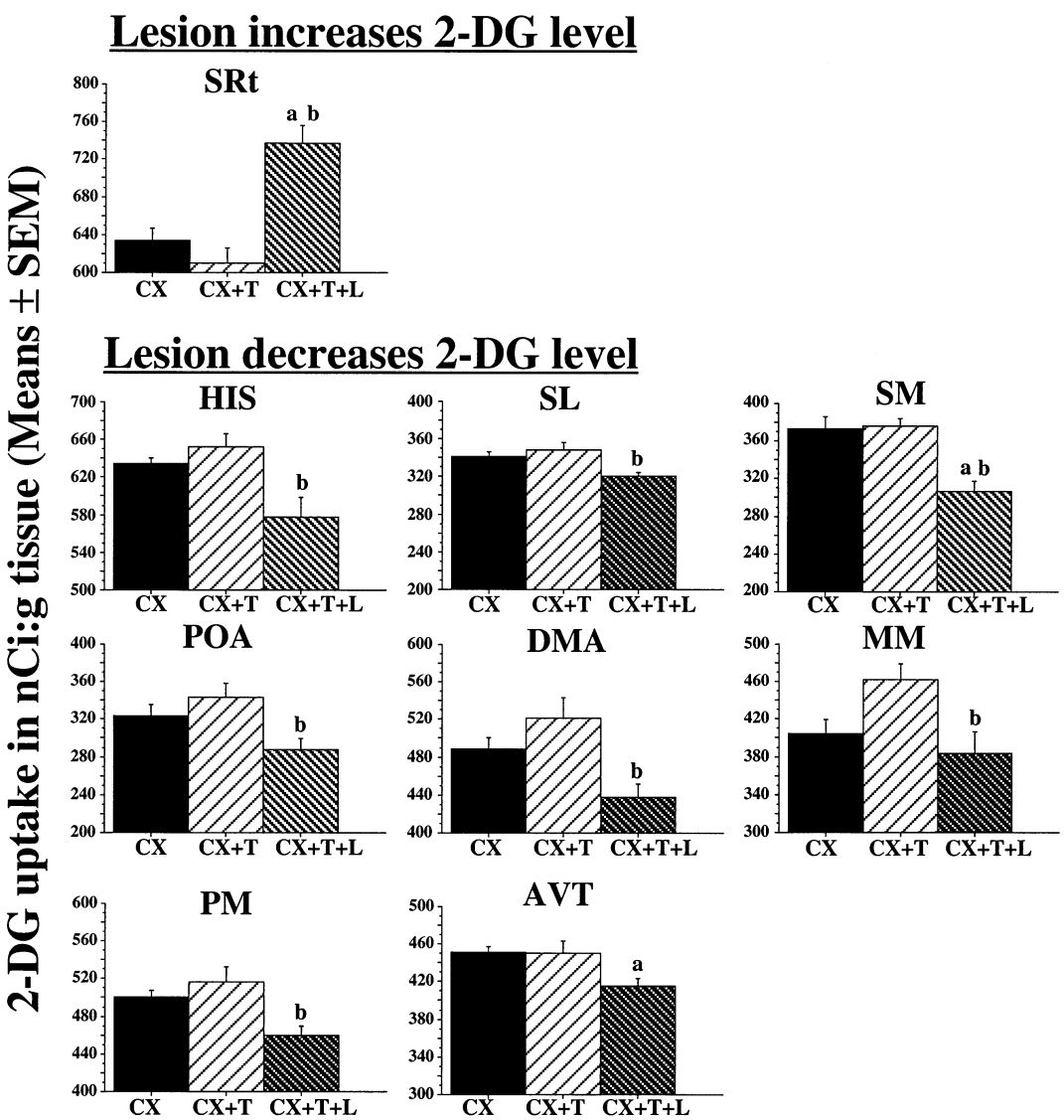

Fig. 4. Bar graphs illustrating the effects of POM lesions on the 2-DG uptake in brain nuclei where statistically significant effects of the lesions were observed. The scale used in each panel is identical to permit a direct comparison of the magnitude of effects but the basis of the vertical axis has been truncated differentially from one panel to the other to facilitate these comparisons. See Table 1 for abbreviations of the names of nuclei.

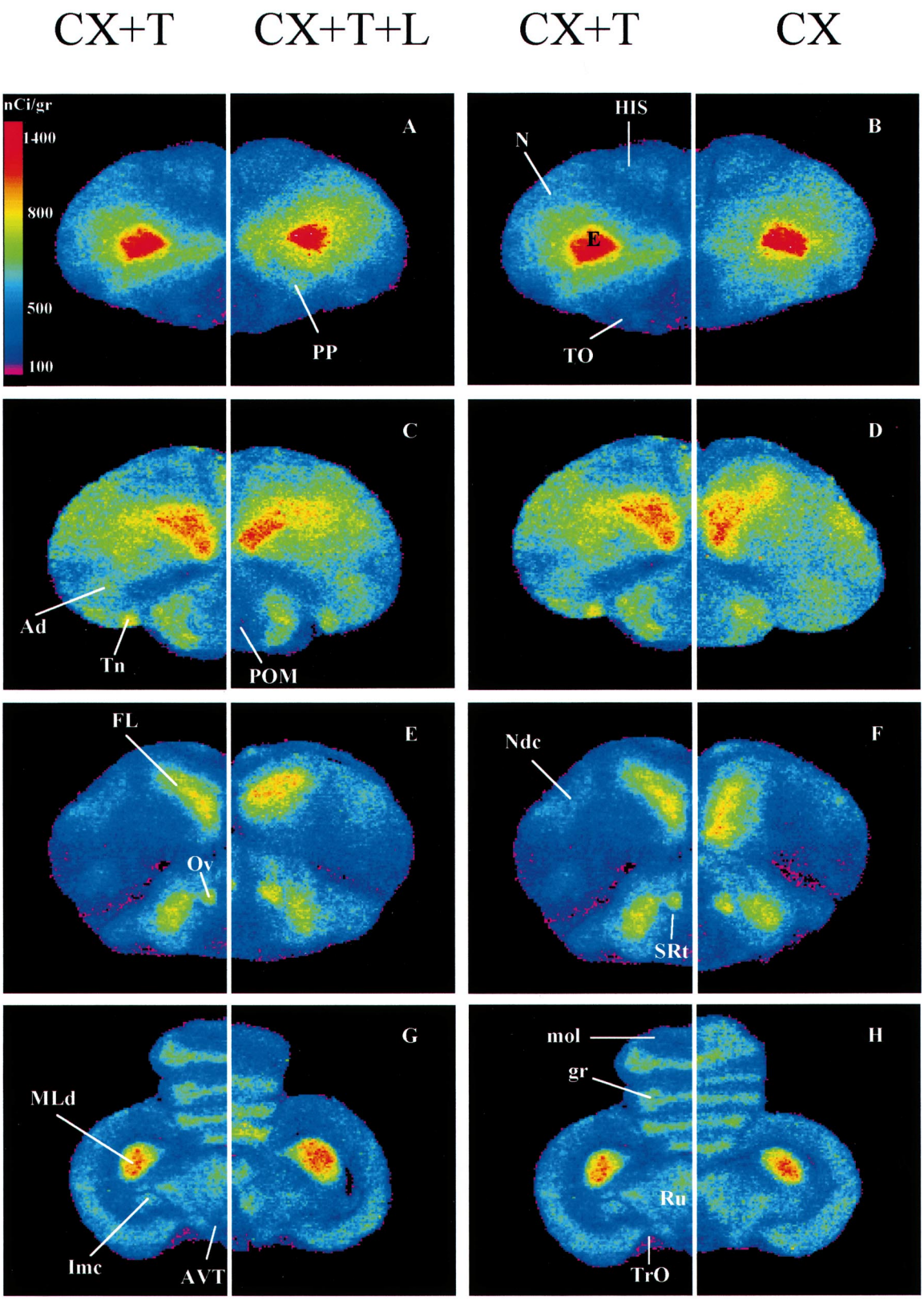

Fig. 5. Color coded autoradiograms illustrating the neuroanatomical distribution of 2-DG uptake in coronal sections of the quail brain and some of the localized changes induced by the treatment with testosterone (CX+T) of castrated control birds (CX) or by lesions of the POM (CX+T+L). The color scale in the top left panel provides calibration of colors in nCi of radioactive 2-DG per g of tissue.

The difference remained non-significant when the CX+T group alone was compared with CX birds ($t = 0.571$, $P = 0.5885$).

Pattern of local 2-deoxyglucose uptake in the quail brain

As previously observed in similar conditions, a highly heterogeneous 2-DG uptake was detected by autoradiography in the quail brain (Table 1). The pattern of uptake was similar to the one observed in our previous study (Dermon et al., 1999). The highest levels of 2-DG accumulation were observed in the ectostriatum. Very high activities (> 700 nCi/g in the CX group) were detected in the field L (FL), paleostriatum augmentatum (PA), nucleus rotundus (ROT), nucleus ovoidalis (Ov), nucleus mesencephalicus lateralis pars dorsalis (MLd) and nucleus cerebellaris internus (CbI). Still high but slightly lower uptakes (> 600 nCi/g in the CX group) were present in the hyperstriatum intercalatum supremum (HIS), neostriatum frontale, intermedium and caudolaterale (Nf, NI and Ndc), in the fasciculus prosencephali lateralis (FLP), nucleus pretectalis (PT), nucleus subrotundus (SRt), nucleus ruber (Ru), nucleus tegmenti pedunculo-pontinus pars compacta (TPc), nucleus lemnisci lateralis pars intermediate (LLi) and in the granular layer of the cerebellum (gr). Lower levels of uptake were detected in the other brain regions but a high level of heterogeneity was still detectable in the less active brain sites (see Table 1 for detail).

Lateralization of 2-deoxyglucose incorporation

The 2-DG uptake in each brain region was first analyzed by a two-way mixed design ANOVA with the three experimental groups representing the independent factor and the two brain sides (left and right) representing the repeated factor. Results of these two-way ANOVAs identified only a small number of structures where a lateralization of metabolic activity could potentially exist.

Significant main effects of the repeated factor (brain side) were only observed in eight brain regions out of 77: the FL [left (L) > right (R)], archistriatum anterior (AA; L > R), hippocampus (Hp; L > R), cortex pyriformis (Cpi; L > R), nucleus anterior medialis hypothalami (AM; L > R), nucleus paraventricularis magnocellularis (PVN; R > L), area ventralis tegmentalis of Tsai (AVT, R > L) and stratum griseum et fibrosum superficiale (SGFS; L > R). These differences were significant at the level $P < 0.05$ but had a small amplitude.

Because this lateralization of activity could have been induced, at least in part, by the limited asymmetry of the preoptic lesions in the CX+T+L group, the same analyses were repeated after exclusion of all corresponding data (i.e. on data from the CX and CX+T groups only). In these conditions, a significant effect of the brain side was observed in only four brain areas: the FL, AM, PVN and AVT. In each case, the direction of these differences was the same as in the general analysis.

Given (a) the small magnitude of the observed lateralizations, (b) their limited reproducibility, (c) that the number of lateralized brain regions is barely above what could be expected by chance (eight or four out of 77 areas) and (d) that lateralization usually concerns areas where no effect of testosterone or of the lesions was observed, we decided to carry out all subsequent analyses of the effects of experimental treatments on the means of 2-DG uptake in the left and right side of the brain (see also Discussion).

Effects of testosterone and medial preoptic nucleus lesion on 2-deoxyglucose uptake

Analysis by one-way ANOVA (three levels: CX, CX+T and CX+T+L) of the effects of treatments (testosterone associated or not with a POM lesion) identified significant experimental effects in 22 of the 77 brain areas that were considered (see Table 1 for details of F and P values). For the brain areas demonstrating significant effects, additional post hoc tests (Tukey–Kramer compromise test) were conducted to further determine the groups that were different. These tests identified changes in 2-DG uptake in both directions (increases as well as decreases) that were associated with the systemic treatment of castrates with testosterone as well as with the lesion of the POM in CX+T birds. These effects are graphically presented in Figs. 3 and 4 respectively. All data are in addition presented in full detail in Table 1. Representative autoradiograms can be found in Fig. 5.

Testosterone-induced changes in local cerebral metabolic activity

Treatment with testosterone was found to increase the 2-DG uptake in four brain areas: two of them are steroid-sensitive (i.e. they contain androgen or estrogen receptors): the nucleus taeniae (Tn) and the stratum cellulare externum (SCE). The two other brain areas are in contrast not known to contain sex steroid receptors; they are the visual nucleus Edinger–Westphal (EW) and the nucleus nervi oculomotori (OM).

In addition, testosterone induced a significant decrease in the oxidative metabolism in seven distinct brain regions. Once again, some of these regions are steroid-sensitive (PVN and VMN) but others are not [stratum album centrale (SAC) and nucleus isthmi pars parvocellularis (Imc), neostriatum (N), area temporo-parieto-occipitalis (TPO), tractus opticus (TrO)].

Effects of electrolytic lesion of the POM

POM lesion produced 5 days before metabolic measures induced marked distal metabolic changes in 12 brain areas by comparison with the testosterone group. In four nuclei, an increase in 2-DG uptake was observed: N, TPO, TrO and SRt. Interestingly, testosterone had been found to decrease the oxidative metabolism in three of these areas (N, TPO, TrO) by comparison with the castrated group. In these three cases, the lesioned testosterone-treated birds had therefore a metabolism that was no longer different from the metabolism measured in the control castrated birds. In SRt, no

effect of testosterone had been observed so that the 2-DG uptake in the CX+T+L group was significantly higher than in the CX+T group but also than in the CX group (see Table 1).

The POM lesions also induced decreases in 2-DG uptake in eight brain areas: the HIS, the nucleus septalis lateralis and medialis (SL and SM), POA, the nucleus dorsomedialis anterior thalami (DMA), the nucleus mamillaris medialis (MM), the nucleus pontis medialis (PM) and EW. In most of these brain regions (seven out of eight), no significant effect of testosterone had been detected, but in many cases a small numerical increase was present. As a consequence, the metabolism after lesion was significantly lower than in the CX+T but was in general not different from the metabolism in the CX group (HIS, SL, POA, DMA, MM, and PM). In one brain region (SM), the decrease induced by the lesion was so large that 2-DG uptake in the CX+T+L group was significantly lower than in both the CX+T and the CX groups. In EW testosterone significantly increased 2-DG uptake so that the decrease caused by the POM lesion lowered the metabolism back to the level seen in CX birds (no significant difference between these two groups).

Finally, another pattern of results was seen in three brain regions: the nucleus habenularis medialis (HM), AVT and the tuberculum olfactorium (TO). In each of these nuclei, the ANOVA indicated the presence of a significant treatment effect. In HM no significant difference was however detected by the post hoc tests. Treatment with testosterone alone slightly decreased but did not significantly modify 2-DG uptake (by comparison with the CX group) in AVT and TO. However in birds exposed to both testosterone and to the lesion, a significant decrease in oxidative metabolism was observed in these two nuclei (CX+T+L significantly lower than CX at $P = 0.05$).

DISCUSSION

The present results indicate that localized changes in oxidative metabolism take place in specific brain areas of castrated quail that are either treated with testosterone or treated with testosterone and subjected to a bilateral lesion of the medial preoptic nucleus, a brain area that is critical for the activation of sexual behavior by testosterone. It was also confirmed that, in parallel, these experimental treatments affect the performance of male copulatory behavior as expected based on previous research. Specifically, systemic testosterone treatment activated the performance of an active male-typical copulatory behavior and this effect was completely blocked in birds bearing a lesion in the POM. These data provide new information on the functional organization of brain circuits that presumably underlie the activation of male sexual behavior by testosterone.

Behavioral and morphological results

Previous studies (Beach and Inman, 1965; Sachs, 1969; Adkins and Adler, 1972; Adkins, 1975; Adkins and Pniewski, 1978; Balthazart et al., 1983) had shown that a systemic treatment with testosterone activates within one or two weeks the expression of an active male-typical sexual behavior. This effect was fully confirmed here in the two groups (CX+T and CX+T+L) that were exposed to testosterone. All subjects in these groups regularly displayed during the two behavioral tests the full sequence of copulatory behavior (from NG to cloacal contact movements).

The bilateral lesions of the POM completely suppressed, as already observed previously, the expression of all these testosterone-dependent behaviors. This confirms that this nucleus plays a critical role in the expression of male copulatory behavior. Lesions of this area are sufficient to completely block the activational effects of testosterone (Balthazart and Surlemont, 1990b). It has also been shown that stereotaxic implantation of testosterone in the POM is sufficient to activate the expression of the full copulatory sequence in castrated male quail (Balthazart and Surlemont, 1990b; Balthazart et al., 1992b). This does not mean, however, that testosterone acts only in the POM to increase the expression of these behaviors. Other brain areas must also be implicated as indicated namely by the fact that the intensity and frequency of the behaviors displayed by birds bearing testosterone implants in the POM are always smaller than in birds that are treated with testosterone systemically.

Testosterone-treated birds also expressed the androgen-dependent vocalization crowing and most of them performed the precopulatory display, strutting. For unexplained reasons, this behavior was however infrequent and irregularly observed before the lesions in the testosterone-treated birds (present in only one subject in the CX+T and in two subjects in the CX+T+L groups). Previous research has indicated that strutting is observed only unreliably even in male quail that have adequate levels of circulating testosterone (Alexandre and Balthazart, 1986). The expression of this behavior is presumably affected more markedly by various factors in the environment such as the behavior of the stimulus female that cannot be adequately standardized. Accordingly, for unexplained reasons the strutting frequency increased between the pre- and post-lesion tests in the CX+T group. Interestingly also strutting was not completely abolished by the POM lesions contrary to the other androgen-dependent behaviors. This suggests that the brain areas that control the activation of this behavior are at least partly different from those that are implicated in the control of copulatory behavior *sensu stricto*. This difference in neuroanatomical control has previously been suggested in studies analyzing strutting in cockerels (Barfield, 1965, 1969). The brain nuclei that control this behavior remain however unknown at present (see Barfield, 1969 for discussion).

General pattern of 2-deoxyglucose uptake in the quail brain and its potential lateralization

The overall pattern of brain metabolic activity observed here in male quail is similar to the pattern

previously described for chicks and quail (chicks: Kossut and Rose, 1984; quail: Dermon et al., 1999) with the visual and auditory brain areas showing the highest levels of metabolic activity. The *in vivo* 2-DG autoradiographic method provides a well established strategy for mapping functional poly-synaptic events in the CNS. Neuronal activity is reflected in glucose utilization since function-related energy requirements are almost exclusively met from the oxidative catabolism of glucose supplied by brain blood flow. The 2-DG method provides information on the rate of glucose phosphorylation in specific brain regions, which in turn is indicative of energy production. Dynamic alterations in glucose utilization reflect mainly energy requirements for electrical activity in nerve terminals (ion transport; Na^+, K^+-ATPase) of neuronal pathways rather than the biochemical processes taking place in the cell body (Nudo and Masterton, 1986). The contribution of neuronal terminals to overall glucose utilization is likely to be related to the greater surface area to volume ratios compared to perikarya. It must also be noted that increases or decreases in glucose use cannot be assigned to neuronal excitation or inhibition, because the energy requirements for synaptic inhibition are similar to those for synaptic excitation.

It is therefore not unexpected that the highest levels of oxidative metabolism were detected in brain areas that continuously process environmental information. Quail like other birds primarily rely on visual and auditory information for the organization of their social interactions as well as in their maintenance behavior. The analysis of these auditory and visual stimuli is a complex work-intensive process and it is therefore understandable that the brain areas involved in this information processing require more energy.

The quantitative densitometric analysis of the 2-DG accumulation in cells allowed us to determine the specific neuroanatomical localization of relative metabolic changes. The present experiments did not however provide absolute values for local rates of glucose utilization. The determination of these values would require the knowledge of specific kinetic parameters that are available for only a few species of mammals (Sokoloff et al., 1977). These parameters could be obtained in quail by serial measures of radioactivity in the blood but the repeated blood sampling that would be required to reach this goal would be difficult to perform in a relatively small bird and would obviously interfere with the performance of copulatory behavior. Therefore, only relative changes in incorporation could be assessed in the absence of knowledge of specific kinetic parameters for the 2-DG distribution in quail. These relative changes are however the only values that really matter for the purpose of the present experiment that was designed to investigate the changes in local energy requirement induced by two specific experimental treatments.

The comparison of metabolic activities in the left and right parts of the brain revealed a number of statistically significant differences. This small number of differences that were observed almost corresponds to what would be expected by chance given the large number of regions that were compared. It is therefore tempting to conclude that these results provide no conclusive evidence for a marked functional lateralization. A significant lateralization was detected in eight out of the 77 areas that were studied but only four of these differences remained significant when the analysis was restricted to the CX and CX+T groups. This suggests that part of these differences (four out of eight) could be due to the slight asymmetry that was detected in the POM lesions. Alternatively it is also possible that the decreased power of the analysis (*n* number reduced from 12 to eight) is responsible for the loss of five significant differences when the CX+T+L group is excluded.

The few differences between left and right side of the brain could have no biological significance. We believe however that this statement may not be entirely true at least for some brain regions. It is indeed interesting to note that in a previous study utilizing the 2-DG method in quail (Dermon et al., 1999) a small number of differences between the left and right brain (six out of 94 areas) was similarly observed but two of these differences were related to the same regions as in the present study (AM and Cpi). Furthermore the direction of the differences ($L > R$) is similar in both studies. It is therefore possible that brain activity is slightly different in the left and right sides at specific locations. This may relate to the fact that the control of some instinctive behaviors such as attack and copulation is also lateralized to some extent at least in domestic chicks (Bullock and Rogers, 1992), possibly as a result of the differential exposure of the two eyes to light during ontogeny (Rogers and Krebs, 1996; Rogers and Deng, 1999). During the incubation period, the right eye of chicks is indeed exposed to the light coming through the top of the egg shell whereas the left eye is covered by the head and wing and thus protected from the light. It has been shown that this differential exposure has long-lasting consequences on the development of the brain (in particular the visual pathways) and its functioning (Rogers and Deng, 1999). Additional metabolic studies should be performed to further explore these interesting differences.

Effects of testosterone and medial preoptic nucleus lesions on 2-deoxyglucose uptake

The 2-DG *in vivo* autoradiographic method provided detailed anatomical evidence on the brain loci where changes in metabolic activity are specifically associated with bilateral lesions in the POM nucleus and/or increases in plasma testosterone levels. The four types of changes in 2-DG uptake that could theoretically be expected after exposure to two different experimental treatments were actually observed in the quail brain: testosterone increased (Tn, OM, SCE, EW) or decreased (PVN, VMN, SAC, Imc, N, TPO, TrO) the local metabolism in specific brain areas and similarly, the lesions of the POM increased 2-DG uptake in some brain regions (N, TPO, TrO, SRt) but decreased this uptake in other areas (EW, HIS, SL, SM, POA, DMA, MM, PM, AVT). Interestingly there were brain regions affected by both

types of treatments. In EW for example an increase in 2-DG uptake was observed after exposure to testosterone but a decrease followed after POM lesions. Conversely in N, TPO and TrO testosterone decreased the 2-DG uptake but an increase was observed after POM lesions in these nuclei. In these four brain regions (EW, N, TPO, and TrO) the metabolic activity measured in the CX+T+L group was thus equivalent to the activity seen in castrates not treated with testosterone. These effects and their interactions provide important information about the physiology of the brain circuitry mediating sexual behavior in male quail.

Effects of testosterone

After a 2-week treatment with testosterone, significant increases in 2-DG uptake were detected here in four brain areas (Tn, OM, SCE, EW) whereas decreases were seen in seven separate nuclei (PVN, VMN, SAC, Imc, N, TPO, TrO; see Fig. 3). Several of these brain regions are known to contain androgen receptors (AR) or estrogen receptors (ER) (Watson and Adkins-Regan, 1989; Balthazart et al., 1989, 1992a, 1998b). This is namely the case for Tn, for PVN and VMN and possibly for SCE. Testosterone could therefore affect the metabolic activity in these brain regions by a direct local action as an androgen or after transformation into an estrogen by aromatization. The enzyme aromatase is indeed known to be present in the perikarya in three brain nuclei and/or in immediately adjacent regions (Foidart et al., 1995). The fact that testosterone either increased or decreased energy metabolism may be taken as evidence suggesting that different types of steroid receptor (androgens or estrogens) participate in cellular events controlling energy requirements. Moreover, since the precise location of the aromatase in dendrites, axons and terminals is not yet precisely known, it is possible that the presynaptic or postsynaptic localization of receptors plays an important role in the cellular processes influenced by the hormonal signal. Aromatase expression is differentially regulated in the POA or Hp of adult male zebra finches, suggesting a different role for aromatization in the pre- or postsynaptic function in steroid-sensitive areas (Saldanha et al., 2000).

The other brain regions where oxidative metabolism was affected by testosterone are not known as classical sex steroid targets. AR and ER have not been observed in these areas. There are therefore two possible ways through which testosterone could affect these nuclei. It is first possible that AR and/or ER are located in these regions but have escaped detection so far because they are either not present in sufficient numbers or present in a conformation that prevents their detection by methods used so far (e.g. occupied receptors not detected by *in vivo* autoradiography, receptors present in a conformation that does not allow immunocytochemical detection). Many brain regions in mammals that were not previously thought to be steroid targets have recently been shown to contain ER with the use of a method with higher sensitivity (e.g. *in situ* hybridization). It must also be noted that the quail brain has recently been shown to express, like the mammalian brain (Kuiper et al., 1996, 1998), a second form of ER called ER beta (ERβ) (Foidart et al., 1999; Ball et al., 1999). This receptor has a broader distribution than the classical ER now renamed ERα and it is therefore possible that it is also present in some of the areas where we detected here effects of testosterone on the 2-DG uptake. The original survey of the quail brain (Foidart et al., 1999) did not detect high densities of ERβ in these nuclei but additional work is certainly needed on this topic.

Secondly, it could also be argued that the effects of testosterone on the energy metabolism of specific brain nuclei do not result only from a direct local action but also from the modulation of synaptic activity in projections originating from classical sex steroid-sensitive neurons. Effects of steroid hormones on cerebral metabolism in areas devoid of steroid-sensitive neurons have accordingly been described in the rat brain (Nehlig et al., 1985). The 2-DG uptake method primarily detects changes in oxidative metabolism that reflect electrical activity in nerve terminals (Nudo and Masterton, 1986). Therefore if a steroid-sensitive region sends an important projection to a given area, changes in oxidative metabolism could be detected in this target area after treatment with testosterone, even if the area itself is not androgen-sensitive. Moreover, projections originating in a steroid-sensitive area could also affect trans-synaptically the activity of neurons in brain areas that contain no AR or ER. Such a mode of action could for example be invoked for an area such as the TPO that is known to receive direct projections from both the POM and the BST (Balthazart and Absil, 1997), two of the major testosterone targets for the activation of male sexual behavior (Balthazart et al., 1998a).

The present data therefore demonstrate that testosterone affects energy metabolism in brain areas that are not related in a direct manner to reproductive behavior. This statement is in agreement with a recent study, suggesting that castration results in a decrease in spine density in secondary sensory and association areas (Lieshoff et al., 2000) by affecting the action of testosterone on energy demands that control the mechanism of spine density.

Effects of medial preoptic nucleus lesions

Bilateral lesions of the POM applied 5 days before the 2-DG experiment also modified (increase or decrease) the 2-DG uptake in a variety of brain nuclei. Changes in oxidative metabolism presumably result in this case from changes in the activity in projections emanating from the POM or from adjacent areas. One could alternatively speculate that the lesion-induced changes are the consequence of an overall modification in the activity of the birds but this interpretation would then not be consistent with the fact that the overall condition of the subjects did not appear to be affected after the lesions and more importantly that the changes in brain metabolism are highly specific from an anatomical point of view. Electrolytic lesions of the POM may have damaged local neurons as well as fibers en passage. Therefore, the highly specific changes in brain metabolism reflected

both anterograde and retrograde events that are discussed below in relation to known anatomical pathways.

It is important to note that in four brain regions where the POM lesions affected the 2-DG uptake, a change had also been detected after treatment with testosterone but in the opposite direction (testosterone increases and lesion decreases 2-DG uptake in EW; testosterone decreases and lesion increases 2-DG uptake in N, TPO and TrO; Fig. 3). At a phenomenological level, this suggests that in these areas, the lesions blocked the effects of testosterone which in turn may be taken as an indication that testosterone was indeed affecting the metabolism in these nuclei mainly through its action in the POM. The simplest model that could explain this interaction implies that the POM directly projects to these four areas and that the metabolic changes are confined in axon terminals that originate in the POM (see above). As already mentioned, we have evidence for a direct projection of POM to TPO but not for the other three brain regions and tract-tracing studies should be performed to test this interpretation. Alternatively indirect projections including one or several interneurons could also mediate these metabolic changes at remote sites (e.g. in TrO where the presence of axons originating in POM appears unlikely). This interpretation could only be tested with the use of tracers that can be transported trans-synaptically such as the pseudorabies virus that has been recently used to study brain connectivity in mammals (Daniels et al., 1999; Aston-Jones and Card, 2000).

In a number of brain areas, changes in 2-DG uptake were also observed whereas no effect of testosterone had been detected (Fig. 4). Many of these nuclei are known to be mono-synaptically connected to POM. This is namely the case of the SL, SM, DMA and MM. In each of these four cases, the lesions were found to inhibit the oxidative metabolism. It is therefore likely that the areas receive tonic inputs from the POM. A high level of spontaneous electrical activity is known to be present in the medial POA of the quail (Cornil, C., Balthazart, J. and Seutin, V., unpublished data) as previously shown in the rat (Sakuma, 1994; Kato and Sakuma, 2000). This electrical activity is likely to be reflected in axon terminals originating in this nucleus and located in the areas of interest. Destruction of a large fraction of the POM should therefore suppress activity in these terminals.

Most of these areas are reciprocally connected with POM. Thus, in addition to the anterograde events described above, the observed metabolic depression could additionally reflect retrograde changes in the parent neurons projecting to the damaged field. Indeed distal fiber and terminal degeneration has been reported after archistriatal lesions within a 3-day post-operative period (Zeier and Karten, 1971).

POM lesions also induced metabolic changes in a few other areas that are not known to receive projections from the POM (e.g. SRt, HIS, PM). Based on the present results, one could then assume that either these connections are still waiting to be described or that the changes are mediated by poly-synaptic pathways.

It must also be mentioned that in three additional brain nuclei (HM, AVT and TO) significant effects of the experimental treatments were detected by the overall ANOVA but the post hoc tests that were performed to compare groups two by two did not identify clear changes that could easily be assigned to the effects of testosterone or of the lesion. In HM no significant difference at all was detected by the Tukey–Kramer post hoc tests. No conclusion can therefore be made although the numerically lower values of 2-DG uptake in the CX+T+L group suggest that this group is the main source of variance that leads to a significant result in the ANOVA. This would fit well with the fact that HM is known to receive direct projections from the POM (Balthazart et al., 1994; Balthazart and Absil, 1997).

In AVT, the avian homologue of the mammalian ventral tegmental area (dopaminergic cell group A10), the post hoc tests detected a statistically lower 2-DG uptake in the CX+T+L group by comparison with the CX group but not with the CX+T group. These two latter groups had however a very similar metabolism (marginally smaller in the CX+T than in the CX group) so that the difference with the CX group is just below the critical 5% level whereas the difference with the CX+T group is just above that value. From a biological perspective, it can be assumed that the two groups that did not bear a lesion were very similar and that the POM lesion decreased the oxidative metabolism in AVT. This conclusion would indeed be supported statistically if a less stringent post hoc test had been selected. The Fisher protected least significant difference test for example indicates the presence of significant differences between the CX+T+L group and both the CX and the CX+T groups.

It is important to note that the metabolic activity was decreased by POM lesions in the dopaminergic cell group AVT whereas, in contrast, no effect could be detected in the noradrenergic locus ceruleus that also projects to POM. POM contains significant densities of dopamine beta hydroxylase immunoreactive fibers (Bailhache and Balthazart, 1993) and tract-tracing studies have demonstrated direct connections between these nuclei (Balthazart and Absil, 1997). The lack of change in the locus ceruleus may relate to the wide collateralization of noradrenergic neurons. The metabolic changes in AVT could reflect both orthodromic degeneration of POM terminals and antidromic degeneration of AVT neurons due to the damage of their field of projection. They are highly relevant to the control of male sexual behavior.

The same pattern of changes as in AVT was also observed in TO. Metabolic activity in this brain region thus also appears to be affected by brain lesions but not by testosterone. The functional significance of this observation is discussed below.

Functional interpretation of the changes observed after testosterone treatment or medial preoptic nucleus lesion

We previously used the 2-DG uptake technique to identify changes in brain oxidative metabolism that are related to the performance of male appetitive and consummatory sexual behavior (Dermon et al., 1999). This

study revealed the existence of localized changes in brain metabolism specifically associated with the performance of these behaviors. Interestingly several of the areas where these changes were detected (POA, OM, TO, TeO) were also found here to be affected by testosterone and/or by lesions of the POM, a key site for the activation of male sexual behavior by testosterone. The two studies addressed however very different aspects of the brain activity and it is therefore not unexpected that the metabolic changes detected here concerned for the most part specific brain regions. These two sets of data clearly reinforce the notion that the POA plays a key role in the control of male sexual behavior. These data also draw attention to the involvement of visual areas in the control of sexual behavior. In addition, the indication that TO is functionally related to the POM and thus to the neuronal circuits that control reproductive behavior, also suggests a possible involvement of olfaction in male sexual performance. The significance of these metabolic changes for the control of male sexual behavior is discussed below in an integrated manner with reference to specific functional circuits.

Preoptic area

The fact that the POA metabolism was affected both by lesions of the POM (decreased 2-DG uptake) and by the performance of male copulatory behavior (increased 2-DG uptake) is fully consistent with the idea that this brain region plays a key role in the control of male sexual behavior. Based on studies carried out in rats, it has been suggested that the POA could play a tonic inhibitory role on the expression of male sexual behavior through its projections to the pre-motor and motor relays located in the mesencephalic central gray and spinal cord. Testosterone action on the preoptic dopaminergic system would remove this tonic inhibition and in this way facilitate the expression of the behavior (Hull, 1995; Hull et al., 1999). The removal of this tonic inhibition could be an active process in itself as suggested by the fact that expression of male sexual behavior increases 2-DG uptake in the POA and also promotes the expression of the immediate early gene *c-fos* in the same area. Lesions of this structure would completely disrupt the afferent and efferent connections of this region which would be the direct cause of the behavioral inhibition. The bilateral electrolytic lesion of POM affected the POA metabolic activity in the vicinity but clearly outside of the lesioned area. This decrease in metabolic activity in the POA and in the rostral POM in the subject in which this part of the nucleus was not destroyed could represent a loss of synaptic activity in interneurons connected to the POM following the lesion-induced death of the POM neurons. Taken together the present study and studies measuring brain activity in behaving animals (measures of 2-DG uptake and of *c-fos* expression mentioned above) provide converging evidence for the involvement of the POA in the control of male mating behavior. This conclusion is directly supported by the facts that, in quail, lesions of the POA severely disrupt the expression of male copulatory behavior and that implants of testosterone in the POA activate male-typical copulation in castrated male quail.

Visual system

Testosterone action in the brain is thought to produce physiological changes that increase the likelihood that a male will react by showing copulatory behavior when presented to a suitable stimulus (i.e. a receptive female). Quail like other birds are highly visual species and extensively use visual information to identify females (Domjan and Ravert, 1991; Domjan and Nash, 1988). They are also capable of recognizing specific individual females based on distant cues and it is likely that these cues are visual in nature (Riters and Balthazart, 1998). It is in this respect interesting to note that treatment with testosterone alone affected the metabolism of many brain areas that are directly or indirectly implicated in the acquisition and processing of visual information. The steroid indeed increased 2-DG uptake in the EW and in the OM whereas it decreased this uptake in the TrO, in the SAC and in the Imc. This latter decrease could be related to the Imc function since Imc is GABAergic (Veenman and Reiner, 1994) and it has been suggested that Imc-tectal fibers form symmetrical synapses in the tectum (Tombol et al., 1995) participating in an inhibitory feedback pathway. This tectal–Imc-tectal loop may be metabolically depressed under testosterone treatment and could reflect a more focussed attention on specific objects (see Andrew, 1972 for effects of testosterone on persistence of behavior) leading to a decreased influx of information at that level in the visual pathway.

The fact that some of these effects were blocked when POM lesions were applied (e.g. in EW and TrO) further suggests that these nuclei have functional and probably anatomical connections with the median part of the POA even if these connections have not been described so far. The exact nature of the testosterone-induced changes in metabolic activity in these different parts of the visual system is unclear at present and so is their precise function. It is however worth pointing out that in contrast to Imc-TeO depressions, testosterone increased 2-DG uptake in parts of the system (EW, OM). The increased activity in EW and OM could be related to the increase in eye movements potentially associated with the search of a sexual partner. Independent of the exact meaning of the changes observed, the present study clearly indicates that testosterone markedly affects the processing of visual information in the quail brain and that the POA directly interferes with these processes.

Moreover, it is significant to note that metabolic activity in the OM and layers of the TeO was modified both following testosterone treatment (this study) and during performance of copulatory behavior (Dermon et al., 1999). This may relate to the fact that sexual behavior is largely driven by visual stimuli in quail (Mills et al., 1997; Balthazart and Ball, 1998a).

Limbic system

POM lesion induced distal complex responses in tel-

encephalic areas including parts of the limbic system. A metabolic depression was observed in the lateral septal nucleus, a serotoninergic area (Challet et al., 1996) known to be reciprocally connected with POM, that is functionally related to photoperiodic responses (Kiyoshi et al., 1998). The observed SL metabolic depression possibly represents anterograde and retrograde degenerative events due to the lesion of POM neurons and terminals.

In addition, POM lesions induced metabolic depressions in olfactory areas known to receive projections from olfactory bulb, such as the medial septal region, TO, and Tn (Reiner and Karten, 1985). The area including TO, ventral to the PP and LPO is considered visceral/limbic in birds. TO contains a dense accumulation of GABAergic and catecholaminergic fibers and receives inputs from the olfactory bulb, LPO and lateral septum. TO has also been shown to project to the septum, archistriatum, lateral corticoid area, CPi, TPO, Hp, medial HV including IMHV, dorsomedial thalamic nucleus, entire habenular region and ventral hypothalamic area, but these connections have not been fully characterized (Szekely et al., 1994). It is therefore likely that either there is a direct connection of TO with the POA or that the metabolic depression observed in TO following POM lesions is a poly-synaptic event, related to the lack of activity of its lateral septal afferents. Previous studies have indicated that TO is involved in functional circuits related to reproductive behavior: metabolic activity as measured by the 2-DG method was decreased in birds after they had expressed either in the anticipatory or the consummatory phases of male sexual behavior (Dermon et al., 1999). In the present study metabolic depressions were additionally observed in projection areas of TO such as SM, SL, HM and DMA. It is also important to point out that the alterations of metabolic activity found in diencephalic limbic areas, such as the depressions of the medial habenula, dorsomedial thalamic nucleus and medial mammillary nucleus are located in structures that are known to have connections with preoptic and septal areas (Balthazart et al., 1994; Balthazart and Absil, 1997). Similarly the activation of SRt may be related to the connection of this structure to Tn (Thompson et al., 1998; Cheng et al., 1999). Taken together, the data described above strongly support the notion that the POM extensively influences the activity of the medial diencephalic and basoventral telencephalic limbic systems in addition to the activity of the visual system. The metabolic changes observed in the olfactory pathway and in particular in Tn (homologous of parts of the mammalian amygdala) also suggest that POM may control the processing of olfactory stimuli that could play a role in reproduction.

Moreover, changes in metabolic activity following POM lesions were seen in telencephalic association areas, such as N, TPO, and HIS. These effects were possibly mediated via thalamic limbic nuclei. The enhanced activity in N and TPO could be related to the metabolic activation of the thalamic SRt (comparable to mammalian posterior intralaminar nucleus), that sends wide projections to neostriatal regions, that in turn projects to TPO (Miceli and Repérant, 1985; Metzger et al., 1996). In addition, DMA (presumably homologous to mammalian midline and MD nuclei) sends non-dopaminergic projections to the TO, LPO, septal area, and N (Metzger et al., 1996) that were possibly inhibited by POM lesions. DMA has been shown to project to the visceral/limbic striatum and it has been suggested that its medial part is equivalent to the mammalian midline thalamic nuclei and its lateral part to the mediodorsal nucleus (Veenman et al., 1997). The present study indicates that limbic thalamic nuclei (homologous of intralaminar, midline, MD) are involved in the circuits influenced by POM activity and possibly influence the activity of their telencephalic targets. An alternative but not mutually exclusive explanation could be that the activity of the telencephalic areas discussed above is influenced by the withdrawal of AVT dopaminergic efferents (see discussion of dopaminergic system below).

Dopaminergic system

It is finally worth noting that the present data also indicated the existence of a decreased metabolic activity in the AVT that constitutes the avian homologue of the ventral tegmental area of mammals. Previous tract-tracing studies have demonstrated the existence of bi-directional connections between AVT and POM (Balthazart et al., 1994; Balthazart and Absil, 1997). Furthermore, the combination of tract-tracing techniques with immunocytochemistry for tyrosine hydroxylase, the rate limiting enzyme in catecholamine synthesis, indicates that a significant proportion of the inputs from AVT to POM are dopaminergic in nature (Balthazart and Absil, 1997). AVT also sends massive projections to the bed nucleus striae terminalis (BST; Balthazart and Absil, 1997) another steroid-sensitive nucleus that is implicated in the activation of male reproductive behavior (Balthazart et al., 1998a). Bi-directional connections between AVT and a large number of hypothalamic, thalamic and mesencephalic nuclei have indeed been demonstrated (Balthazart et al., 1994). Dopamine is known to play a major role in the control of male sexual behavior in mammals (reviews: Blackburn et al., 1992; Hull, 1995; Hull et al., 1999) and similarly, injections of agonists or antagonists of the D1- or D2-like dopaminergic receptors have profound and rapid effects on the expression of appetitive and consummatory sexual behavior in male quail (Absil et al., 1994; Castagna et al., 1997; Balthazart et al., 1997a).

Moreover, immunohistochemical studies have shown that the septal nucleus of pigeons contains a dense plexus of catecholaminergic fibers and terminals (Dubé and Parent, 1981), that partly originates from AVT (Kitt and Brauth, 1986). Therefore the major projections of the mesolimbic component (Ac, TO, septum, BST) and mesodiencephalic pathway (POA, POM, nucleus medialis dorsalis) of the ventral tegmental area (VTA) in mammals (Oades and Halliday, 1987; Beckstead et al., 1979) are comparable to the projections of AVT in birds.

The present lesion data suggest that the POM directly controls the metabolic activity in AVT and it is possible that a behaviorally relevant part of the dopaminergic

activity in AVT is affected in this way. This notion is supported by the hodological data described above and by the recent demonstration that performance of copulatory behavior in quail increases the expression of the immediate early genes *c-fos* and *zenk* specifically in dopaminergic (tyrosine hydroxylase immunoreactive) neurons of AVT (Balthazart et al., 2000). This study therefore suggests that one important way through which the POM may be regulating sexual behavior expression is through the control of the dopaminergic AVT inputs to a large number of nuclei including the POM itself but also the BST, PVN, VMN and intercollicular nucleus, to just cite a few of the nuclei that are known to receive AVT afferents. Because all these nuclei presumably play a role in the activation of behavior, the control by POM of the dopaminergic activity in AVT may represent one of the major ways through which POM regulates behavior. Functional studies should be carried out to test directly this functionally important conclusion.

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