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Appetitive and Consummatory Male Sexual Behavior in Japanese Quail Are Differentially Regulated by Subregions of the Preoptic Medial Nucleus

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Central testosterone aromatization is required for the activation of both appetitive (ASB) and consummatory (CSB) male sexual behavior in Japanese quail. There are two major clusters of aromatase immunoreactive (ARO-ir) cells in the rostral forebrain; these outline the nucleus preopticus medialis (POM) and the nucleus striae terminalis (BST). We investigated the role of these nuclei in the regulation of ASB and CSB. Appetitive male sexual behavior was measured with the use of a learned social proximity procedure that quantified the time spent by a male in front of a window with a view of a female who was subsequently released into the cage, providing an opportunity for CSB. Males first acquired the response and then received bilateral electrolytic lesions aimed at the POM or BST, followed by retesting for ASB and CSB. Brain sections were stained for ARO-ir, and lesions to the two ARO-ir cell groups were quantitatively characterized. Lesions damaging the POM completely abolished CSB and also significantly decreased ASB. Lesions of the rostral BST had no effect on ASB, but moderately decreased CSB. Detailed anatomical analysis revealed that lesions of a subdivision of the POM just rostral to the anterior commissure specifically impair CSB, whereas lesions that are more rostral to this subdivision induce a severe deficit in ASB. These data indicate that different subregions of the POM regulate ASB and CSB in a somewhat independent manner, whereas the BST is only important in the regulation of CSB.

Key words: appetitive sexual behavior; consummatory sexual behavior; medial preoptic area; bed nucleus striae terminalis; testosterone aromatization; electrolytic lesions; learned social proximity response; preoptic area subdivisions

Male sexual behavior includes appetitive and consummatory components (Beach, 1956; Balthazart and Ball, 1997, 1998). Some stereotyped behaviors result in a functional outcome that is associated with a reduction in motivation; these constitute consummatory behaviors such as copulation. Other more variable behaviors allow an individual to converge on this functional outcome; these are appetitive behaviors such as seeking out a female (Timberlake and Silva, 1995). Experimental studies have revealed dissociations between aspects of the neural system regulating appetitive and consummatory components of male sexual behavior. In rats, lesions to the medial preoptic area (mPOA) eliminate male-typical copulatory behavior (Heimer and Larsson, 1966; Meisel and Sachs, 1994). These animals still exhibit appetitive behaviors in that they pursue females and acquire learned responses to gain access to females (Everitt, 1990, 1995). Damage to the mPOA does not impair other appetitive measures such as penile erections in response to remote cues from estrous females (Liu et al., 1997). Lesions to the medial amygdala or the bed nucleus of the stria terminalis (BST) decrease noncontact erections or the ability of males to acquire learned responses that are rewarded with access to females (Everitt, 1990, 1995; Kondo et al., 1997; Liu et al., 1997). These studies suggest that the mPOA integrates sensory inputs needed for the activation of copulation but appetitive components of the male sexual response are regulated by pathways independent of the mPOA (Everitt, 1995; Liu et al., 1997). There are some inconsistencies, in that there is evidence that lesions to the mPOA can impair measures of appetitive male sexual behavior (Edwards and Einhorn, 1986; Paredes et al., 1993). An alternative hypothesis suggests that dopamine acting in the mPOA enhances the processing of sensations from the genitals as well as olfactory cues from estrous females and redirects behavior in favor of sex-related activities (Hull, 1995; Hull et al., 1995).

In quail, appetitive and consummatory aspects of male sexual behavior are activated by estrogens produced in the brain via the local aromatization of testosterone (Balthazart and Surlemont, 1990b; Balthazart et al., 1995, 1997a). Therefore, the localization of brain aromatase can guide one to components of the neural circuit regulating these behaviors. The nucleus preopticus medialis (POM) and the BST contain a high number of aromatase-immunoreactive cells (Panzica et al., 1996). Lesioning the POM profoundly impairs copulatory behavior (Balthazart and Surlemont, 1990a), but appetitive behavior has not been investigated. In this study, electrolytic lesions were directed at the POM or BST, and male sexual behavior was measured. Appetitive male sexual behavior was assessed by a learned social proximity response (Balthazart and Ball, 1997) and by measuring rhythmic cloacal sphincter muscle movements in reaction to the visual presentation of a female (Seiwert, 1994). Consummatory sexual behavior

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was assessed by observing copulatory behavior. These studies demonstrate that the POM is involved in the activation of consummatory and appetitive aspects of male sexual behavior. Lesions to the BST also impaired copulatory behavior, but there were no effects on appetitive aspects of male sexual behavior.

MATERIALS AND METHODS

Subjects. Experiments described in this paper involved male Japanese quail (*Coturnix japonica*), age ~3 weeks, bought from a local breeder (Dujardin Farms, Liernu, Belgium). After their arrival in the laboratory, the young birds were housed in groups in brooders. All subjects were always provided with food and water *ad libitum* and were maintained on a photoperiod simulating long summer days (16 hr light/8 hr dark) throughout the experiments. Sexually mature stimulus females used during the behavioral tests were purchased at the same dealer and were housed under the same conditions as the males.

All experimental procedures were in agreement with the Belgian laws on the Protection and Welfare of Animals and 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 Supervisor of Animal Care for the University of Liège.

Castration and endocrine treatments. All male subjects were castrated under complete anesthesia (15 mg/kg Hypnodil; Janssen Pharmaceutica, Beerse, Belgium) within 1 week after their arrival in the laboratory. The two testes were removed through a unilateral incision behind the last rib as described previously (Balthazart and Schumacher, 1984). Two weeks later, approximately three-quarters of the birds received one SILASTIC implant (catalog #602–252; Dow Corning, Midland, MI) (1.57 mm inner diameter; 2.41 mm outer diameter; length = 20 mm) filled with crystalline testosterone. The rest of the subjects served as controls and were implanted with a similar capsule that was left empty. Capsules were implanted subcutaneously in the neck region. At that time, all subjects were transferred to individual cages where they remained until the end of the experiments. Throughout the experiment, birds were periodically weighed to the nearest gram, and the size of their cloacal gland was measured with calipers (cloacal gland area = largest length × largest width in square millimeters). This gland is an androgen-sensitive structure (Sachs, 1967), and its surface therefore provides a sensitive measure of the endocrine state of the birds (Follett and Maung, 1978; Delville et al., 1985). These data confirmed the efficacy of the testosterone replacement (the species-typical enlargement of the cloacal gland was regularly observed in all subjects) as well as the absence of adverse effects of the experimental treatments on the general health condition of the subjects (all birds slowly gained weight with time, as normally observed in this age range). No further mention of these data are made in this paper.

Behavioral screening and acquisition of the social proximity response. Two weeks after the implantation of the SILASTIC capsules, all birds were tested four times for the presence of male-typical copulatory behavior using behavioral procedures that have been described previously in detail (Balthazart and Schumacher, 1984). Briefly, each male was introduced into a small arena (50 × 60 cm) that contained a sexually mature female, and the occurrence of copulatory behaviors [neck grabs, mounts, and cloacal contact movements (for descriptions, see Adkins and Adler, 1972; Hutchison, 1978)] was recorded. Testosterone-treated birds that failed to exhibit mounts and cloacal contact movements were excluded from the study at this point. As expected, most of the castrated birds possessing empty implants remained behaviorally inactive during these tests. The few subjects that exhibited low levels of copulatory behavior, indicating an incomplete castration and gonadal regeneration, were also excluded.

During the next 10 d period, all remaining subjects experienced, in a two-compartment test cage, eight associative learning trials, each taking place on a different day (tests 1–8). During these trials, visual access to a sexually mature female, who was in an adjacent chamber and could be seen through a small window, was paired with the subsequent free access to that female, allowing sexual interactions and copulation. In these conditions, all male subjects who engaged in copulatory behavior acquire the learned social proximity response (this involves standing in an area in front of the window and looking though it at the female) that is used in our experiments to test appetitive behavior. This procedure is based on experimental protocols originally developed by Dr. M. Domjan at the University of Texas (Domjan and Hall, 1986a,b; Domjan et al., 1986; Crawford et al., 1993; Domjan, 1994), and the specific modifications introduced in our laboratory have been validated and described in detail (Balthazart et al., 1995, 1997a,b; Balthazart and Ball, 1997; Castagna et al., 1997). They are only briefly summarized below.

Two-compartment test cages. Four, two-compartment cages were used in this study. The larger of the two compartments was 90 cm wide × 90 cm deep × 50 cm high. A smaller compartment, for stimulus females, was 20 cm wide × 26 cm deep × 24 cm high and was centered on the left lateral wall of the main cage and separated from it by a vertically sliding door, 20 cm wide × 20 cm high, that could be controlled remotely by strings and pulleys. A small narrow window (consisting of a vertical slit, 1 cm wide × 15 cm high, cut in the plywood) was located in the middle of this door and provided the male with limited visual access to the female. This window could be closed by an opaque swinging plywood panel attached by a hinge just above the door. The lower part of that panel was attached to a string and pulley system that allowed remote lifting of the panel. One square area of the floor (30 × 30 cm), located in the middle of the lateral left wall (in front of the door/window), represented the test area for bird position. When the window was open, the male located in the main chamber could see the female located in the lateral chamber only if he stood in front of the window in this area. This square area was mounted on four springs and four microswitches (one in each corner) wired in parallel and powered by a 4.5 V battery so that depression of any of these switches generated a positive signal. The output signals were digitized and sent to a MacIntosh computer using commercially available hardware and software. A computer program written in Basic recorded during the observation periods (5 min periods; see below) the total time spent by the bird in the test area and the number of times that the bird entered this area. The presence of a bird in the test area (i.e., microswitches activated) was sampled in the test area of each cage once every second.

Behavioral tests: general procedure. Four tests were always run in parallel in the four experimental cages. Each lasted a total of 25 min. At the beginning of the test, one male was introduced into the main chamber and one stimulus female was placed in the adjacent smaller cage. The window between the two compartments was closed at that time. Birds were given 5 min to habituate to the new environment. The position of the male was then recorded continuously during the next 5 min period with the window still closed (pretest). The window was then opened, and the position of the male was again recorded for 5 min (time at the window). During these 5 min, a beeper was activated and emitted a weak sound every 5 sec. At each beep, the observer recorded whether the subject was actually looking through the window. Looking behavior was defined as a stereotyped positioning of the head that allows the subject to focus on the female through the window. This point sampling (Martin and Bateson, 1986) provided a score for the looking behavior ranging from 0 (never observed) to 60 (behavior present at every beep). It has been demonstrated previously that the social proximity response (time spent at window and frequency of looks through the window) develops only in birds that are permitted to copulate during the 5 min interaction with the female and that this response is steroid-dependent (Domjan, 1987; Balthazart et al., 1995). These observations support the notion that the social proximity response is a valid measure of appetitive male sexual behavior in quail.

At the end of that period, the door separating the two compartments was lifted, and the two birds were allowed to interact freely for 5 min. During that time, the frequency and latency of the first occurrence of male sexual behaviors were recorded. The following behavior patterns were systematically noted: strut, neck grab (NG), mount attempt (MA), mount (M), and cloacal contact movements (CCM) [for a detailed description, see Adkins and Adler (1972) and Hutchison (1978)]. These data provided a measure of the consummatory sexual behavior of the birds. The female was then removed from the experimental chamber where the male stayed for another 5 min before he was returned to his home cage.

Stereotaxic brain lesions. As expected on the basis of our previous studies, most of the testosterone-treated males acquired the social proximity response during these eight tests, whereas castrated control subjects were never observed to spent a significant amount of time in front of the window. The few testosterone-treated birds that had not acquired the response were discarded at this time. The remaining testosterone-treated birds were then randomly assigned to one of two groups that were balanced on the basis of the cumulative time that birds had spend in front of the window during the last two training tests. Subjects in one of these groups received in the following days a bilateral electrolytic brain lesion,

whereas the other testosterone-treated birds and the castrated controls were subjected to a sham operation.

The experiment described here was performed in three replicates during which the electrolytic lesions were aimed at three distinct brain areas characterized by the presence of a dense group of aromatase-immunoreactive (ARO-ir) cells: the nucleus POM, the rostral part of the nucleus striae terminalis, where ARO-ir cells are clustered as a small group dorsal to the anterior commissure (rostral BST), and the caudal part of the nucleus striae terminalis, where ARO-ir cells that were present at more rostral levels in POM and rostral BST merge to form a bilateral V-shaped structure (caudal BST) [for a detailed description, see Foidart et al. (1995); for definitions of the POM and BST in quail, see Panzica et al. (1991) and Aste et al. (1998); also see Fig. 1 for a schematic illustration of these cell groups), respectively. After the actual locations of these lesions were analyzed by histological techniques, however, it appeared that because of the close proximity of these three brain areas, there was an extensive amount of overlap between the lesions aimed at the three theoretically distinct targets. All data were therefore reorganized and analyzed on the basis of the actual position of the lesions (see below).

To produce the desired electrolytic lesions, birds were anesthetized with Hypnodil (15 mg/kg) and placed in a stereotaxic apparatus (with pigeon head holder; Trent Wells, Inc. South Gate, CA), 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 (1.25 mA for 10 sec). 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 1 hr.

Electrode coordinates were 5.0 mm anterior (x) and 2.3 mm above the zero reference point (center of the interaural axis, y) and 0.4 mm lateral to the sagittal midline (identified on each bird by the suture of the frontal bones, z) for lesions aimed at the POM; $x = 5.0$, $y = 3.5$, $z = 1.6$ for lesions aimed at the rostral BST; and $x = 4.9$, $y = 2.5$, $z = 0.5$ for lesions aimed at the caudal BST. These coordinates had been obtained originally from the quail brain stereotaxic atlas (Baylé et al., 1974) and adjusted by trial and error for the heavier body weight of our birds.

Quantification of behavioral deficits. Beginning on the day after surgery, all birds experienced nine behavioral tests to quantify the effect of the experimental procedures of the measures of appetitive and consummatory sexual behavior (tests A–I). These testing procedures were identical to the training tests used to acquire the social proximity response (25 min total duration in the two-compartment test cage), and they were completed within the first 15 d after operation.

Birds from replicates 2 and 3 were subsequently submitted to two additional behavioral tests to control for both the specificity of the acquired proximity response and the potential effects of the lesions on another measure of appetitive sexual behavior in quail: the induction of rhythmic cloacal sphincter movements in the presence of a female.

In the first of these tests, each male (from replicates 2 and 3) was tested once in the two-compartment cage with the same protocol as before except that no female was present in the adjacent small compartment. This procedure was run after the lesioning had occurred to insure that the presence of a male in front of the window providing visual access to the female was still related to the actual presence of the female in the adjacent chamber rather than the result of some other nonspecific effect of the lesion.

Male quail from replicate 3 were also tested once for rhythmic cloacal sphincter muscle movements (RCSMs) in reaction to the visual presentation of a female. These movements are used by males just before copulation to produce the stiff meringue-like foam that is transferred to females during copulation (Seiwert, 1994). It has been shown previously (Seiwert and Adkins-Regan, 1992; Seiwert, 1994; Thompson et al., 1998) that gonadally intact, sexually active males rapidly increase the rate of these movements when they are provided with visual access to a female. These movements thus provide an additional measure of appetitive male sexual behavior in quail. The testing procedure used to assess these movements is as follows. Each male was placed in an aquarium (20 × 40 cm) located on a raised platform. A mirror was placed under the aquarium at a 45° angle and provided an observer with an unobstructed view of the male's cloacal area. Feathers of the experimental subjects were plucked from the cloacal area to facilitate the assessment of cloacal movements. At the beginning of each behavioral test, the aquarium was divided into two chambers by an opaque partition, and RCSMs were quantified for 2.5 min during which the male could not see the female. The opaque partition was then removed so that the male and the female were separated only by a wire-mesh grid and the male had visual access to the female, although he could not physically interact with her. The RCSMs were then quantified for an additional 2.5 min under these stimulus conditions.

Histological verification of the lesion sites and aromatase immunocytochemistry. At the completion of behavioral tests, all birds were injected intravenously with 150 μl of heparin solution (Sigma H-7005, 20 mg/ml; Sigma, St. Louis, MO) and deeply anesthetized with Hypnodil (Janssen Pharmaceutica; 50 mg/kg body weight). They were perfused through the heart initially with a saline solution (9 gm/l; 0.15 M NaCl) until the return blood was clear and then with 400 ml of fixative (4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2). Brains were dissected out of the skull and placed overnight in a 20% sucrose solution in 0.1 M phosphate buffer. The next day they were frozen on powdered dry ice and stored in a freezer at −75°C until they were processed. At the time when the perfusion was performed, the birds were checked for the completeness of castration and for the presence of subcutaneous testosterone SILASTIC implants in the lesion experiments. All birds showing testicular remnants or who had lost their testosterone implant were discarded before any data analysis.

Frozen brains were cut with a cryostat into 50-μm-thick coronal sections, and sections located between the level of the tractus septomesencephalicus and the level of the bed nucleus of the supraoptic decussation were collected in PBS (PBS, 0.01 M; NaCl, 0.125 M, pH 7.2). The plane of section was adjusted to match as closely as possible the plane of the quail brain atlas (Baylé et al., 1974). Alternate sections were distributed in two series that were either stained by toluidine blue for the Nissl substance or by immunocytochemistry for aromatase.

A standard peroxidase–antiperoxidase (PAP) procedure using diaminobenzidine as the chromogen was used to visualize immunoreactive aromatase as described previously (Foidart et al., 1995). Briefly, sections were rinsed three times in PBS containing 0.1% Triton X-100 (PBST) and then in 0.6% hydrogen peroxide PBS for 20 min to block endogenous peroxidases. After three additional rinses in PBST, they were incubated for 30 min in normal goat serum (5% in PBST) and then overnight at 4°C in the primary polyclonal antibody against aromatase (1:200 in PBST). This antibody has been raised in rabbit against a preparation of recombinant quail aromatase and then purified by affinity chromatography. This antibody specifically recognizes aromatase-containing cells in the quail brain [for details on preparation and validation of the antibody, see Foidart et al. (1995)].

On the next day, sections were processed according to the PAP technique. The goat anti-rabbit (dilution 1:100 for 120 min) and PAP complex (1:500 for 120 min) were both diluted in PBST. Extensive rinses in PBST were made between each step. The peroxidase was finally revealed by immersing sections for 6–7 min in a solution of diaminobenzidine (20 mg in 50 ml of PBST containing 20 μl of hydrogen peroxide at 30%). Sections were then mounted on microscope slides and coverslipped.

Data analysis. On the basis of sections stained by toluidine blue and sections stained by immunocytochemistry for aromatase, the extent and location of each lesion was first drawn for each bird on a series of schematic drawings of the preoptic area–anterior hypothalamus. These plots were made while paying maximal attention to the position of the lesion with respect to the ARO-ir cell groups.

Sections stained by immunocytochemistry were then digitized by means of a CCD camera connected to a Macintosh CI computer, and the lesions and groups of ARO-ir cells were drawn with the mouse on each image. The corresponding areas were calculated by the program NIH Image (Version 1.52, Wayne Rasband, Bethesda, MD), and volumes of the lesions and the remaining ARO-ir cell groups (POM, rostral and caudal BST) were subsequently reconstructed by multiplying areas by the distance between consecutive sections (in this case 100 μm). Because in

most cases the lesions destroyed only a portion of the POM or BST and the shape of these nuclei changes only gradually in the rostrocaudal axis, it was often possible to reconstruct, with reasonable precision, the contours of the nuclei as they would be in the absence of a lesion. These putative areas that would have been occupied by the intact nuclei were then used to calculate the estimated total volumes of the nuclei.

Five behavioral variables were selected for systematic analysis. They consisted of three measures of appetitive sexual behavior, namely, the time spent in front of the window (time), the frequency of looks through the window (looks), and the number of times that birds entered the test area in front of the window (entrances) during the 5 min test period, and two measures of consummatory sexual behavior, namely, the frequencies of MAs and of CCMs. Other behavioral measures of copulatory behavior (e.g., frequencies of neck grabs or mounts) are highly correlated with these two measures and therefore redundant. These behavioral data were analyzed by one- or two-way ANOVA, which were followed, when appropriate, by *post hoc* Fisher's protected least significant difference test (PLSD). An α level of 0.05 was used for all statistical tests.

RESULTS

A total of 73 birds completed the entire behavioral experiment and had their brain lesions analyzed with enough detail so that their behavior could be related to the actual site and extent of the lesion. Included in this total sample of 73 are 30 castrated, testosterone-treated birds with a lesion that destroyed to varying degrees either the POM ($n = 21$), or the BST ($n = 9$), as well as 22 castrated, testosterone-treated birds (CX+T) and 21 castrated birds (CX) with empty implants that had no lesion (but had undergone the sham operation). In addition, 11 subjects had lesions that could not be fully reconstructed because of various technical problems and are therefore not included in the analyses. Three other birds had lesions that did not affect POM or BST and were not considered further because they were not available in sufficient number to drawn any conclusion. It must be noted here that although lesions had been specifically aimed at the caudal portion of the BST (bilateral V-shaped structure caudal to the anterior commissure) during the third replicate of the experiment, histological examination of these lesions indicated that this part of the nucleus had never been touched exclusively; all lesions aimed at the caudal BST were in reality placed in the caudal POM. This can be explained by the fact that this structure extends only in a very short portion of the rostrocaudal axis (100–150 μm) so that it is very difficult to hit this structure specifically. All lesions to BST described in this study therefore refer to the rostral and dorsal part of the nucleus, above the anterior commissure (Fig. 1). It must be noted that, as illustrated in Figure 2*D,E*, these lesions to the dorsorostral part of the BST very often affected the adjacent nucleus accumbens, which also contains a scattered population of ARO-ir cells. With this exception, lesions aimed at the given nucleus always affected this nucleus specifically.

Initial data reduction

Similar numbers of the CX and CX+T birds were run as controls from the three replicates of the experiments ($n = 6$–9 for each replicate). The behavior of these three subsets of subjects was first compared to test whether these data could be pooled in further analyses. Two-way ANOVAs with one independent variable (three subgroups of CX birds) and one repeated variable [17 behavioral tests, i.e., eight learning trials (tests 1–8) plus nine post-surgery tests (tests A–I)] indicated no overall group difference and no interaction between repeated testing and groups for the five behavior-dependent variables that were considered (time, look, entrances, MA, and CCM; $p > 0.05$ in each case). The same analysis performed to compare the three subgroups of CX+T birds similarly failed to detect overall group differences ($p > 0.05$ for the five behaviors) but indicated the presence of a significant interaction between subgroups and repeated testing for all behaviors except MA. Additional analyses were performed to identify the origin of these differences. One-way ANOVAs comparing the mean behavior of the three subgroups during the last two acquisition tests (tests 7 and 8) revealed no group difference ($p > 0.05$ for the five behaviors). Similarly, the same analysis on the mean behaviors exhibited during the experimental phase (mean of tests A through I) revealed no groups difference except in the case of LOOK, where larger behavioral scores in one of the three subgroups produced a significant group difference in the ANOVA ($F_{2,19} = 8.039$; $p = 0.003$). However, this difference occurred in only one of many analyses and concerned only one subgroup. Overall, it was concluded, therefore, that the interactions identified previously in the two-way ANOVA resulted from short-term random variations in the behavior of the three subgroups of CX+T birds, but that in general these three groups behaved in a similar manner. This was particularly the case at the end of the training period and during the experimental phase. Therefore, it was decided to pool data from the three CX and the three CX+T groups in all subsequent analyses, and from now on these groups are referred to as the CX and CX+T birds.

Qualitative and quantitative evaluation of lesions

As already indicated above, a substantial number of subjects were found at the end of the experiment to have lesions overlapping extensively with the POM ($n = 21$) or the rostral BST ($n = 9$). These lesions typically destroyed a significant part of the nucleus, as visible in a Nissl stain and more precisely in sections stained for aromatase. Photomicrographs of typical examples of these two types of lesions are shown in Figure 2.

It has been demonstrated in previous work on the role of the preoptic area in the control of reproduction that only bilateral lesions are able to produce significant deficits in male sexual behavior. Subjects bearing a unilateral lesion usually show little or no impairment (Numan, 1988; Yahr, 1995). Therefore, we first quantified the degree of symmetry of the electrolytical lesions that had been produced. Quantitative reconstructions indicated that the volume of lesions was very similar on both sides of the brain, for lesions targeting the POM (0.145 ± 0.013 vs 0.144 ± 0.008 mm^3; mean ± SEM; $t = 0.083$; df = 18; $p = 0.935$) as well as for those targeting the BST (0.164 ± 0.038 vs 0.161 ± 0.024 mm^3; mean ± SEM; $t = 0.079$; df = 8; $p = 0.939$). Because the total volume that would have been occupied by the POM in the absence of a lesion could almost always be reconstructed, it was possible to calculate in a large number of subjects the percentage of the nucleus that remained after the lesion. This percentage was also nearly identical on both sides of the brain (46.21 ± 5.96 vs $46.77 \pm 4.35\%$; mean ± SEM; $t = 0.144$; df = 15; $p = 0.887$), and on an individual basis the volume of a nucleus remaining on one side was highly correlated with the volume remaining on the other side ($r = 0.759$; $n = 16$; $p < 0.001$). This analysis could not be performed in a pertinent way for the BST because the lesion and nucleus volume could be reconstructed on both sides of the brain in only a limited number of subjects. Given the high degree of symmetry of all lesions, all subsequent analyses considered only the total volume (or size) of lesions without paying any attention to their very low degree of asymmetry.

The volume of lesions and of the ARO-ir cell groups (POM, rostral and caudal BST) could be reconstructed in most of the 73 experimental subjects in which the exact position of the lesions had been observed, although quantitative studies were occasion-

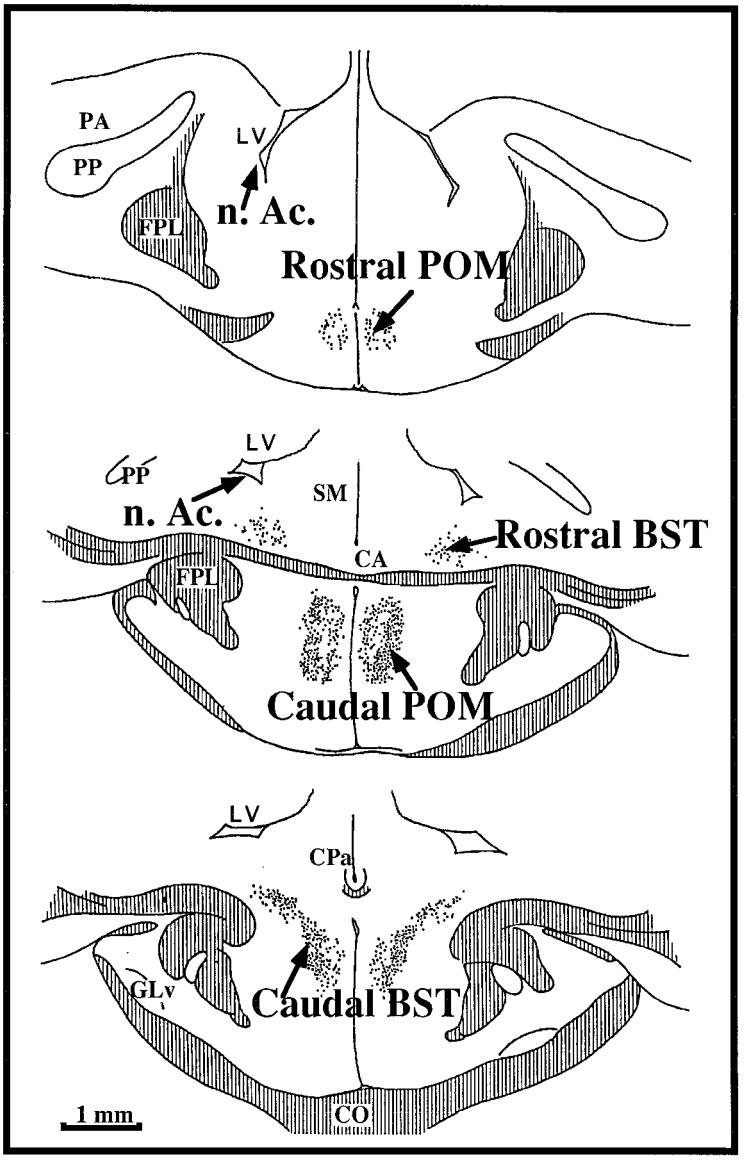

Figure 1. Schematic drawings of coronal sections through the quail brain illustrating the distribution of aromatase-immunoreactive cells in the medial preoptic nucleus (*POM*) and in the bed nucleus striae terminalis (*BST*)/nucleus accumbens (*n. Ac.*). Plates are arranged in a rostral to caudal order from the top to the bottom.

ally impossible to complete because of accidental loss of intermediate sections.

A one-way ANOVA comparing the total lesion volumes in the two experimental groups that had actually been lesioned (Fig 3*A*, *POM* and *BST*) indicated the presence of no significant difference ($F_{1,26} = 30.651$; $p = 0.427$): lesions in POM or in BST were overall very similar in size (Fig. 3*A*).

The volumes of the POM, rostral BST, and caudal BST, as measured based on the dense clusters of ARO-ir cells, are presented in Figure 3*B–D*. When a lesion actually destroyed a part of a given nucleus, a double column is presented indicating the remaining volume of the nucleus as well as the estimated total volume that would be occupied by the nucleus if the lesion were not present (volume extrapolated from the remaining part of the boundaries).

The remaining and total volume of the POM was significantly affected by the experimental treatments ($F_{3,51} = 63.378$, $p < 0.001$ and $F_{3,48} = 26.812$, $p < 0.001$, respectively). As indicated by Fisher's PLSD tests presented in Figure 3*B*, the POM volume was larger in CX+T than in CX males, and it was smaller in birds bearing a POM lesion than in CX+T or even in CX birds. This latter difference was present in the volume of the remaining POM (obviously decreased by the lesion) but also in the total extrapolated volume. Birds bearing a BST lesion had POM volumes that were intermediate between those observed in CX and in CX+T birds, and they did not differ statistically from these two groups.

In contrast, the one-way ANOVAs comparing the remaining or estimated total volumes of the other two ARO-ir cell groups (rostral and caudal BST) did not identify overall significant effects of the treatments ($p > 0.05$ in each of these four cases). This lack of overall significant effect makes it statistically invalid to conduct any further *post hoc* comparisons. However, a visual examination of Figure 3*C,D* suggests that the In BST groups are lower than some of the comparison groups, and it should be noted that Fisher's PLSD tests comparing all groups two by two suggested that the volume of remaining rostral BST was smaller in the In BST group than in the CX+T group, and the caudal BST was smaller in the In BST group than in the CX+T group.

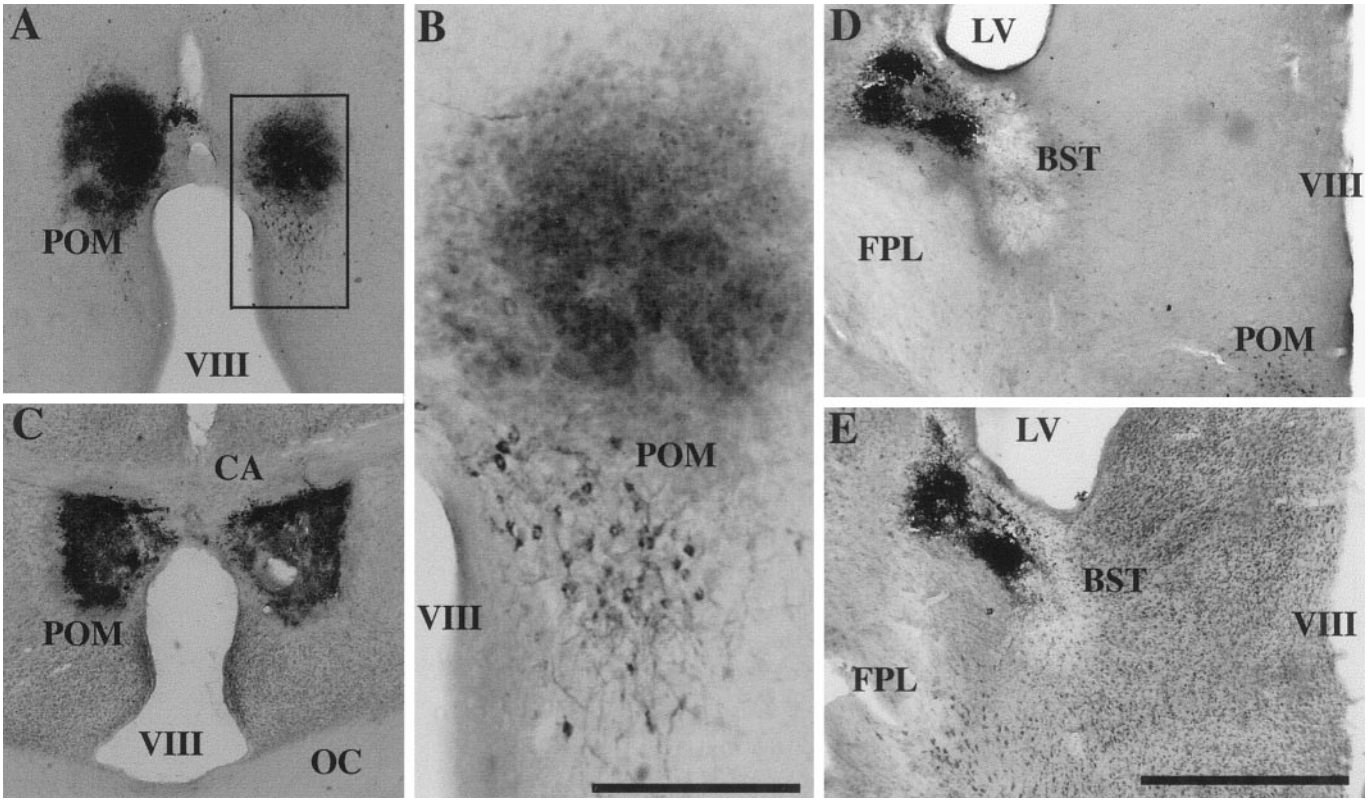

Figure 2. Photomicrographs illustrating the extent of a typical lesion of the POM (*A–C*) and of the rostral part of the BST (*D, E*) as seen in Nissl stain (*C, E*) and in sections stained by immunocytochemistry for aromatase (*A, B, D*). *A*, Lesion in the dorsal part of the rostral POM as illustrated in sections stained for aromatase. *B*, Higher magnification of the box in *A* showing the remaining aromatase-immunoreactive cells in the ventral part of the POM. *C*, Lesion in the caudal part of the POM at the level of the anterior commissure (*CA*) from a Nissl-stained section. *D*, Lesion of the rostral BST as seen in a section stained for aromatase. *E*, Adjacent section illustrating the same lesion after staining for Nissl material. This lesion of the BST partly destroys the group of aromatase-immunoreactive cells located dorsal to the commissure (BST proper) but also extends to a cell group just ventral to the lateral ventricles that is identified as the nucleus accumbens in the stereotaxic atlas of the chicken brain (Kuenzel and Masson, 1988). Scale bar (shown in *E*): *A, C, D, E*, 1 mm; *B*, 250 μm. *FPL*, Fasciculus prosencephali lateralis (lateral forebrain bundle); *LV*, lateral ventricle; *VIII*, third ventricle.

Effect of POM and BST lesions on appetitive and consummatory sexual behavior

Data illustrating the acquisition and maintenance of the response indicative of appetitive sexual behavior (time at window) and of the frequency of consummatory sexual behavior (cloacal contact movements) after stereotaxic surgery are presented in Figure 4 for the four experimental groups (In POM, In BST, CX+T, CX).

The analysis by two-way ANOVA (independent variable = four experimental groups; repeated variable = 17 behavior tests) of these data indicated for the five behavioral dependent variables (Time, Look, Entrances, MA, and CCM) the presence of significant group differences, of significant changes between the different tests, and of significant interactions between the two factors ($p < 0.001$ for each of these 15 F ratios, i.e., three tests for five behaviors). The same analyses were then repeated on data accumulated separately during the eight acquisition trials and during the nine postoperative tests to identify better the origin of these experimental effects.

During the eight acquisition trials (Fig. 4, *Pretests*), the two-way ANOVAs indicated the existence of significant group difference and effects of repeated testing for all variables ($p < 0.001$ for each of the five dependent variables). A significant interaction between groups and repeated testing ($p \leq 0.05$) was also observed for two measures of appetitive (Time, Look) and one measure of consummatory (CCM) sexual behavior. The group differences and interactions occurred primarily because of the comparison of the CX birds with the three other experimental groups that were all treated with testosterone. When the same analyses were repeated after exclusion of the CX group, all the group differences disappeared in the ANOVA ($p > 0.10$), and all interactions of groups by repeated testing became nonsignificant ($p > 0.10$ in each case). Significant effects of the repeated testing conducted during the acquisition phase were still observed for the three measures of appetitive sexual behavior (time, look, and entrances; $p < 0.0001$ in each case). The frequencies of MA and CCM also significantly changed during the acquisition period ($F_{7,343} = 3.244$, $p = 0.002$, and $F_{7,343} = 3.683$, $p < 0.001$, respectively) because the effects of the testosterone treatment were not yet fully established and birds were still developing their copulatory skills. Overall, however, it can be concluded that the three groups of testosterone-treated birds (In POM, In BST, and CX+T) behaved in a very similar manner during the acquisition phase (Pretests) as illustrated in Figure 4 for Time at Window and Cloacal Contact Movements.

During the experimental postoperative phase (Fig. 4, Tests), two-way ANOVAs comparing the four groups (independent variable) across the nine behavior tests (repeated variable labeled by letters *A–I* in Fig. 4) demonstrated the presence of significant group differences for each dependent variable ($F_{3,69} > 14.9$; $p < 0.001$ in each case). Significant changes in behavior as a function of repeated testing was also observed for two of the dependent variables (Look, $F_{8,552} = 6.793$, $p < 0.001$; MA, $F_{8,552} = 2.699$,

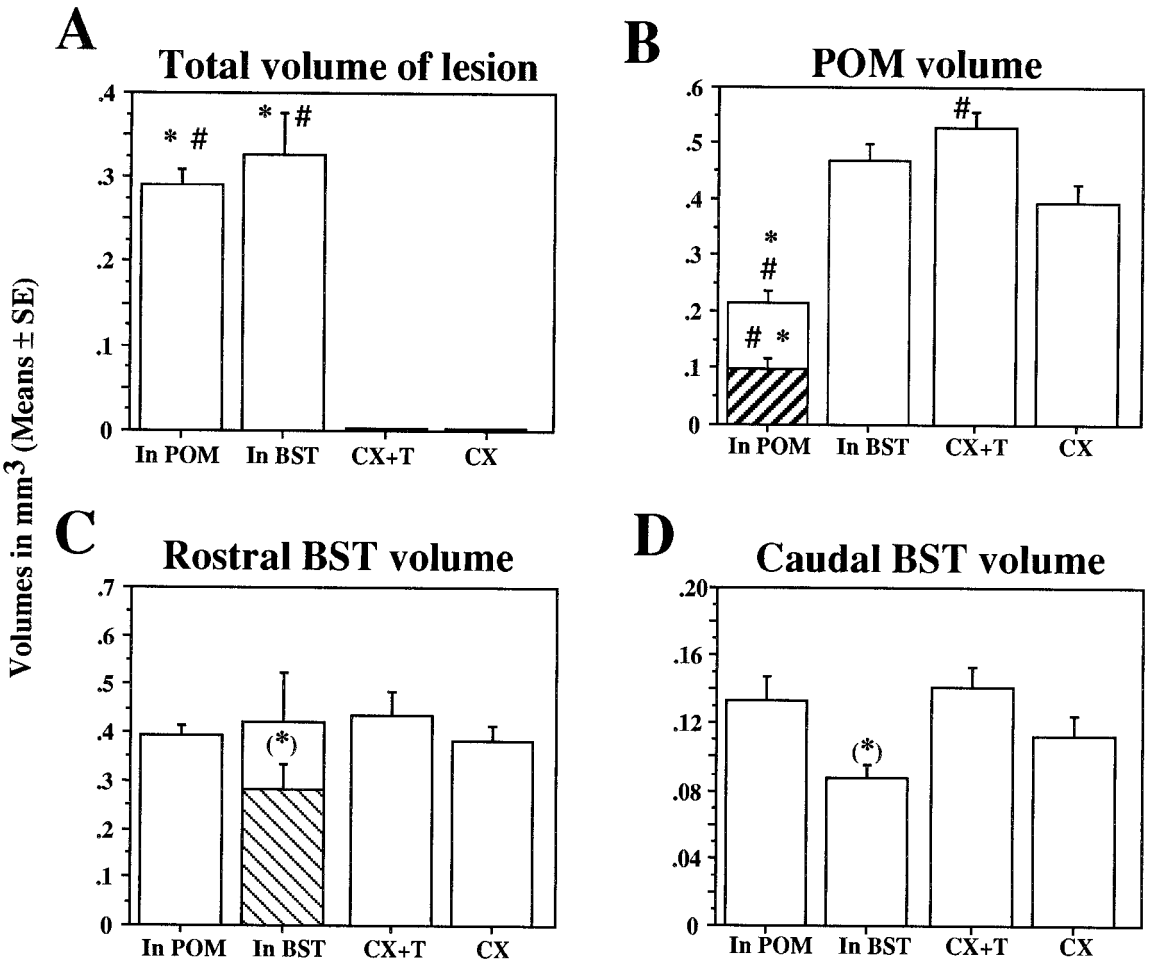

Figure 3. Reconstruction of the volumes (means and SEM) of the electrolytic lesions (*A*) and of the volumes of the POM (*B*), rostral BST (*C*), and caudal BST (*D*) in the four experimental groups. When the lesion actually destroyed a part of a given nucleus (*POM* in the *In POM* group and *Rostral BST* in the *In BST* group), the corresponding *bar* has been divided into a *hatched bar* that indicates the volume of the nucleus remaining after lesion and an *open bar* that indicates the total extrapolated volume that would be occupied by the nucleus if no lesion were present. Experimental groups were compared two by two by Fisher's PLSD tests whose results are indicated at the *top* of the bars as follows: $^{*}p < 0.05$ by comparison with the CX+T group and $\#p < 0.05$ by comparison with the CX group. *Parentheses* around a symbol indicate that the corresponding general ANOVA comparing the four groups did not detect a significant effect, so that results of Fisher's PLSD tests can only be considered as indicative (see Results for more detail).

$p = 0.006$) but not for Time at Window ($F_{8,552} = 1.818$, $p = 0.071$), Entrances ($F_{8,552} = 0.890$, $p = 0.525$) or Clocal Contact Movements ($F_{8,552} = 1.508$, $p = 0.151$). Interactions between groups and repeated testing were present for Look ($F_{24,552} = 1.542$, $p = 0.049$) and Mount Attempts ($F_{24,552} = 1.801$, $p = 0.012$) but not for the three other dependent variables (Time, $F_{24,552} = 1.173$, $p = 0.260$; Entrances: $F_{24,552} = 1.448$, $p = 0.078$; Cloacal Contact Movements: $F_{24,552} = 0.706$, $p = 0.847$).

The origins of the group differences were then investigated by comparing the behaviors of the five experimental groups, two by two, by means of Fisher's PLSD tests. To facilitate the presentation, these comparisons of groups are illustrated in Figure 5, which represents the mean behavioral activity (time in seconds or behavior frequency) observed across the nine postoperative tests.

Significant overall effects of the experimental treatments were detected for each of the behaviors that were considered ($p < 0.001$ for the ANOVA comparing the four experimental groups; see details concerning the results in Fig. 5). Fisher's PLSD tests comparing all groups two by two indicated that lesions of the POM almost completely abolished the two consummatory sexual behaviors (frequencies of mount attempts and cloacal contact movements were significantly lower in the In POM than in the CX+T groups and are not different from these frequencies in the CX group). These same lesions also significantly decreased the measures of appetitive sexual behavior (time at window and looking through window) in comparison with the CX+T group, but the inhibition was only partial in this case (20–30% decrease) so that behavioral scores were still significantly higher than in the CX group. Lesions destroying a part of the BST significantly decreased, but did not abolish, measures of consummatory sexual behavior (MA and CCM frequencies lower than in CX+T group but higher than in the CX group), but these same lesions had absolutely no effect on appetitive sexual behavior. However, the number of entrances in the test area in front of the window was strongly decreased by both types of lesions (for detail of statistical comparisons, see Fig. 5). It should also be pointed out that the behavioral deficits produced by the lesion became apparent immediately after the stereotaxic surgery, and there was no apparent recovery during the duration of the experiment (Fig. 4). The significant effects of repeated testing detected in the general ANOVA of the data (see above) therefore appear to reflect

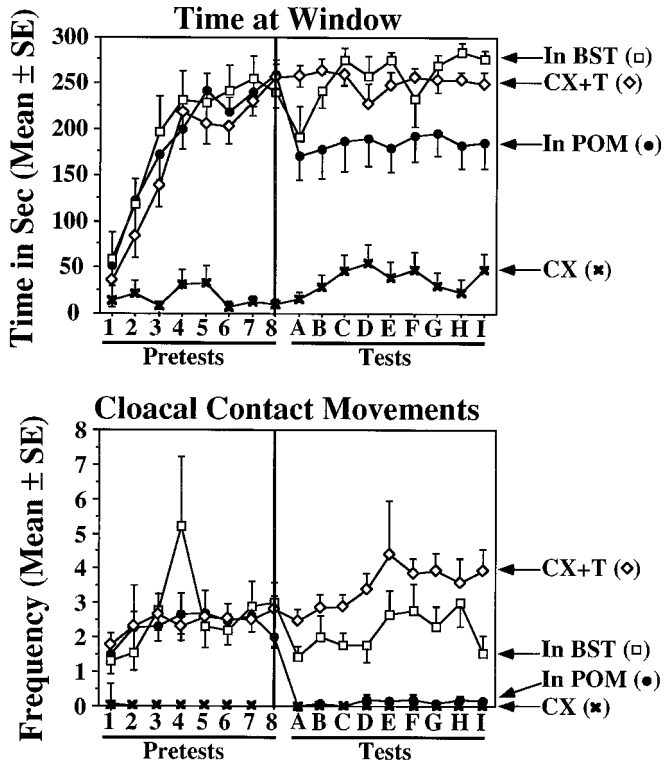

Figure 4. Effects of lesions of the POM or BST for one of the measures of appetitive (*Time at Window*) and for one of the measures of consummatory (*Cloacal Contact Movements*) male sexual behavior in castrated male quail treated with exogenous testosterone. Data for CX birds not treated with testosterone are also illustrated. The data shown (means ± SEs) represent the acquisition of behaviors during the eight preoperative tests (*Pretests*) and then the effects of the experimental manipulations observed during the nine postoperative tests (*Tests*) in the four experimental groups.

random fluctuations in behavior rather than a progressive decline or recovery after lesion.

Dissociation between lesions to different subregions of the POM and appetitive and consummatory sexual behavior

The results described above indicated a first level of dissociation between the two components of sexual behavior measured in this study. On the one hand, lesions of the POM almost completely abolished our measures of consummatory sexual behavior, but they only partly suppressed our measures of appetitive aspects of this behavior. On the other hand, lesions of the BST significantly decreased the frequency of consummatory sexual behaviors (MA and CCM), but they had absolutely no effect on the appetitive component. A closer inspection of the experimental results suggested additional levels of dissociation.

A qualitative analysis of the individual results indicated that within the group of subjects that were bearing a lesion affecting the POM, behavioral deficits independently affected the appetitive and consummatory aspects of male sexual behavior. This notion is illustrated in Figure 6, which presents the individual correlation between the measures of these two components of the behavior in the CX+T and In POM groups. As can be readily observed, all birds of the In POM group had substantial deficits in copulatory behavior by comparison with those of the CX+T group (MA and CCM frequencies are zero or very low compared with scores of nonlesioned birds). By contrast, there was a very broad range of scores for the two measures of appetitive sexual behavior, and the limited decrease that was observed in the mean values (Figs. 4, 5) resulted from the averaging of scores that differed widely. In some cases individuals with POM lesions were not affected at all, whereas in other cases a nearly complete inhibition was observed. We therefore decided to investigate the origin of this marked variation in the effects of POM lesions on appetitive sexual behavior with the goal being the identification of a brain area that would be specifically related to this behavioral deficit.

A first qualitative attempt to subdivide the lesions according to whether they were primarily affecting the rostral versus caudal or the lateral versus medial POM did not provide meaningful insight into this question. We therefore decided to assess the extent and location of these lesions in a more sophisticated manner. Four equally spaced levels in the rostrocaudal axis extension of the POM were selected for analysis. The most caudal of these levels corresponds to the caudal end of the nucleus located under the anterior commissure; the most rostral level is ~600 μm more rostral (~200 μm between each level). At each of these levels, each side of the POM was subdivided into four quadrants (dorsomedial, dorsolateral, ventrolateral, and ventromedial), and in each of these quadrants the extension of the lesion was scored on a five-point ordinal scale ranging from 0 (quadrant totally destroyed by the lesions) to 4 (quadrant intact, no lesion hitting this part of the nucleus). Intermediate values (1–3) corresponded to decreasing lesions, so that ~25, 50, and 75% of this part of the nucleus was still intact. Eight of these scores were therefore collected at each of the four rostrocaudal levels of the POM [four on the left side (*a–d*) and four on the right side (*e–h*), always in the order dorsomedial, dorsolateral, ventrolateral, and ventromedial]. In the end, the overall lesion of the nucleus could be described by a series of 32 scores (four rostrocaudal levels × two sides × four quadrants) on a 5-point scale. The average postoperative behavioral scores [values that generated the means plotted in Fig. 5 (Time at Window, Looking through Window, Entrances in Test Area, Mount Attempts, and Cloacal Contact Movements)] were added to this data table to form a matrix of 37 columns (32 lesions scores plus five behavioral measures) by 16 rows (individuals that had a lesion in POM that could be assessed entirely by the method described above). A correlation analysis produced a 37 by 37 correlation matrix, and this entire set of results was submitted to factor analysis by the principal component method (Statview 4.0 program, Abacus Concepts, Calabasus, CA) to summarize the major relationships between these different variables (Fruchter, 1954).

The factor analysis extracted six meaningful factors that had an eigenvalue superior to 1. However, the magnitude of these eigenvalues decreased rapidly so that the first three factors alone already explained >74% of the total variance. Therefore only these three factors are considered in the following data presentation. The unrotated factor loadings are plotted in a three-dimensional graph in Figure 7*A* and as two combinations of these three factors/axes (Level 1 vs Level 2 and Level 1 vs Level 3) in Figure 7*B*,*C*. These plots all identified four discrete groups of variables that were found to correspond precisely to the lesion scores obtained at the four rostrocaudal levels. The two measures of consummatory sexual behavior (MA and CCM) were found to be closely associated with lesion scores for Level 3, whereas the measures of appetitive sexual behavior (TIME, LOOK, and

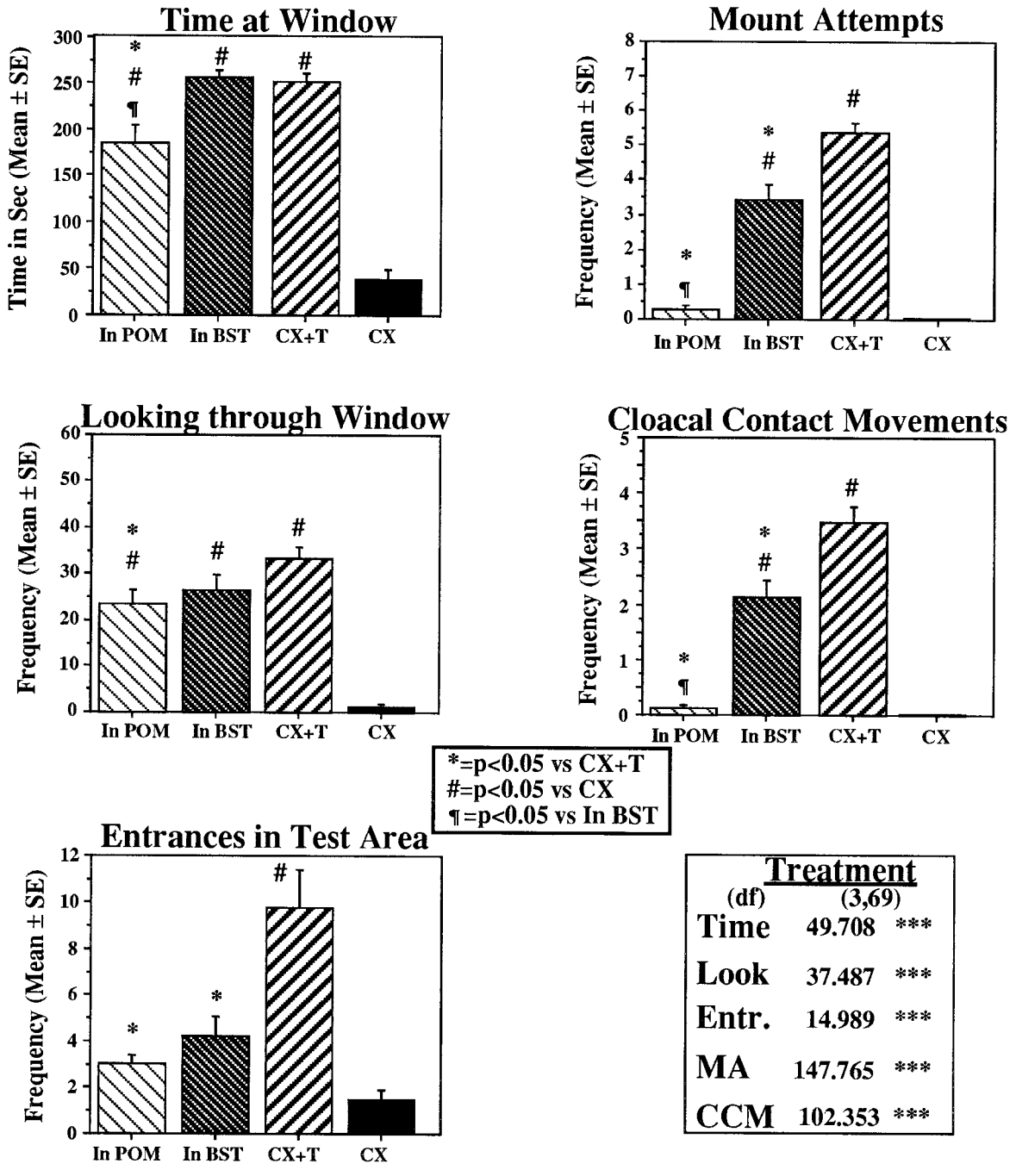

Figure 5. Means of the behavioral scores for all the behavioral measures taken for both appetitive and consummatory male sexual behavior observed in the four experimental groups during the postoperative phase of the experiment. Data presented are the means ± SEs of the mean of the behavioral frequencies or of time spent in front of the window during the nine separate tests. For each dependent variable, results corresponding to the four experimental groups were compared by a one-way ANOVA, and these results are summarized in the bottom right panel (*F* values, df, and associated probabilities; $^{***}p < 0.001$). Experimental groups were then compared two by two by Fisher's PLSD tests whose results are indicated at the *top* of the bars.

ENTR) were located in a position intermediate between lesion scores at levels 2 and 3.

This analysis therefore suggested a differential relationship between lesion scores and measures of ASB and CSB. An additional characterization of these relationships was therefore performed. Birds of the In POM group were subdivided into three subgroups based on the behavioral effects of their lesion, namely a group that had retained a weak consummatory sexual behavior and an apparently normal appetitive sexual behavior (ASB+/CSB+; $n = 5$), birds experiencing a complete suppression of consummatory sexual behavior (postoperative CCM frequency equal to zero) but with an apparently normal appetitive sexual behavior (ASB+/CSB−; $n = 7$), and finally birds with a complete suppression of consummatory sexual behavior (postoperative CCM frequency equal to zero) and an inhibited appetitive sexual behavior (i.e., Looking through Window frequency below 15;

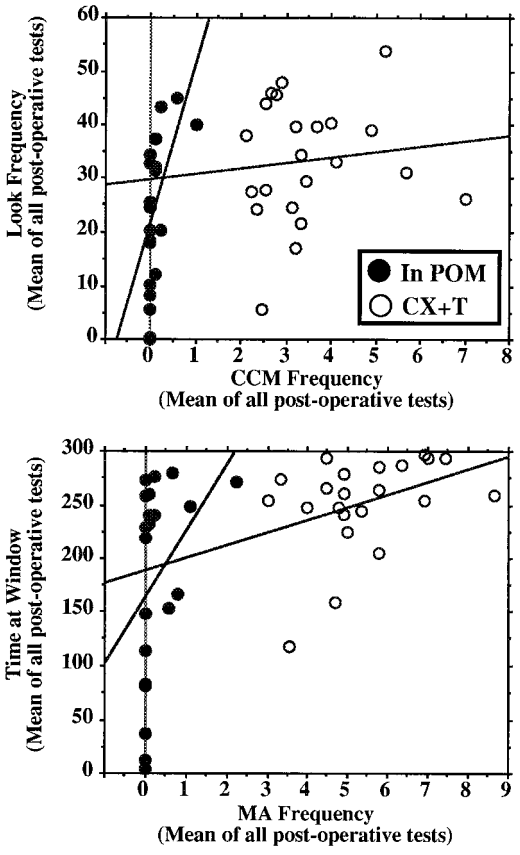

Figure 6. Relations between the two measures of appetitive sexual behavior and the two measures of consummatory sexual behavior in birds bearing a lesion of the POM and in their control group (CX+T). Correlation coefficients associated with the four regression lines indicated in the figure are not significant ($p > 0.05$) except for *CCM Frequency* versus *Look Frequency* in the *In POM* group ($r = 0.536$; $p = 0.012$). These data clearly illustrate the nearly complete inhibition of consummatory sexual behavior but the quite variable inhibition of appetitive sexual behavior in the lesioned group.

ASB+/CSB−; $n = 4$). As would be expected on the basis of these definitions, all aspects of the ASB and CSB in these three subgroups of subjects were significantly different (Time, $F_{2,13} = 16.843, p < 0.001$; Looking through Window, $F_{2,13} = 17.693, p < 0.001$; Mount Attempts, $F_{2,13} = 7.916, p = 0.006$; Cloacal Contact Movements, $F_{2,13} = 10.013, p = 0.002$) except for the Entrances in Test Area (Number, $F_{2,13} = 0.071, p = 0.932$). Comparisons of groups two by two by Fisher's PLSD tests also indicated that a group with an inhibited component of behavior always significantly differed from the corresponding group(s) in which this component was not inhibited (for details of these two by two comparisons, see Fig. 8*A*).

The mean lesion scores in these three subgroups of birds were first computed for the 32 subdivisions of the POM that had been analyzed separately. These scores were then further averaged on the basis of the eight subdivisions that were present at each of the four different rostrocaudal levels. This was valid because at a given rostrocaudal level, the analysis indicated no obvious relationship between the position of the lesion in a specific subdivision and behavior (in general, the eight subdivisions are similarly affected at a given level; data not shown). The data based on these analyses are displayed in Figure 8*B*. Birds that had maintained an apparently normal appetitive sexual behavior and exhibited weak consummatory behavior (ASB+/CSB+) possessed a lesion that destroyed primarily the rostral part of the POM (low scores at levels 1 and 2). By contrast, birds in which CSB had been suppressed completely (ASB+/CSB− and ASB−/CSB−) had an extensive lesion of the level 3 in POM. These last two groups, however, were clearly separable, and it appeared that the inhibition of ASB (group ASB−/CSB−) was specifically associated with a large lesion at level 2 that was not observed in the other two groups [(Fig. 8*B*) compare lesion score for level 2 in the ASB+/CSB− with ASB−/CSB−].

These differences between subgroups were confirmed statistically. A general ANOVA comparing the four levels in the three subgroups indicated no overall difference between the groups ($F_{2,13} = 2.654$, $p = 0.108$) but indicated significant differences between levels ($F_{3,39} = 5.171$, $p = 0.004$) and significant interactions between levels and subgroups ($F_{6,39} = 5.122$, $p < 0.001$). Furthermore, separate ANOVAs comparing the three subgroups at each level separately indicated significant or nearly significant differences in lesions between the three groups at each level (level 1, $F_{2,13} = 3.726, p = 0.053$; level 2, $F_{2,13} = 3.042, p = 0.082$; level 3, $F_{2,13} = 7.238$, $p = 0.008$; level 4, $F_{2,13} = 4.078$, $p = 0.054$). Comparisons of the subgroups two by two at each rostrocaudal level by Fisher's tests are summarized in Figure 8*B*. The specific inhibition of consummatory sexual behavior (compare ASB+/CSB+ with ASB+/CSB−) was associated with significantly larger lesions at levels 3 and 4 (and smaller lesions at level 1). Only one significant difference was observed between the ASB+/CSB− and ASB−/CSB− groups: birds in the latter category had significantly larger lesions at level 2.

Some additional analyses were also performed to investigate whether the deficits in consummatory sexual behavior that were induced by lesions in the BST were associated with a specific rostrocaudal position of these lesions. The smaller number of available subjects and more diffuse nature of the BST (ARO-ir cells form a dense cluster at the caudal level but are more scattered at the rostral pole of the nucleus) prevented us from establishing any clear-cut anatomical relationships.

Tests in the absence of the female

It was noted during behavioral observations that some of the birds bearing lesions in the POM entered the test area in front of the window less frequently during the post-lesion tests, and this effect of the lesion was confirmed in the analysis described above. Because this behavioral effect was not associated in all subjects with a decrease in the time spent in front of and looking through the window, we wanted to investigate whether the social proximity response displayed by these lesioned subjects still reflected appetitive sexual behavior (i.e., the animals were still sensitive to the stimulus they were viewing through the window) or perhaps reflected only the stereotyped repetition of a learned response. Alternatively, it was also possible that the decrease in the number of times that birds entered the test area reflected a general nonspecific effect on the mobility of the subjects.

In an attempt to discriminate between these possibilities, birds from replicates 2 and 3 of this experiment (In POM, $n = 8$; In BST, $n = 9$; CX+T, $n = 16$; CX, $n = 14$) were submitted after the ninth postoperative test (Fig. 4, test *I*) to an additional test performed under the exact same conditions, except that no female was placed in the small lateral cage of the two-compartment chamber. The appetitive sexual behaviors (Time at Window, Looking through Window, and Entrances in Test Area) displayed during these tests without female and during the last test per-

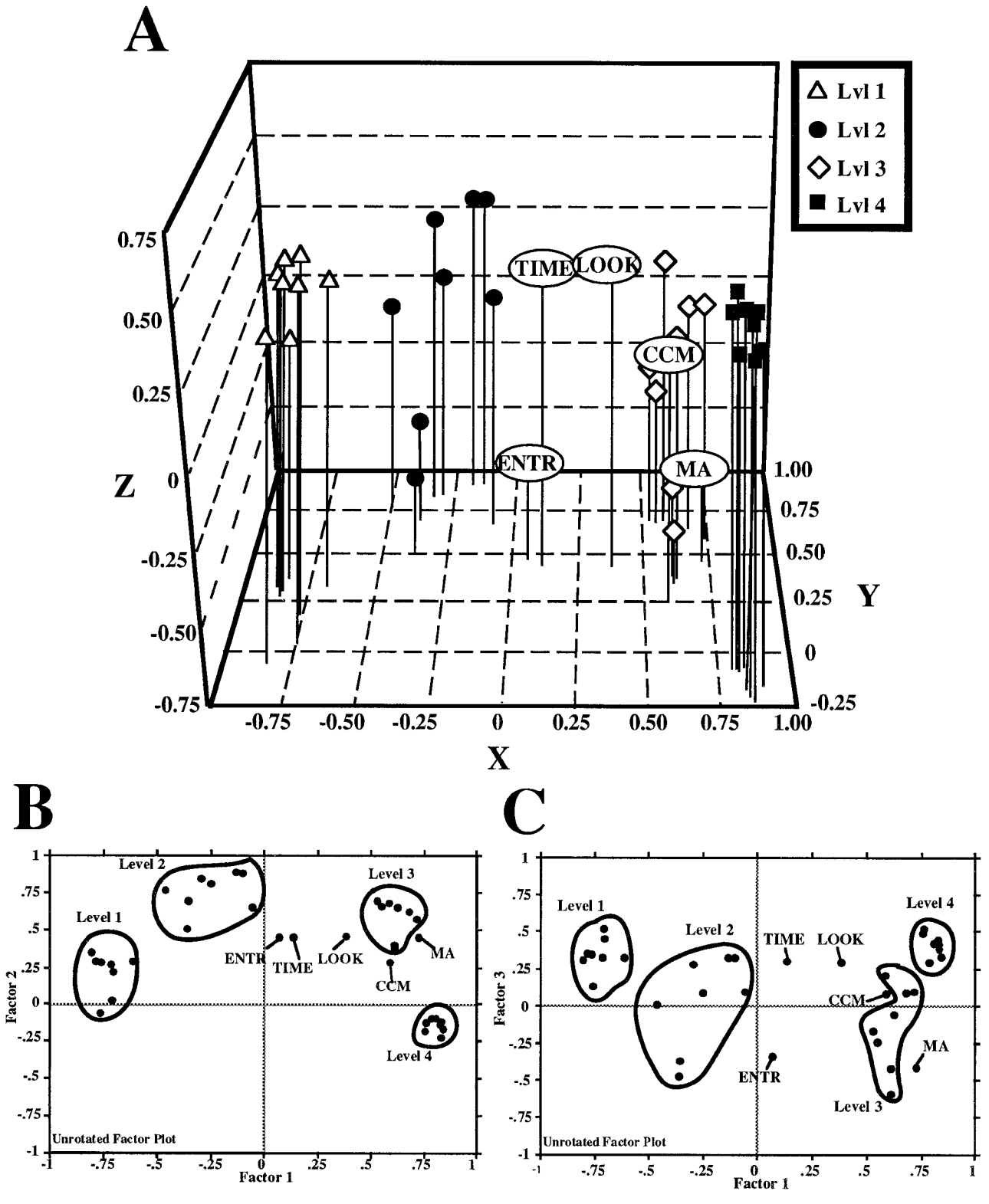

Figure 7. Factor analysis of the dissociation between appetitive and consummatory aspects of male sexual behavior in birds bearing a POM lesion and of the relationship of the behavioral deficit to the lesion location within POM. *A*, Results of the factor analysis of the correlations between behavioral measures and the associated scores of the POM lesion. The figure presents a three-dimensional plot of the factor loadings relative to the first three factors extracted by the analysis that explain 74% of the total variance present in the data. Four clusterings of scores associated with the lesion scores at four different levels are apparent. The position of the behavioral scores for the measures of appetitive and consummatory male sexual behavior is also plotted. *B, C*, Projection in two different planes defined by vectors 1 and 2 or 1 and 3 of the factor loadings for the different variables. In both cases, four clusters of data points representing the lesion scores at the four different rostrocaudal levels are clearly present. The two measures of consummatory sexual behavior are clearly associated with lesion scores at *Level 3*, whereas the measures of appetitive sexual behavior (*TIME, LOOK*, and *ENTR*) are located in a position intermediate between lesion scores at levels 2 and 3.

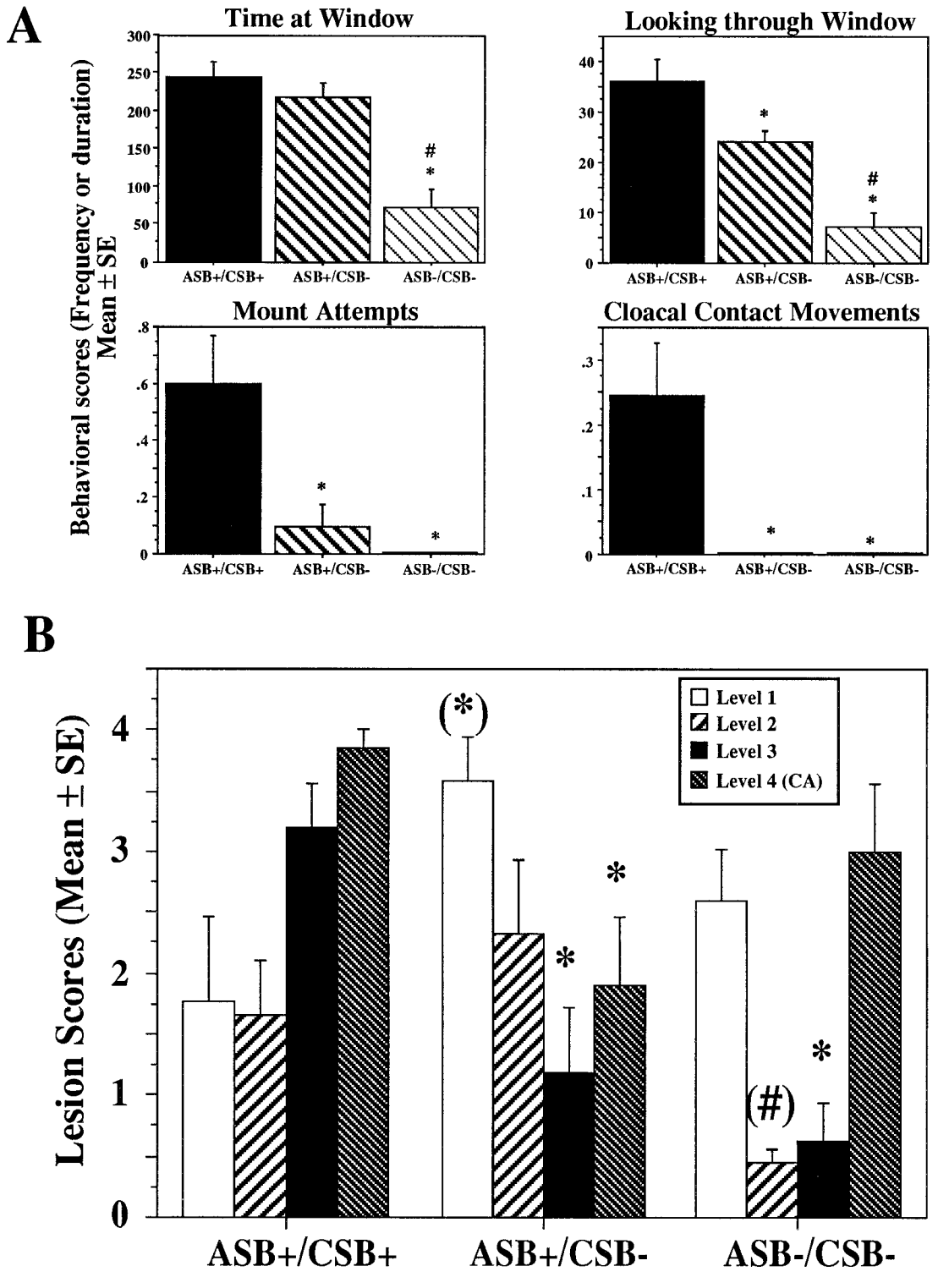

Figure 8. *A*, Mean ± SE of the behavioral scores (frequencies of behavior or time at window) computed for three subgroups of birds bearing a POM lesion (In POM group) defined by the presence of appetitive sexual behavior and a low level of consummatory sexual behavior (*ASB+/CSB+*), by the presence of appetitive sexual behavior and the complete absence of consummatory sexual behavior (*ASB+/CSB−*), or by the strong inhibition of appetitive sexual behavior and the complete absence of consummatory sexual behavior (*ASB−/CSB−*). Data were analyzed by one-way ANOVA (see Results) followed by Fisher's PLSD tests comparing groups two by two. The results of the statistical analyses are illustrated at the *top* of the bars as follows: $^{*}p < 0.05$ by comparison with ASB+/CSB+ subgroup and $\#p < 0.05$ by comparison with ASB+/CSB− subgroup. *B*, Means of the lesions scores observed in eight quadrants of the POM (four quadrants on each side of the POM) at the four rostrocaudal levels in the three subgroups of birds defined by their behavior. Data were analyzed by a separate one-way ANOVA for each rostrocaudal level (see Results) followed by Fisher's PLSD tests comparing groups two by two. The results are shown at the *top* of the bars as follows: $^{*}p < 0.05$ by comparison with ASB+/CSB+ subgroup and $\#p < 0.05$ by comparison with ASB+/CSB− subgroup. *Parentheses* around a symbol indicate that the corresponding general ANOVA comparing the three subgroups did not detect a significant effect, so that results of the Fisher PLSD tests can only be considered as indicative.

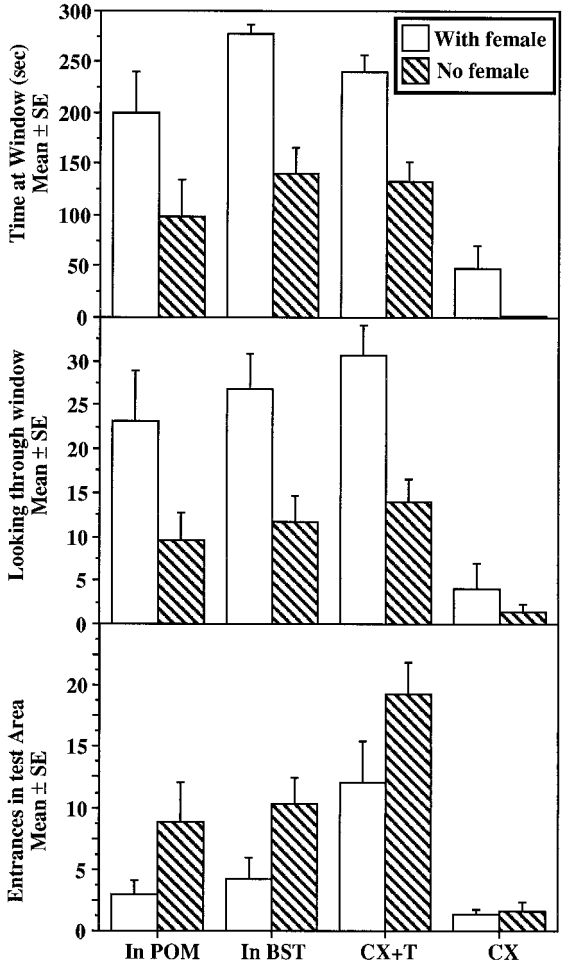

Figure 9. Comparison of the behavioral responses observed in castrated birds treated or not treated with testosterone and in birds with a lesion in POM or BST when tested for appetitive sexual behavior after the completion of the nine postoperative tests. In this case, the cage adjacent to the main chamber either contains or does not contain a stimulus female. In all groups, in the absence of the females the time spent in front of the window and the frequency of looks through the window decreased. At the same time, there was an increase in the number of times that birds entered the test area in front of the window relative to tests when the female was present.

formed in the presence of a female (test I) are summarized in Figure 9.

These data were analyzed by two-way ANOVAs with one independent (four groups of birds) and one repeated (tests with and without female) variable. Highly significant group differences were observed in the three responses (Time, $F_{3,43} = 22.501$, $p < 0.001$; Look, $F_{3,43} = 12.793$, $p < 0.001$; Number, $F_{3,43} = 10.740$, $p < 0.001$), therefore confirming, in a subset of the experimental subjects, the effects of the experimental treatments on these behaviors. A significant effect of the test repetition (comparison with and without female) was also detected on each response (Time, $F_{1,43} = 75.389$, $p < 0.001$; Look, $F_{1,43} = 37.538$, $p < 0.001$; Number, $F_{1,43} = 17.155$, $p < 0.001$). A significant interaction between the experimental groups and the test situation was also observed in the analysis of two of the three behaviors (Time, $F_{3,43} = 3.084$, $p = 0.037$; Look, $F_{3,43} = 3.425$, $p = 0.025$; Number, $F_{3,43} = 2.239$, $p = 0.097$), but overall it was clear that Time and Look were decreased in all groups in the absence of the female. These results support the idea that all birds, including those bearing a POM lesion, were attracted in the test area by the stimulus female. Consistent with this interpretation, the number of times that birds entered the test area increased in all groups in the absence of the female as if birds were actively searching for the sexual stimulus.

Effects of POM lesions on rhythmic cloacal sphincter muscle movements

Tests performed in the two-compartment chamber demonstrated that lesions of the POM slightly but significantly decrease several measures of the learned social proximity response (Time, Look, Number) used to assess appetitive male sexual behavior. Because this response includes a learned component, the behavioral deficit induced by the lesion could be interpreted as an indication of a decrease in appetitive sexual behavior but also as a loss of the learned component of the response. To provide an experimental test allowing us to discriminate between these alternatives, birds from the third replicate (CX, CX+T, and In POM groups) were submitted to an additional test that quantifies appetitive sexual behavior in male quail, the RCSMs. We therefore measured in the experimental subjects the frequency and number of bouts (rapid sequences of muscle contractions separated by interruptions of at least 1 sec) of RCSMs during the 2.5 min preceding and immediately after the visual access to a female in standardized conditions. The latency between the beginning of each experimental period and the first RCSM was also recorded. A latency of 150 sec was recorded if no RCSM was observed during one test. These data are summarized in Figure 10.

These data (number of RCSM, number of bouts, and latency) were first analyzed by two-way ANOVAs with one independent variable (three experimental groups) and one repeated variable (before vs after visual presentation of the female, i.e., pretest vs test). These analyses revealed for each dependent variable (number of RCSM, number of bouts, and latency) significant group differences, effects of the presence of the female (in Fig. 10 pretest refers to no female being present and test refers to the female being present), and effects of the interaction between groups and test condition ($p < 0.001$ in each case except for the interaction groups by test condition in the analysis of latencies where $p = 0.002$). The nature of these effects is obvious in Figure 10. Testosterone treatment increased the number of RCSMs and RCSM bouts and decreased the latency to the first movement, and this effect was strongly inhibited by the POM lesion. In addition these behaviors were stimulated by the view of a female but more so in the CX+T than in the other groups. Fisher's PLSD tests comparing the overall scores of the three experimental groups indicated for each dependent variable a significant difference between the CX and CX+T groups as well as between the CX+T and In POM group. The analysis of the latencies also indicated a significant difference between the In POM and the CX groups. Separate Fisher's tests were additionally performed to compare specifically the behavior of the three groups of birds in the presence and absence of the female. These results are noted by symbols at the top of the bar graphs in Figure 8. In general, testosterone increased and lesions in the POM decreased RCSM in both the presence and absence of the female.

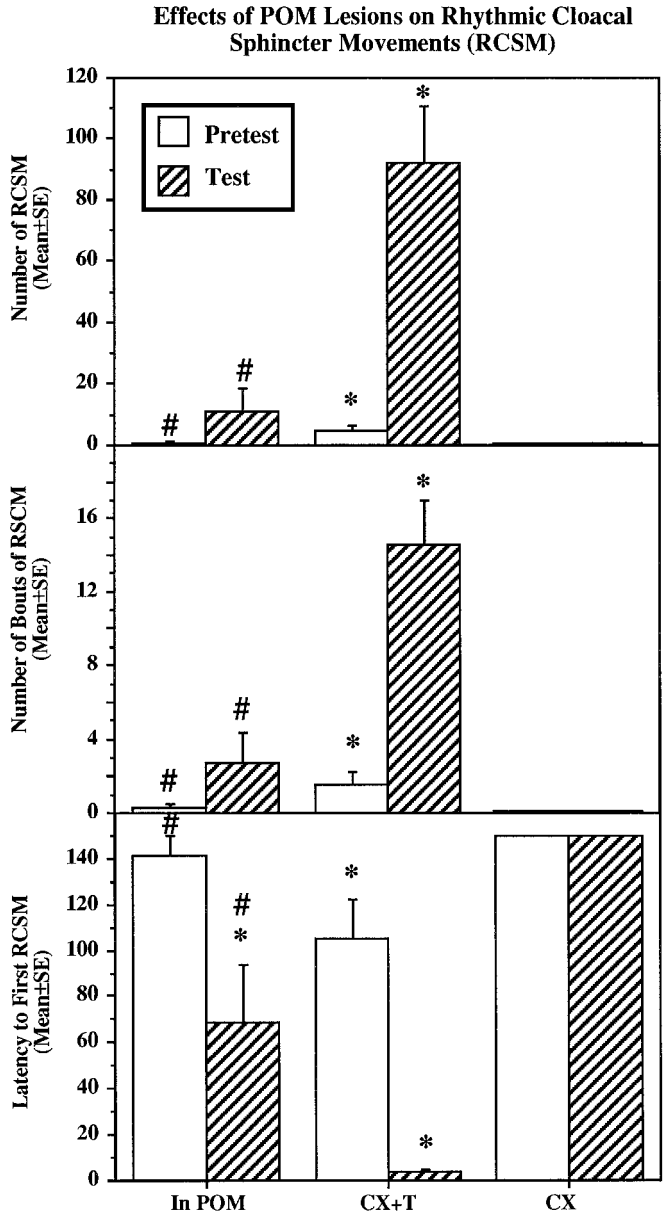

Figure 10. Effects of testosterone treatment and of a lesion in the POM on the frequency of rhythmic cloacal sphincter muscles movements (*RCSM*), on the number of bouts of RCSM, and on the latency to initiate RCSM in the absence (*Pretest*) or presence (*Test*) of a stimulus female. Fisher's PLSD tests were performed to compare the behavior of the three groups of birds in the absence (*Pretest*) and presence (*Test*) of the female. The results are reported by *symbols* at the *top* of the corresponding bar in the graph (*$p < 0.05$ by comparison with the CX group; #$p < 0.05$ by comparison with the CX+T group).

DISCUSSION

Lesions to different subregions of the POM revealed a dissociation in the neural sites regulating appetitive and consummatory components of sexual behavior in male quail. In particular, damage to a portion of the POM just rostral to the anterior commissure resulted in the complete disappearance of CSB, whereas damage to parts of the POM just rostral to this quadrant selectively impaired ASB. In contrast, lesions to the rostral BST had no effect on appetitive behavioral measures, but such lesions moderately decreased consummatory sexual behaviors. These data indicate that the preoptic region is involved in the regulation of both components of male sexual behavior.

Lesions to the POM inhibit both appetitive and consummatory male sexual behavior

The mPOA is important for the activation of male sexual behavior in every vertebrate species in which it has has been investigated (Meisel and Sachs, 1994; Yahr, 1995). In quail, a sexually dimorphic nucleus in the preoptic region, the POM, is necessary, and testosterone action in this nucleus is sufficient for the activation of copulatory behavior in adulthood (Panzica et al., 1996). In the present study, a factor analysis of the behavioral effectiveness of lesions sites within POM indicated that damage to the subdivision of the POM just rostral to the anterior commissure was the most effective in inhibiting copulatory behavior. This observation agrees with a previous study also suggesting that damage to a similar subregion of the POM was the most efficacious in inhibiting copulatory behavior (Balthazart et al., 1992). Testosterone implants in the POM are most effective in stimulating copulatory behavior when located in the same subregion of the nucleus (Balthazart et al., 1992). This subregion of the POM is also the site of a sex difference in a number of ARO-ir cells (Balthazart et al., 1996). These data suggest that the lesions of this subregion are effective because they destroy particular cells (containing aromatase or not) within the POM rather than because of the disruption of fibers of passage, although this needs to be tested explicitly. Lesion studies in rats also indicate that cells intrinsic to the mPOA are essential for the occurrence of male sexual behavior (Liu et al., 1997). It is not known whether there is a similar specificity in the effectiveness of preoptic region lesions in mammalian species, but in hamsters, damage to caudal portions of the mPOA are most effective in blocking copulatory behavior (Powers et al., 1987).

Lesions to the POM attenuated appetitive measures of sexual behavior including the learned social proximity response and the RCSM, an unlearned response that occurs in males just before copulation. These marked effects of POM lesions on our measures of ASB are at variance with some studies on rodents suggesting that the preoptic region is preferentially involved in the activation of copulatory performance and plays little or no role in appetitive aspects of male sexual behavior (Everitt, 1995; Liu et al., 1997). Cells within the second and third rostrocaudal subdivisions of the POM seem to be particularly important for the control of appetitive aspects of quail male sexual behavior. Chemical neuroanatomical studies have revealed no obvious reason why this cell group would be preferentially important in the control of ASB (Panzica et al., 1996).

Lesions to the BST only impair measures of consummatory aspects of male sexual behavior

Lesions to the rostral BST did not decrease most measures of ASB (Time and Look), but the number of times a male would enter and leave the test area did decrease. However, when these BST-lesioned males were tested under stimulus conditions with the female not present in the adjacent chamber, they *increased* the number of times they entered the area providing visual access. The sham-lesioned controls also did this, indicating that both of these groups of birds were still engaging in behaviors associated with seeking out the female. Although measures of ASB were not affected by BST lesions, measures of CSB were affected, although not as dramatically as in birds receiving POM lesions.

Moderate decreases in copulatory behavior after lesions to the

BST have been reported in rodents, although results are somewhat inconsistent among different species. However, large and consistent effects of such lesions on measures of appetitive sexual behavior such as chemoinvestigatory behaviors have been reported consistently (Powers et al., 1987; Meisel and Sachs, 1994; Everitt, 1995; Wood, 1997). Thus, the quail BST lesion data suggest that there may be differences between quail and rodents in the organization of the neural circuit regulating male sex behavior.

Implications of studies of appetitive and consummatory behavior in Japanese quail for current views of the neural circuit mediating male sexual behavior

Male quail engage in stereotyped copulatory behaviors, as mammals do, but the motor patterns used and the effector organ used are quite different (e.g., quail have no penis). Also the sensory pathways used by male quail when searching out and identifying a female to copulate with are quite different from what occurs in most mammalian species. Quail rely primarily on visual and auditory cues rather than olfactory cues, which are paramount in rodents (Domjan and Nash, 1988; Balthazart and Ball, 1992; Meisel and Sachs, 1994; Panzica et al., 1996). The neuroanatomical organization of the preoptic area and the hypothalamus appears to be quite similar between the two taxa, and even the organization of sensory inputs and motor outputs is more similar than previously thought by many comparative neuroanatomists (Kuenzel and VanTienhoven, 1982; Reiner et al., 1984; Butler and Hodos, 1996). Therefore, comparing the neural circuit mediating masculine sex behavior in the two taxa may help distinguish aspects of the circuit described for mammals that are peculiar to the life history of rodents (i.e., primary reliance on chemosensory cues for social interactions) and other mammals (e.g., extensive use of an intromittent organ such as the penis when copulating). These then can be distinguished from aspects that are common among all vertebrate species (the integration of sensory cues relevant to the sexual stimulus in question and the generation of an appropriate, organized motor response). Studies of fos and another immediate early gene, egr-1 (called ZENK in birds), have already provided evidence that both the POA and BST are important in the regulation of male reproductive behavior in quail (Ball et al., 1997; Meddle et al., 1997), as they are in mammals (Baum and Everitt, 1992; Wood and Newman, 1995b; Heeb and Yahr, 1996; Pfaus and Heeb, 1997), .

The major deficits in appetitive measures of rodent male sexual behavior that have been identified after BST lesions have been related to the role played by the BST in the integration of chemosensory cues (Powers et al., 1987; Wood, 1997). The lack of an effect of lesions to the BST on ASB in quail may be because quail primarily use visual rather than olfactory inputs when responding to females. It is not known how visual stimuli get to the POM, but the existing data do not suggest that it is via the BST (Panzica et al., 1996).

Nearly all investigators agree that the mPOA is an important part of the neural circuit that integrates sensory inputs needed for the activation of male sexual behavior, especially those relevant to male copulatory behavior (Baum, 1992; Meisel and Sachs, 1994; Hull, 1995; Wood and Newman, 1995a,b; Yahr, 1995). There is still some disagreement about whether this brain area is also involved in the regulation of appetitive components of male sexual behavior. The data presented here on quail are consistent with the idea that the preoptic region is central to the regulation of both aspects of reproductive behavior. These results therefore support the concept that the preoptic region coordinates appetitive and consummatory aspects of male reproductive behavior via the removal of a tonic inhibition (Hull, 1995; Hull et al., 1997), although this idea was not tested in the present study. The release of this inhibition may involve the action of dopamine in the mPOA. Dopamine is also important in the control of male mating behavior in quail, although the precise anatomical sites of its action are unknown (Absil et al., 1994; Balthazart et al., 1997b; Castagna et al., 1997).

In conclusion, these data bolster the substantial body of evidence supporting the role of the mPOA in the regulation of male reproductive behavior and support the view that the preoptic region is involved in both appetitive and consummatory aspects of male sexual behavior, although different subregions of this area are important for these two different aspects of behavior. The BST does not appear to be important in the regulation of appetitive sexual behavior. Perhaps its prominence in the regulation of rodent sexual behavior represents a neural specialization limited to taxa that rely heavily on chemosensory cues for the identification of sexually attractive conspecifics. Further studies of the circuit in quail and a comparison with other species will help generate a general theory of the regulation of vertebrate sexual behavior that can apply to all species, including humans.

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