Effects of Lesions of Nucleus taeniae on Appetitive and Consummatory Aspects of Male Sexual Behavior in Japanese Quail

Philippe Absil[a,b]   Jean Baptiste Braquenier[a]   Jacques Balthazart[a]
Gregory F. Ball[c]

[a]University of Liège, Center for Cellular and Molecular Neurobiology, Research Group in Behavioral Neuroendocrinology, Liège, [b]University of Antwerp, U.I.A., Department of Biology, Ethology Research Group, Wilrijk, Belgium, [c]The Johns Hopkins University, Department of Psychological and Brain Sciences, Baltimore, Md., USA

Abstract

Neurochemical, hodological and functional criteria suggest that the nucleus taeniae and parts of the adjacent archistriatum represent the avian homologue of parts of the mammalian amygdaloid complex. It has been proposed in particular that the nucleus taeniae is the homologue of the mammalian medial amygdala. In male quail, relatively large lesions to the posterior/medial archistriatum selectively decrease the expression of appetitive sexual behavior in a manner reminiscent of similar manipulations involving the medial amygdala in mammals. We investigated the effects of discrete lesions restricted to nucleus taeniae and of lesions to an adjacent part of the archistriatum (pars intermedium ventralis, Alv) on the expression of appetitive (ASB) and consummatory (CSB) aspects of male sexual behavior. ASB was measured by a learned social proximity response (after copulation a male quail stands in front of a window providing visual access to a female) and by the frequency of rhythmic cloacal sphincter movements. CSB was assessed by the frequency of mount attempts (MA) and cloacal contact movements (CCM). Lesions confined to nucleus taeniae and to Alv did not influence the acquisition or the maintenance of the two responses indicative of ASB. In contrast, lesions of nucleus taeniae significantly increased the occurrence frequencies of MA and CCM when administered before the beginning of behavior testing and increased the frequency of MA only when performed on sexually experienced subjects. No effect of Alv lesions could be detected. The discrepancy between these results and previous experiments in quail might reflect procedural differences, but more probably differences in locations of the lesions that were restricted in the current study to the anterior part of taeniae. Those in the Thompson study were in the posterior part of this nucleus. These findings indicate that there is a larger degree of functional heterogeneity in the nucleus taeniae than previously thought. The effects of taeniae lesions suggest that this nucleus, similar to the medial amygdala in mammals, might be implicated in the control of sexual satiety.

Copyright © 2002 S. Karger AG, Basel

Dr. J. Balthazart
Center for Cellular and Molecular Neurobiology
Research Group in Behavioral Neuroendocrinology, University of Liège
17 place Delcour (Bat. L1), B–4020 Liège (Belgium)
Tel. +32 4 366 59 70, Fax +32 4 366 59 71, E-Mail jbalthazart@ulg.ac.be

Introduction

Male sexual behavior can be usefully divided into two different components [e.g., Beach, 1956; Pfaus, 1996; Balthazart and Ball, 1998]. One component involves seeking and courting an appropriate female and is referred to variously as appetitive sexual behavior, sexual motivation or anticipatory sexual behavior [e.g., Beach, 1956; Pfaus, 1996; Balthazart and Ball, 1998; Pfaus et al., 2001]. The second component consisting of the sequence of behaviors that involve contact with the female and results in copulation, is referred to as consummatory male sexual behavior or sexual performance [Beach, 1956; Pfaus, 1996; Balthazart and Ball, 1998]. The exact terminology that is appropriate is a matter of some discussion because, as is not surprising, it is often difficult to distinguish definitively between these two necessarily overlapping aspects of male sexual behavior [e.g., Sachs, 1983; Hull et al., 2002]. However, using a behavioral distinction related to these two different aspects of male sexual behavior has been valuable in clarifying the function of the neural circuit that regulates male sexual responses [e.g., Everitt, 1990, 1995].

We adopt the terms appetitive and consummatory in our analysis of the neural circuit regulating male sexual behavior in Japanese quail [*Coturnix japonica*; Balthazart and Ball, 1998] because they capture aspects of male sexual responding, such as the fact that the appetitive responses are usually, but not always, less stereotyped than the consummatory responses and that these responses are not necessarily sex-specific, although they might be, as is the case for consummatory responses [see Timberlake and Silva, 1995; Pfaus, 1996; Balthazart and Ball, 1998 for discussion].

Studies of male sexual responding in rats have suggested that the amygdaloid complex plays a particularly important role in the regulation of appetitive aspects of male sexual behavior [Everitt, 1990, 1995]. This conclusion has been questioned by several investigators who observed that the preoptic complex, well known to be important for consummatory male sexual behavior [Hull et al., 2002], is also involved in the regulation of certain measures of appetitive responding [e.g., Baum, 1995; Hull, 1995; Hull et al., 1997; Paredes et al., 1998]. However, in hamsters the medial amygdala clearly plays an important role in the approach behavior of males to female-typical odors [Wood and Swann, 2000] that is a precusor to copulation. Furthermore, in rats stimulation of the medial amygdala enhances dopamine release in the preoptic area [Dominguez et al., 2001], an event that is essential for copulation and is normally stimulated by the presence of a female [Hull et al., 1997; Du et al., 1998]. Thus there continues to be evidence that the medial amygdala is a key part of the neural circuit mediating male sexual responses and might play a particularly important role in regulating appetitive responses.

In birds, the nucleus taeniae is a structure that expresses androgen receptors [Balthazart et al., 1992, 1998a] and estrogen receptors of both the alpha and beta type [Balthazart et al., 1989; Foidart et al., 1999]. It has recently been suggested that this structure is the avian homologue of the mammalian medial amygdala [Thompson et al., 1998; Cheng et al., 1999]. In particular, Thompson et al., based on electrolytic lesions, claimed that in quail this structure is involved in several components of male responses to females [Thompson et al., 1998]. We tested the effects of precise lesions of this structure on appetitive and consummatory sexual responses of male quail. Studies of the function of this structure in male sexual responses in quail can provide a valuable contrast to rodent studies because the sensory basis of the response in quail involves visual and auditory cues, whereas in rodents olfactory cues are paramount [Balthazart and Ball, 1998; Thompson et al., 1998]. Thus by comparing studies in these taxa one can potentially discern behavioral functions for a brain structure independent of the species-typical sensory basis of the appetitive sexual response.

Materials and Methods

Two experiments were conducted with very similar protocols. In a first experiment, we quantified the effects of a bilateral lesion aimed at the nucleus taeniae (Tn) on the maintenance of sexual responses. In a second experiment, birds were submitted to bilateral electrolytic lesion of Tn or to a sham operation before they had acquired the learned social proximity response that is used in quail to quantify the appetitive sexual behavior. The experiment thus assessed effects of the lesions on both the acquisition and expression of this social proximity response and copulatory behavior. Methods common to both experiments are described below. The specific protocol of each experiment will then be briefly summarized.

Subjects

Male Japanese quail *(Coturnix japonica)* were bought from a local breeder (Degros-Louppe Farm, Rechrival, Belgium) at the age of 3 weeks. Throughout their life at the laboratory, the birds were exposed to a photoperiod simulating long days (16 h light and 8 h dark per day) and had food and water available ad libitum. All experimental procedures were in compliance 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 Ethics Committee for the Use of Animals at the University of Liège.

The Quantification of Appetitive Sexual Behavior in Quail

In male Japanese quail, two useful behavioral measures have been developed to assess appetitive male sexual behavior. One is an unlearned response that involves rhythmic movements of the cloaca in response to females. Male quail produce a meringue-like foam that is transferred to females during copulation and might enhance the probability that the sperm transferred will fertilize the female [Cheng et al., 1989a, b]. This foam is produced by rhythmic movements of a sexually dimorphic striated cloacal sphincter muscle interdigitated with the proctodeal gland [Seiwert, 1994; Seiwert and Adkins-Regan, 1998]. These movements are greatly facilitated in males, including sexually naive males, when the stimulus animal is a female rather than a male [Seiwert, 1994; Seiwert and Adkins-Regan, 1998; Thompson et al., 1998]. We found that these rhythmic cloacal sphincter muscle movements are facilitated nearly 20-fold in castrated males treated with testosterone and provided with visual access to a female as compared to castrated males receiving no testosterone [Balthazart et al., 1998b]. These cloacal sphincter muscle movements are reminiscent of non-contact erections described in mammalian species [Sachs, 1995] in that they involve muscle movements that control effector organs associated with consummatory aspects of male sexual behavior in response to sensory cues emitted by a female.

A second response indicative of appetitive male sexual behavior is the learned social proximity response first described by Domjan and Hall [Domjan and Hall, 1986a, b]. We modified the procedure developed by Domjan and Hall somewhat and in our test the measured response is a male quail standing in front of a window and focussing his attention on a female in a small adjacent chamber during a 25 min test period. Males acquire this response after a single copulation with a female in the main chamber and further copulatory experience is not necessary to maintain the response [Balthazart et al., 1995]. The sensory basis of the response involves the perception of female-typical visual cues provided by the plumage [Domjan and Hall, 1986a; Domjan and Nash, 1988]. The behavior requires estrogenic metabolites of testosterone for acquisition and maintainance [Balthazart et al., 1997] and lesions to the preoptic region attenuate the response [Balthazart et al., 1998b].

Castration and Testosterone Treatment

About one week after their arrival in the laboratory, all birds were castrated under complete anesthesia (Hypnodil™, Janssen Pharmaceutica, Beerse, Belgium, 15 mg/kg body weight). The two testes were removed through a unilateral incision behind the last rib as described previously [Balthazart and Schumacher, 1984]. Birds were housed in individual cages for about 2 weeks and then they were subcutaneously implanted with two 20 mm-long capsules made of Silastic™ tubing (Silclear™ Tubing number 20301502431; i.d. 1.57 mm; o.d. 2.41 mm; Degania Silicone, Degania Bet 15130, Israel) filled with crystalline testosterone (T; Fluka, cat.nbr. 86500). The efficacy of the T replacement therapy was regularly confirmed by measuring the growth of the cloacal gland with calipers (cloacal gland area = largest length × largest width in mm^2). 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]. Birds were periodically weighed to the nearest gram throughout the experiments to confirm the absence of adverse effects of the manipulations on their general condition.

Behavioral Screening and Acquisition of the Social Proximity Response

Three weeks after the implantation of Silastic capsules, all birds were tested four times for the presence of male-typical copulatory behavior employing behavioral procedures that have been previously described in detail [Balthazart and Schumacher, 1984]. Briefly, each male was introduced into a small arena (50 × 60 cm; see fig. 1A) that contained a sexually mature female and the occurrence of copulatory behaviors [neck grabs, mounts and cloacal contact movements; see Adkins and Adler, 1972; Hutchison, 1978 for description] was recorded for 5 min. Testosterone-treated birds that failed to exhibit mounts and cloacal contact movements were excluded from the study at this point.

All remaining subjects then experienced, in a two-compartment test cage, a series of associative learning trials, each taking place on a different day. 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 through 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 [see Balthazart et al., 1995, 1997; Castagna et al., 1997; Balthazart and Ball, 1998]. They are only briefly summarized below.

Two-Compartment Test Cages

Four identical two-compartment cages were used in this study (see fig. 1B). A large compartment (90 × 90 cm) was next to a smaller compartment for stimulus females (20 × 25 cm) centered on the left lateral wall of the main cage and separated from it by a vertically sliding door, 20 (wide) × 20 (high) cm, that could be controlled remotely by strings and pulleys. A small narrow window (consisting of a vertical slit, 1 × 15 cm, 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 only see the female located in the lateral chamber if he stood in front of the window in this area. This square area was mounted on 4 springs and 4 microswitches (one in each corner) wired in parallel and powered by a 4.5 volt battery so that depression of any switch generated a positive signal. The output signals were digitized and sent to a Macintosh computer using commercially available hardware and software. During the observation periods (5 min periods, see below), a computer program written in Basic recorded 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) were sampled in the test area of each cage once every second.
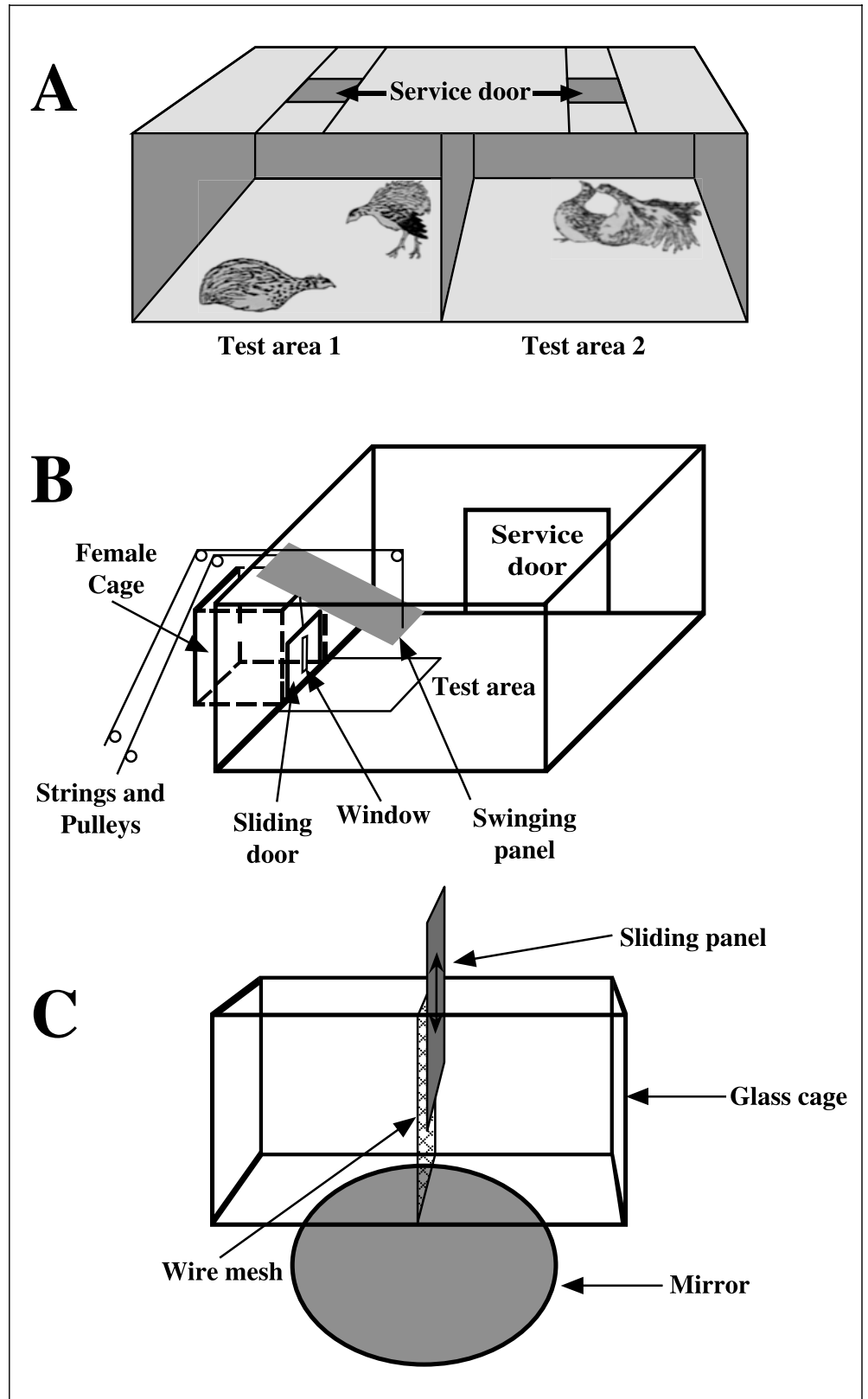
Fig. 1. Schematic presentation of the experimental conditions used to pre-screen subjects for copulatory behavior (**A**), test birds for the learned social proximity response (**B**) or measure the frequency of rhythmic cloacal sphincter movements in response to the visual presentation of a female (**C**). See text for additional description.

Behavioral Tests: General Procedure

Four tests were always run in parallel in the 4 experimental cages. Each lasted a total of 25 min. At the beginning of the test, one male was introduced in 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 (presence/absence in the area in front of the window) was then continuously recorded during the next 5 min period with the window still closed (Pretest-Time). 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 s. At each beep, the observer recorded whether the subject was actually looking through the window or not. 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 (LOOK) 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) only develops in birds that are permitted to copulate during a 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 freely interact for 5 min. During that time, the occurrence frequencies of male sexual behaviors were directly recorded. The following behavior patterns were systematically noted: strut, neck grab (NG), mount attempt (MA), mount (M) and cloacal contact movements (CCM) [see Adkins and Adler, 1972; Hutchison, 1978 for a detailed description]. 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.

Rhythmic Cloacal Sphincter Movements

Birds were submitted to one additional type of behavior test to assess the potential effects of lesions on another measure of appetitive sexual behavior in quail: the induction of rhythmic cloacal sphincter movements (RCSM) in the presence of a female. These movements are used by males just prior to copulation to produce the stiff meringue-like foam that is transferred to females during copulation. It has previously been shown [Adkins-Regan et al., 1994; Seiwert and Adkins-Regan, 1998; 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 described as follows. Each male was placed in an aquarium (20 × 40 cm; see fig. 1C) 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 assessment of cloacal movements. At the beginning of each behavioral test, the aquarium was divided into two chambers by an opaque partition and RCSM 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 female were only separated by a wire-mesh grid and the male had visual access to the female although he could not physically interact with her. The RCSM were then quantified for an additional 2.5 min under these stimulus conditions.

Stereotaxic Surgery

The selected subjects were randomly assigned to one of four experimental groups balanced according to their body weight and behavioral scores during the selection tests (see protocol of specific experiments). Two groups of subjects received a bilateral electrolytic lesion (LES group) aimed at the anterior or posterior portion of the nucleus taeniae (Tn). Two other control groups were submitted to the corresponding sham operations. During each experiment, the surgical procedure took place on two consecutive days.

Subjects were first anesthetized (Hypnodil™, Janssen Pharmaceutica, Beerse, Belgium; 15 mg/kg body weight) and positioned in a stereotaxic frame (Trent Weels, Inc., South Gate, Calif.; pigeon beak holder at 45° below the horizontal axis). 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 (1.0 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 lowering 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 skull opening 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 eat and drink within an hour.

The coordinates of the target were determined based on the quail brain stereotaxic atlas [Baylé et al., 1974] and adjusted by trial and error for the heavier weight of our birds. The coordinates for the anterior or posterior Tn were respectively –4.60/–4.10 mm (antero-posterior axis), ±3.82/±4.15 mm (lateral coordinate = half of the distance between the two electrodes; medio-lateral axis), and +2.10/+3.00 mm (dorso-ventral axis). During experiment 2, the coordinates for the posterior lesions were slightly modified (3.8 mm anterior, 4.5 mm lateral and 2.6 mm above the zero interaural reference point) in an attempt to reach the Tn more precisely. Subsequent histological analyses revealed however that this attempt had not been successful (see results).

Histological Verification of the Position of the Lesions

At the completion of behavioral tests, all birds were killed by decapitation and their brains were dissected out of the skull, frozen on powdered dry ice and stored at –75°C. The birds were then checked for the completeness of castration and the presence of subcutaneous T silastic implants in the lesion experiments. Any bird showing testicular remnants or that had lost its T implant was discarded before any data analysis.

Frozen brains were cut with a cryostat into 50 μm-thick coronal sections. The plane of section was adjusted to match as closely as possible the plane of the quail brain atlas [Baylé et al., 1974]. All sections in the Tn region were collected on gelatin-coated microscope

slides and stained for the Nissl substance by toluidine blue. The extent and location of the lesions were then drawn for each bird on schematic representations of the archistriatal region and these representations were used to assign subjects to their final experimental group (see results).

Sections were digitized by means of a CCD camera connected to a Macintosh CI computer and the lesions 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 were reconstructed by multiplying areas by the distance between consecutive sections. Because the lesions, in most cases, only destroyed a portion of the Tn and the shape of this nuclei only changes gradually in the rostro-caudal axis, it was often possible to reconstruct, with reasonable precision, the contours of the nucleus as it 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 as well as the percentage of the nucleus that had been destroyed by lesions.

Protocol of Experiment 1

Out of a group of 41 castrated males treated with T that were pre-screened for copulatory behavior, 36 subjects were selected and submitted to 8 associative learning trials in a 10-day period during which they learned the social proximity response.

Subjects were then randomly assigned to four experimental groups that were matched based on their behavior during the acquisition phase (time spent in front and looking through the window and frequency of CCM). During the next two days, two groups of subjects received bilateral lesions aimed at the anterior and posterior Tn respectively, and the two other groups underwent the corresponding sham operations.

During the 10 days following surgery, all subjects were subjected to ten additional tests of the learned social proximity response using the same protocol as before the lesion with one exception: during most of these tests, the door between the two compartments was not opened at the end of the test so that birds could not physically interact. In this way the effect of the lesions could be quantified while not exposing the bird to the possible negative effect associated with the lack of sexual reinforcement resulting from its inability to copulate with the female. This procedure was used because it has been previously shown that in already conditioned birds the anticipatory response will persist in the absence of additional reinforcement (extinction tests) [Balthazart et al., 1995]. However, on days 6 and 10 post-surgery, the door between the compartments was opened so that copulatory sexual behavior could be quantified during 5 min of interactions between the male and the female. The frequency of RCSM was assessed 3 days before lesions and on days 5 and 12 post-surgery. The body weight and cloacal gland area of all subjects were measured regularly throughout the experiment on 8 different occasions (5 times before and 3 times after the lesions). Subjects were then killed for histological verification of the lesion sites.

Protocol of Experiment 2

Of the 41 castrated males treated with T that were pre-screened for copulatory behavior, 30 were assigned to four experimental groups that received lesions in the anterior or posterior Tn or had the corresponding sham operations. Starting three days later, all surviving subjects (n = 28) were then subjected in the two-compartment test cage to a series of 13 associative learning trials, each taking place on a different day within a 13-day period (day 4 to 16 post-operation). In this experiment tests were started 3 days after surgery to avoid the possible interference of a transient post-operative behavioral inhibition as suggested by the analysis of TIME spent in front of the window during experiment 1. All tests were carried out with the same procedure as before, except that due to technical problems the TIME spent in front of the window could not be recorded automatically by the micro-switches and computer. Instead, the presence/absence of the male in front of the window was sampled every 5 s and the score obtained in this way was multiplied by 5 so that these values could be compared to those in experiment 1.

The expression of RCSM was quantified on day 0 (before surgery) and on days 6 and 14 after electrolytic lesions. The body weight and cloacal gland area of all birds was measured on 5 different occasions before and after the lesions. Birds were then killed and brains collected for analysis of the lesion sites.

Data Analysis

Eight behavioral measures were analyzed in detail by one-, two- or three-way analyses of variance (ANOVA) as appropriate to the experimental design. Four measures were indicative of the appetitive aspects of male sexual behavior and include: (1) the time spent in front of the window of the compartment containing the stimulus female before (Pretest-Time) and (2) after the window was opened (TIME); (3) the frequency of looks through that window (LOOK); and (4) the frequency of rhythmic cloacal sphincter movements (RCSM). Birds usually spent very little time (less than 50 out of 300 s) in front of the closed window during the pretest and this measure was not affected by the treatment. The corresponding data are therefore not reported here.

Four measures of the consummatory aspects of male sexual behavior were analyzed in detail: (1) the numbers of neck grabs (NG); (2) mount attempts (MA); (3) of mounts (M); and (4) cloacal contact movements (CCM). The analysis of the NG, MA and M frequencies provided similar results so that results only of MA are presented here to avoid redundancy. Struts occurred too rarely in the present experiments to allow any meaningful analysis.

Data relative to specific parts of the experiments (e.g., before or after lesions) were in several cases also analyzed by ANOVA, which were followed, when appropriate, by post-hoc Fisher's protected least significant difference test (PLSD) to compare groups two by two. An α level of 0.05 is used throughout this paper.

Results

Experiment 1: Effect of a Bilateral Lesion of Tn on the Expression of a Learned Social Proximity Response and on Copulatory Behavior

Histological Verification of the Position of the Lesions and Redefinition of the Experimental Groups

Four types of stereotaxic manipulations were originally used in an attempt to cause lesions in the anterior or posterior Tn and the two corresponding control groups of birds. Examination of the lesions indicated that anterior Tn lesions had actually reached their target, but that lesions aimed at the posterior Tn were actually located in
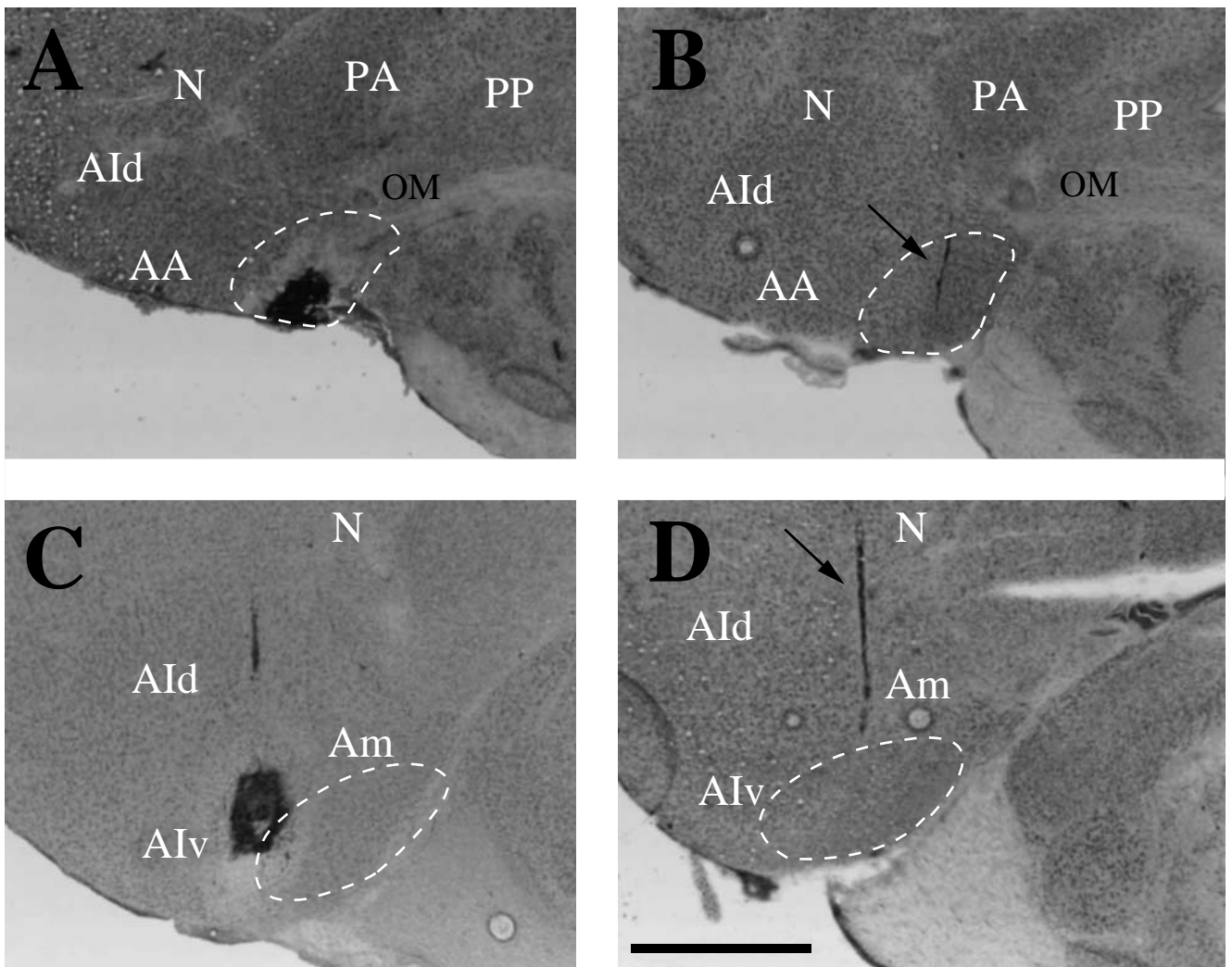
Fig. 2. Photomicrographs illustrating the extent of typical lesions of the Tn (**A**), AIv (**C**), and the corresponding controls (**B**, **D**). The darker areas in **A** and **C** clearly delineate the extent of the lesions. The hatched white line in each panel indicates the limits of Tn as identified in the Nissl-stained sections by the accumulation of neurons and the higher density of their staining as compared to the surrounding areas. The electrode tract is visible in panels **B** and **D** and is indicated by an arrow. Magnification bar: 200 µm. AA: archistriatum anterior, rostrale [Zeier and Karten, 1971]; AId: archistriatum intermedium, pars dorsalis [Zeier and Karten, 1971]; AIv: archistriatum intermedium, pars ventralis [Zeier and Karten, 1971]; Am: archistriatum mediale [Zeier and Karten, 1971]; N: neostriatum; OM: tractus occipitomesencephalicus; PA: paleostriatum augmentatum; PP: paleostriatum primitivum.

the medial part of the ventral archistriatum intermedium (AIv) and barely affected Tn itself (see fig. 2 for representative photomicrographs). The electrode tract in sham-operated birds was located in corresponding archistriatal areas.

Based on these histological analyses, 10 birds were assigned to the Tn-lesioned group and 7 birds to the AIv-lesioned group. The two corresponding control groups contained 7 subjects each.

The morphological and behavioral data relative to these two control groups were originally compared by two-way ANOVAs with one independent factor (the two control groups) and one repeated factor (the successive measures of the body weight and cloacal gland area or of the different behavioral measures). These analyses revealed no significant difference between the two control groups, with the exception of the cloacal gland area that was slightly but significantly smaller in the AIv-control than in the Tn-control group before but not after the ste-
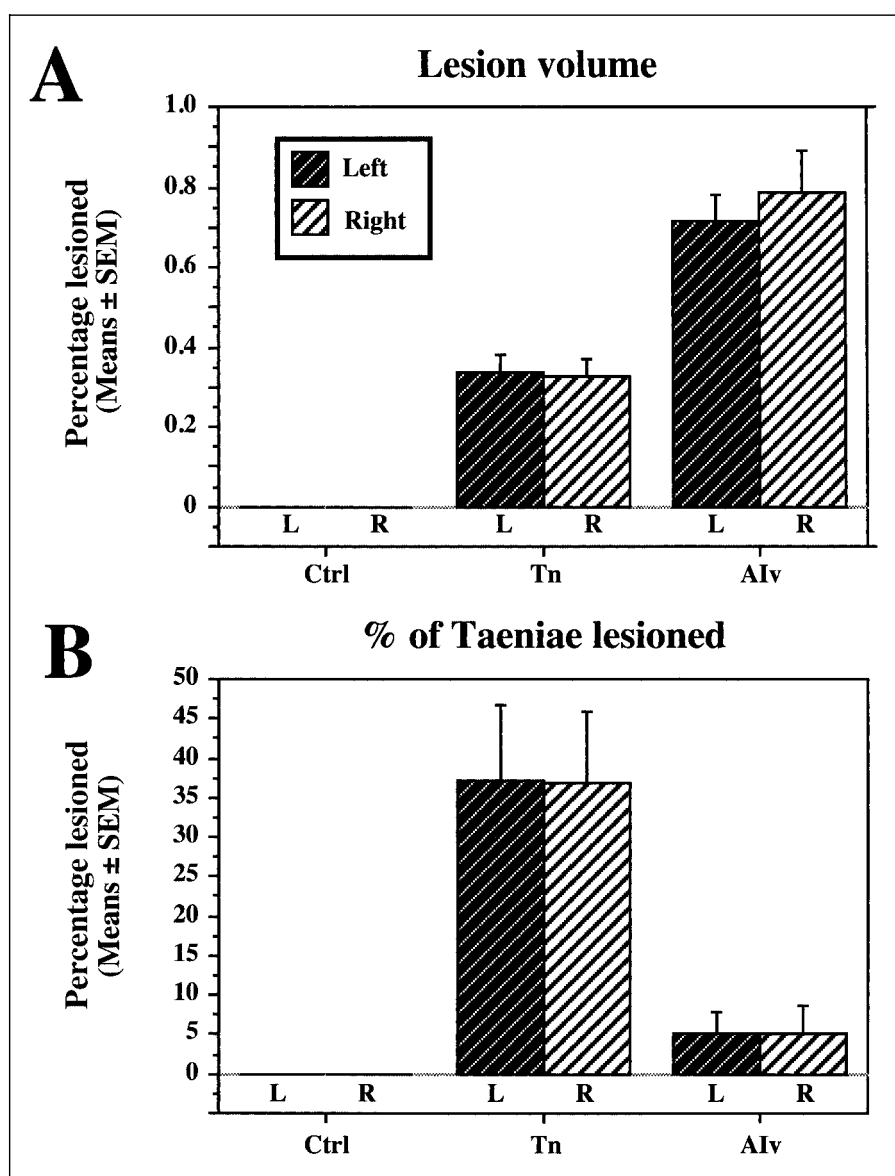
Fig. 3. Reconstruction of the volumes of electrolytic lesions (**A**) and percentage of nucleus taeniae that was destroyed (**B**) in the three experimental groups during experiment 1 (see text for more detail).

reotaxic surgery. It was therefore decided to pool all data from the two control groups for all analyses that are presented here. Thus we compare three experimental groups of subjects that were either bearing a lesion in the rostral part of Tn (Tn group) or bearing a more caudal lesion in the ventral archistriatum intermedium (AIv group) or were submitted to sham operations but had no brain lesion (CTRL group).

Qualitative and Quantitative Analysis of the Lesions

A qualitative evaluation of the electrolytic lesions revealed that in the Tn group a large fraction of the nucleus Tn was symmetrically destroyed throughout the rostral two-thirds of the nucleus. The lesioned tissue was largely confined to the boundaries of the nucleus Tn, with occasionally a limited overlap within the adjacent archistriatal area. Lesions of the AIv affected approximately one half of the region and appeared symmetrical. These lesions were also confined within the AIv, and affected only very marginally or not at all the posterior part of Tn. Lesions affecting other archistriatal regions adjacent to AIv and areas other than Tn were not observed.

Quantitative analysis of the total lesion volumes by two-way ANOVA with the 3 experimental groups as an independent factor and the two brain sides as repeated factors indicated very significant differences in lesion vol-

umes between groups (p < 0.001) but no difference related to brain side and no interaction between the two factors (p > 0.35 in both cases; see fig. 3A). Interestingly, lesion volumes were significantly larger in the AIv than in the Tn group (p < 0.05 by the Fisher PLSD test) despite the fact that similar amounts of electric current had been used to generate lesions at both locations. This difference presumably relates to differences in the electric resistance of the neural tissue in the two affected locations.

Quantitative analysis also confirmed that the percentage of Tn that had been lesioned was very different in the 3 groups (p < 0.0001) but this percentage was similar between the left and right sides of the brain, and there was no interaction between the two factors in this ANOVA (p > 0.90 in both cases; fig. 3B).

Morphological Measures

Two-way ANOVA of the 8 measures of body mass collected throughout the experiment (5 before and 3 after lesion) indicated significant differences between the three groups ($F_{2,28} = 3.558$, p = 0.042) as well as major changes over time ($F_{7,196} = 19.172$, p = 0.0001) reflecting the weight gain that normally occurs in quail at that age. There was, however, no interaction between these two factors ($F_{14,196} = 0.903$, p = 0.5562) indicating that the weight gain was similar in the three different groups. Post hoc Fisher PLSD tests indicated that overall the body mass was lighter in the AIv group (197.4 ± 2.7 g) than in the control (214.7 ± 1.7 g) and Tn (203.8 ± 1.6 g; all means ± SEM) groups, but this difference was only significant in the former case. The separate analysis of body mass before or after lesions similarly indicated that in both periods, the AIv group was slightly and significantly lighter that the CTRL group. This difference was not affected by the stereotaxic lesions.

Similarly, the two-way ANOVA performed on the eight measures of cloacal gland areas collected throughout the experiment indicated no significant difference between groups ($F_{2,28} = 0.532$, p = 0.5932), a highly significant time effect ($F_{7,196} = 323.971$, p < 0.0001) but no interaction between these factors ($F_{14,196} = 1.091$, p = 0.3681). The analysis of the cloacal gland areas before or after lesion also confirmed the absence of group differences in both periods of the experiment. All subjects thus reacted to the treatment with exogenous testosterone and experienced a major growth of this gland (from a mean area of 46.7 ± 2.1 mm^2 at the beginning to 357.9 ± 7.9 mm^2 at the end of the experiment; means ± SEM), but this growth was not affected at all by the experimental manipulations.

Appetitive Sexual Behavior
Social Proximity Response

Two-way ANOVAs performed on the 2 measures of appetitive behavior (TIME, LOOK) collected during the 18 tests performed in the two compartment cages (8 learning trials before lesion and 10 trials after lesion, see fig. 4A, B) identified very significant changes in these behaviors over time (TIME: $F_{17,476} = 5.966$, p < 0.0001; LOOK: $F_{17,476} = 16.251$, p < 0.0001) but no difference between groups (TIME: $F_{2,28} = 1.560$, p = 0.2278; LOOK: $F_{2,28} = 0.863$, p = 0.4328) and no indication of an interaction between these two factors (TIME: $F_{34,476} = 0.759$, p = 0.8369; LOOK $F_{34,476} = 1.097$, p = 0.3282).

Analysis by two-way ANOVAs of the data collected before surgery confirmed the absence of group differences at that time (TIME: $F_{2,28} = 0.972$, p < = 0.3907; LOOK: $F_{2,28} = 1.535$, p = 0.2330) but the existence of very significant differences between sessions, reflecting the acquisition of the social proximity response (TIME: $F_{7,196} = 14.460$, p < 0.0001; LOOK: $F_{7,196} = 34.671$, p < 0.0001). This acquisition was however similar in the three groups of subjects as clearly visible in figure 4 and confirmed by the absence of interactions in the ANOVA (p > 0.38 in both cases).

A similar analysis of the data from the 10 test sessions performed after lesions were placed in Tn or AIv also failed to identify group differences and interactions between groups and sessions (p > 0.10 in all cases). The social proximity response had, however, been fully acquired when these tests were performed and consequently there was no longer any change in the time spent in front of the window ($F_{9,252} = 0.980$, p = 0.1709). For some unexplained reason, the LOOK scores decreased in all subjects independent of their experimental group during the last two tests (see fig. 4B) so that a significant effect of sessions was still present for this measurement in the post-lesion scores ($F_{9,252} = 5.170$, p < 0.0001).

These data were further summarized by computing for each male the average behavioral scores (TIME and LOOK) for the 8 tests before and the 10 tests after surgery (see fig. 4C-D). Analysis of these data by two way ANOVA confirmed the lack of group effects (TIME: $F_{2,28} = 1.584$, p = 0.2229; LOOK: $F_{2,28} = 0.942$, p = 0.4019) and of interaction between groups and periods (TIME: $F_{2,28} = 0.225$, p = 0.800; LOOK: $F_{2,28} = 0.478$, p = 0.6248), but indicated the presence of marked increases in both behavioral scores between the two experimental periods (TIME: $F_{1,28} = 4.933$, p = 0.0346; LOOK: $F_{1,28} = 10.383$, p = 0.0032), which reflects the acquisition of the social proximity response.
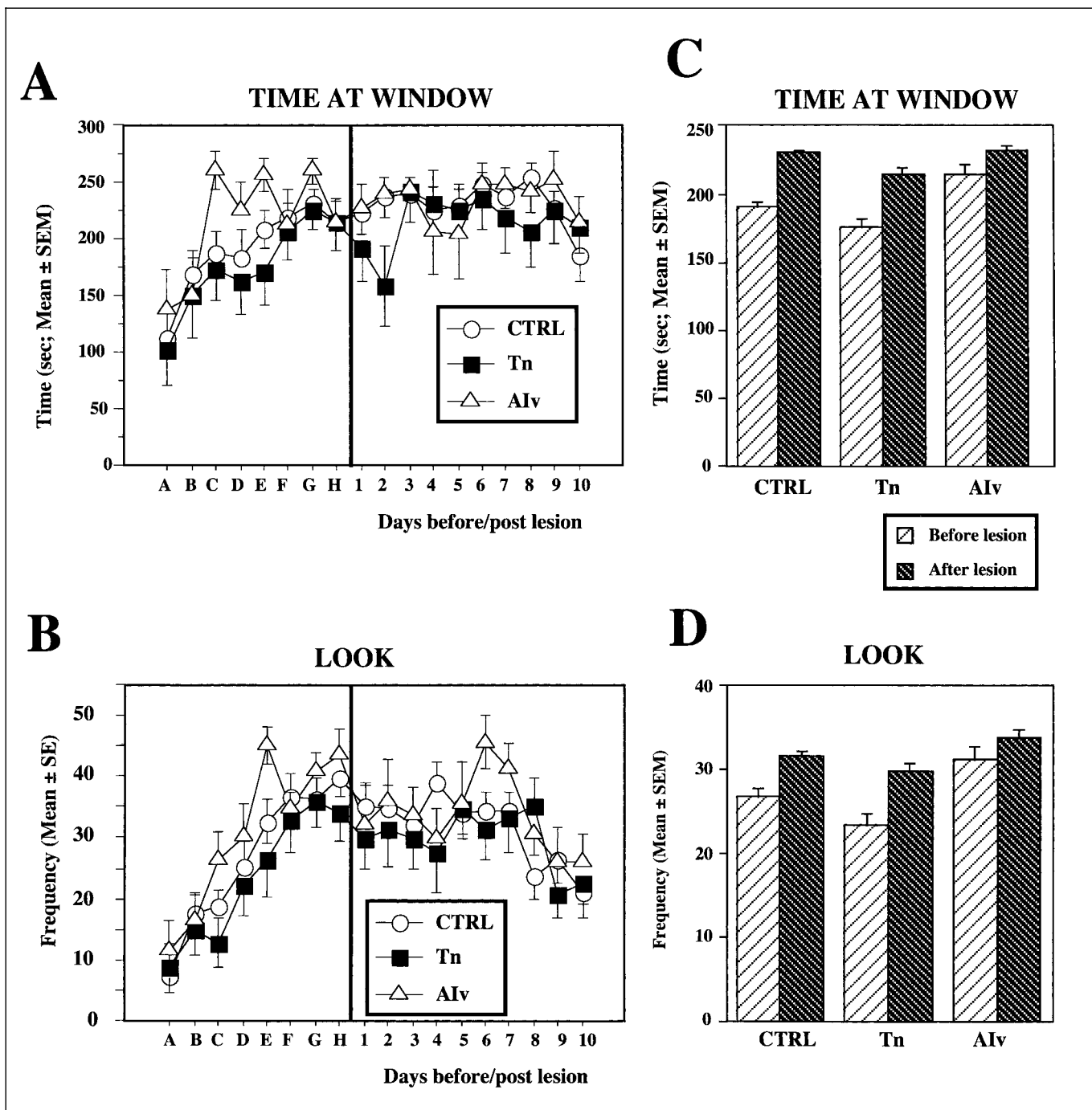
Fig. 4. Effects of lesions of the Tn or AIv on two measures (time at window (**A**, **C**) and look frequency (**B**, **D**)) of the learned social proximity response indicative of appetitive sexual behavior of male quail. Data for control (CTRL) birds bearing sham lesions are also illustrated. Data shown in **A** and **B** represent the acquisition of the responses during the 8 pre-operative tests (labeled A through H in the figure) and then the effects of the experimental manipulations observed during the 10 post-operative tests (labeled 1 to 10 in the figure) in the 3 experimental groups. Data shown in **C** and **D** represent the average responses in the 3 experimental groups before and after the lesions.

Rhythmic Cloacal Sphincter Movements
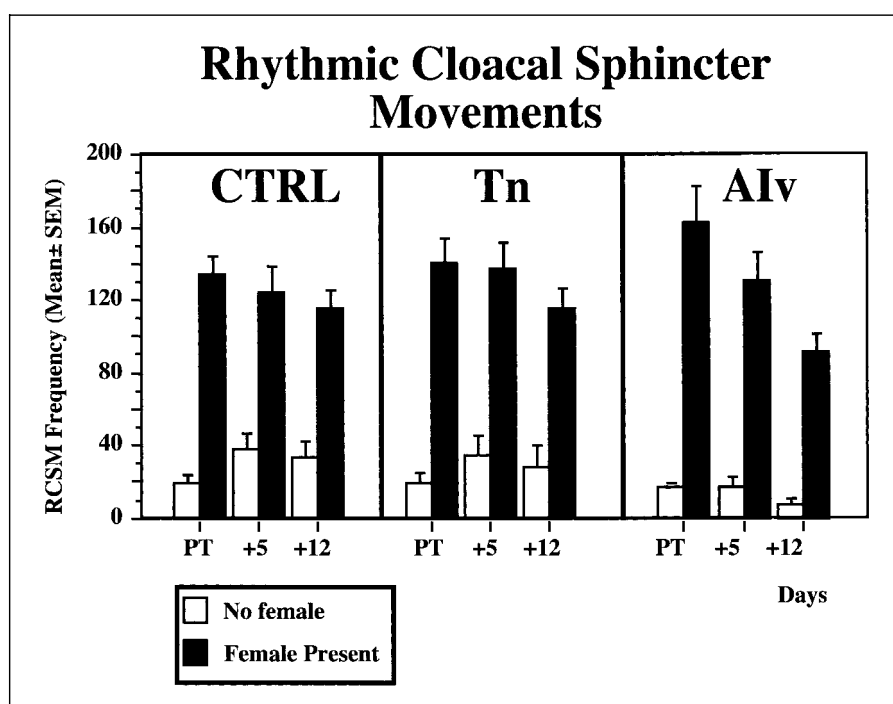
Fig. 5. Effects of lesions of the Tn or AIv on the number of rhythmic cloacal sphincter movements displayed in the absence or in the presence of a stimulus female. Data for control (CTRL) birds bearing sham lesions are also illustrated. The behavior was recorded before the lesions (Pretest, PT) as well as 5 and 12 days after lesions.

Taken together, these analyses indicate that birds from the three experimental groups similarly acquired the social proximity response in anticipation of sexual interaction during the eight pre-surgery tests and maintained to a similar level the expression of this response after lesions aimed at Tn or AIv. It must be noted, however, that a transient numerical decrease in the time spent in front of the window was observed during the first two tests performed immediately after the lesion in the Tn group (see fig. 4A). This effect was not sufficient to result in significant interactions in the ANOVAs described above and was also not sufficient to result in a significant F in a one-way ANOVA specifically analyzing the second test post-lesion data when the effect on the Tn group was numerically maximal ($F_{2,28} = 3.167$, $p = 0.0576$). Post-hoc PLSD Fisher tests, however, suggested that the scores in the Tn group were significantly lower than in controls ($p < 0.05$). Thus there is the suggestion of a transient effect of Tn lesions although it cannot be considered wholly established.

Rhythmic Cloacal Sphincter Movements

The frequency of RCSM was evaluated in the absence and presence of a female once before surgery and twice after birds received stereotaxic lesions (fig. 5). Analysis of these data by a three-way ANOVA with one independent factor (3 groups) and two repeated factors (presence/absence of the female and successive tests) identified significant effects of the female presence or absence ($F_{1,28} = 227.233$, $p < 0.0001$; much higher frequency of RCSM in the presence of the female), the test repetition ($F_{2,56} = 4.885$, $p = 0.0111$; progressive decline of RCSM frequency from the first to the last test), and an interaction between female presence/absence and test repetition ($F_{2,56} = 7.161$, $p = 0.0017$; the decrease in RCSM is more pronounced when the female is present). No effect of groups was present ($F_{2,28} = 0.621$, $p = 0.5448$) and none of the primary or secondary interactions involving the factor group came close to significance ($p \geq 0.15$ in all cases).

Consummatory Sexual Behavior

The analysis by two-way ANOVA of the MA and CCM frequencies observed during the entire experiment (8 tests before and two tests after lesion) revealed as expected no change in these behaviors across time because they did not need to be learned as does the social proximity response (see fig. 6A, B; MA: $F_{9,252} = 0.802$, $p = 0.6151$; CCM: $F_{9,252} = 1.493$, $p = 0.1507$). The overall frequency of CCM did not differ between groups ($F_{2,28} = 0.966$, $p = 0.3930$), but the difference approached significance for MA ($F_{2,28} = 2.639$, $p = 0.0891$). There was no interaction between groups and repeated testing, but a statistical tendency was again observed for MA (MA: $F_{18,252} = 1.621$, $p = 0.0552$; CCM = $F_{18,252} = 0.823$, $p = 0.6719$).
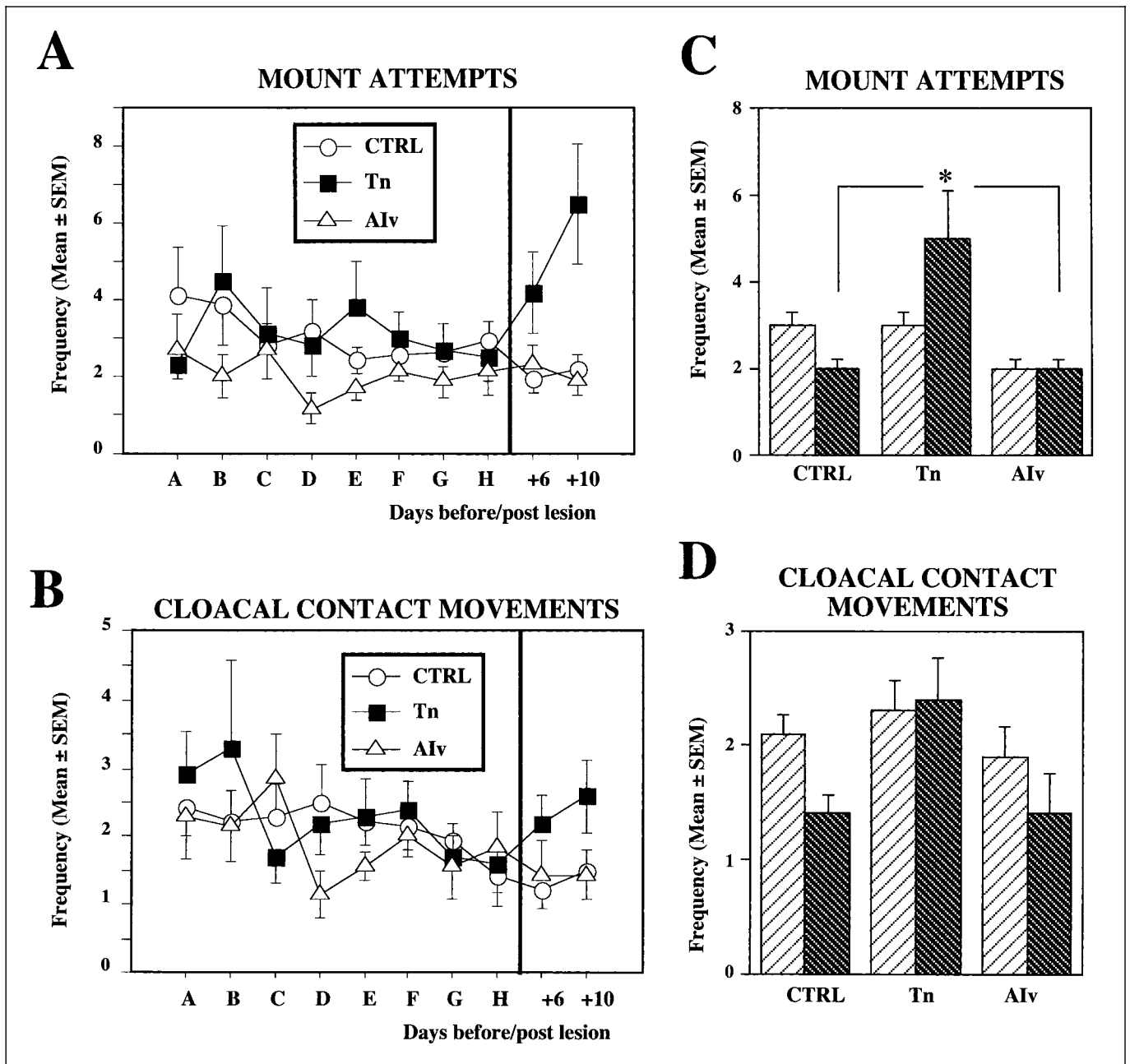
Fig. 6. Effects of lesions of the Tn or AIv on two measures (mounts attempts (**A**, **C**) and cloacal contact movements (**B**, **D**)) of consummatory sexual behavior of male quail. Data for control (CTRL) birds bearing sham lesions are also illustrated. Data shown in **A** and **B** present the behavioral responses during the 8 pre-operative tests (labeled A through H in the figure) and then the effects of the experimental manipulations observed during 2 post-operative tests performed 6 and 10 days after surgery in the 3 experimental groups. Data shown in **C** and **D** represent the average responses in the 3 experimental groups before and after the lesions.

Because the testing of copulatory behavior had been restricted to test 6 and 10 post-lesion in order to avoid possible negative effect associated with the lack of sexual reinforcement that could result from its inability to copulate with the female, data from the first part of the experiment (before lesion) were over-represented in the analysis and this was likely to obscure potential effects of the lesion. The separate analysis by two way ANOVA (groups and repeated testing of data obtained before and after lesion) confirmed this interpretation. Before lesion (8 tests), the frequencies of MA and CCM were similar in the three groups, they did not change over time and there was no interaction between the factors group and repeated testing ($p \geq 0.20$ in each case). In contrast, after lesion (2 tests), MA frequencies were significantly different between groups ($F_{2,28} = 6.634$, $p = 0.0044$) and their change in time was different in the three groups ($F_{2,28} = 3.764$, $p = 0.0357$). Overall, changes in frequency were not fully significant ($F_{1,28} = 3.033$, $p = 0.0926$). These effects were not statistically significant in the analysis of CCM frequencies post lesion (groups: $F_{2,28} = 3.205$, $p = 0.0558$; test repetition: $F_{1,28} = 0.588$, $p = 0.4497$; interaction: $F_{2,28} = 0.133$, $p = 0.8758$).

As in the analysis of appetitive sexual behavior measures, all data in this experiment were also summarized by computing for each male the average behavioral scores (MA or CCM frequencies) for the 8 tests before and the 2 tests after surgery (see fig. 6C-D). Analysis of these average scores confirmed differences between the groups ($F_{2,28} = 5.067$, $p = 0.0132$) and interactions between groups and experimental period (before/after lesion; $F_{2,28} = 5.829$, $p = 0.0077$) at the level of MA, but not at the level of CCM frequencies (Group: $F_{2,28} = 2.504$, $p = 0.0828$; Interaction: $F_{2,28} = 1.397$, $p = 0.2641$). For both behaviors, there was no overall change in frequency between the pre- and post-surgery periods (MA: $F_{1,28} = 0.969$, $p = 0.3333$; CCM: $F_{1,28} = 2.332$, $p = 1.380$). Post hoc analysis of the average MA frequencies during the two tests performed after the lesions indicated that birds in the Tn group performed these behaviors more often than the other 2 groups ($p < 0.05$ in each case; see fig. 6C). These differences were not present before surgery.

Experiment 2: Effect of a Bilateral Lesion of Tn on the Acquisition of a Learned Social Proximity Response and on Copulatory Behavior

Histological Verification of the Position of the Lesions and Redefinition of the Experimental Groups

After histological analysis and reconstruction of the lesions it appeared, as in experiment 1, that lesions aimed at the posterior Tn had usually missed their target and were actually located in the adjacent ventral archistriatum intermedium (AIv). Twenty-eight subjects had completed the entire experiment and, based on the presence/absence and location of their lesion, 20 of them could be clearly assigned to four different experimental groups: (1) bearing a lesion in the rostral part of nucleus taeniae (Tn group; n = 8); (2) in the ventral part of the archistriatum intermedium (AIv group; n = 3); (3 and 4) had been subjected to the two corresponding control manipulations [electrodes were lowered bilaterally in Tn or in AIv but no current was passed; CTRL-Tn (n = 4) and CTRL-AIv (n = 5) groups].

Comparison by two-way ANOVAs with one independent factor (two control groups) and one repeated factor (repeated measures of body mass, cloacal gland area, or of the different behaviors recorded) revealed no significant difference between the two control groups. Therefore, as in experiment 1, they were pooled to form a unique control group (CTRL) containing 9 subjects.

Qualitative Evaluation of the Importance of the Lesion

Microscopic observation of the sections revealed that the lesions were very similar to those obtained in experiment 1. Rostral lesions were almost entirely confined to the Tn, whereas caudal lesions affected mainly AIv with no or very little overlap with Tn. These lesions destroyed approximately one third of the entire Tn or one half of AIv. At its anterior level, Tn was, however, largely destroyed in most subjects.

As in experiment 1, the quantitative analysis of the total lesion volumes by two-way ANOVA revealed very significant differences in lesion volumes between groups ($p < 0.001$), but no difference related to brain side and no interaction between the two factors ($p > 0.45$ in both cases; see fig. 7A). Lesion volumes were again significantly larger in the AIv than in the Tn group ($p < 0.05$ by the Fisher PLSD test).

The percentage of Tn that had been lesioned was also very different in the 3 groups ($p < 0.0001$) but this percentage was similar in the left and right sides of the brain and there was no interaction between the two factors in this ANOVA ($p > 0.25$ in both cases; fig. 7B).
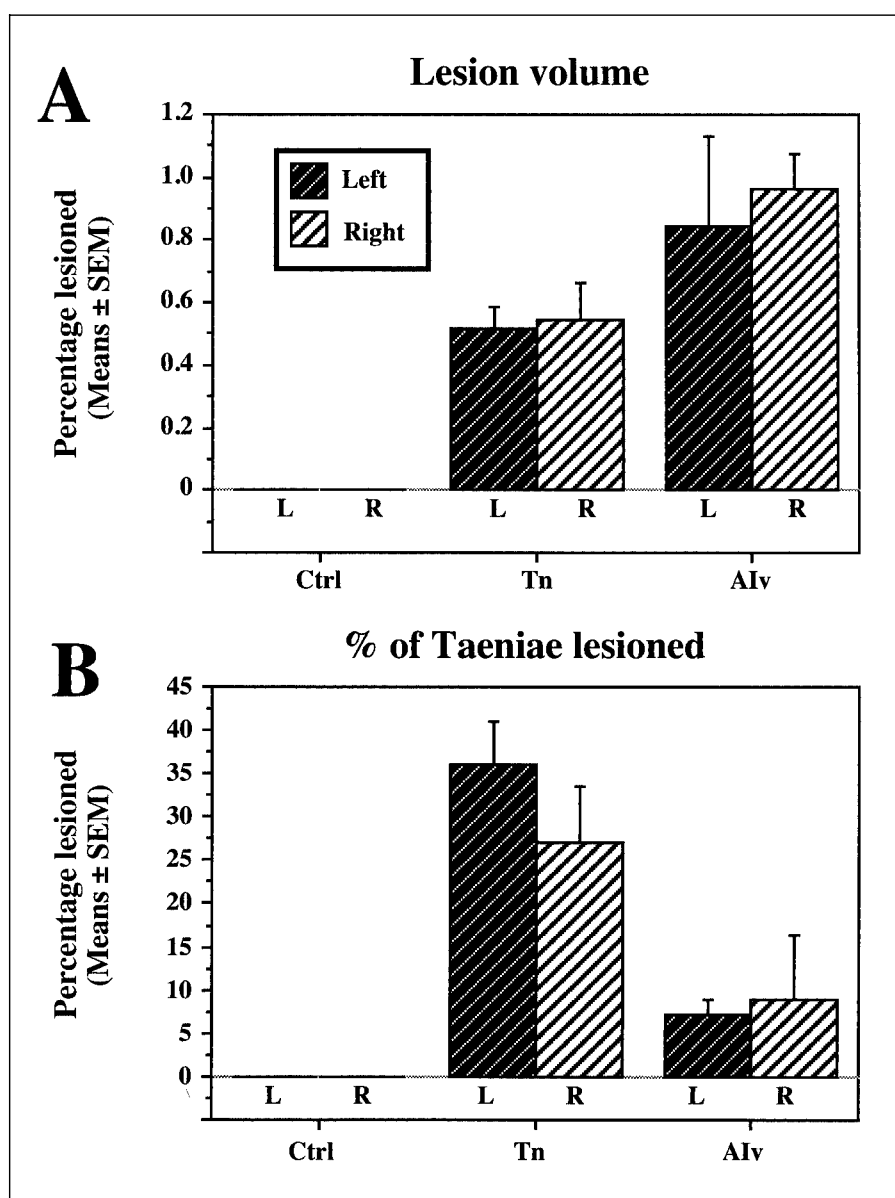
Fig. 7. Reconstruction of the volumes of the electrolytic lesions (**A**) and of the percentage of nucleus taeniae that was destroyed (**B**) in the three experimental groups during experiment 2 (see text for more detail).

Morphological Measures

Two-way ANOVA with the three experimental groups as the independent factor and the five measures of body mass as the dependent variable confirmed that all birds slowly gained weight during the experiments as usually observed in quail at that age (from 193.7 ± 3.9 g at the beginning to 199.2 ± 3.6 g at the end of the experiment, means ± SEM, $F_{4,68} = 4.378$, p = 0.0033). No difference between groups ($F_{2,17} = 0.635$, p = 0.0522) and no interaction ($F_{8,68} = 0.455$, p = 0.8830) could be detected in the ANOVA, which confirmed that the weight gain was similar in the three different groups.

The parallel analysis of cloacal gland areas similarly detected a highly significant effect of time ($F_{4,68} = 260.679$, p < 0.0001) but no group difference ($F_{2,17} = 0.511$, p = 0.6089) and no interaction ($F_{8,68} = 0.483$, p = 0.8642). The cloacal gland area markedly increased immediately after the beginning of exogenous testosterone treatment (from 39.65 ± 2.22 mm^2 to 306.45 ± 9.53 mm^2; means ± SEM), but then reached a maximum value and remained at that level throughout the behavioral observations. There was no sign of an effect of the stereotaxic surgery on the size of this androgen-dependent structure.
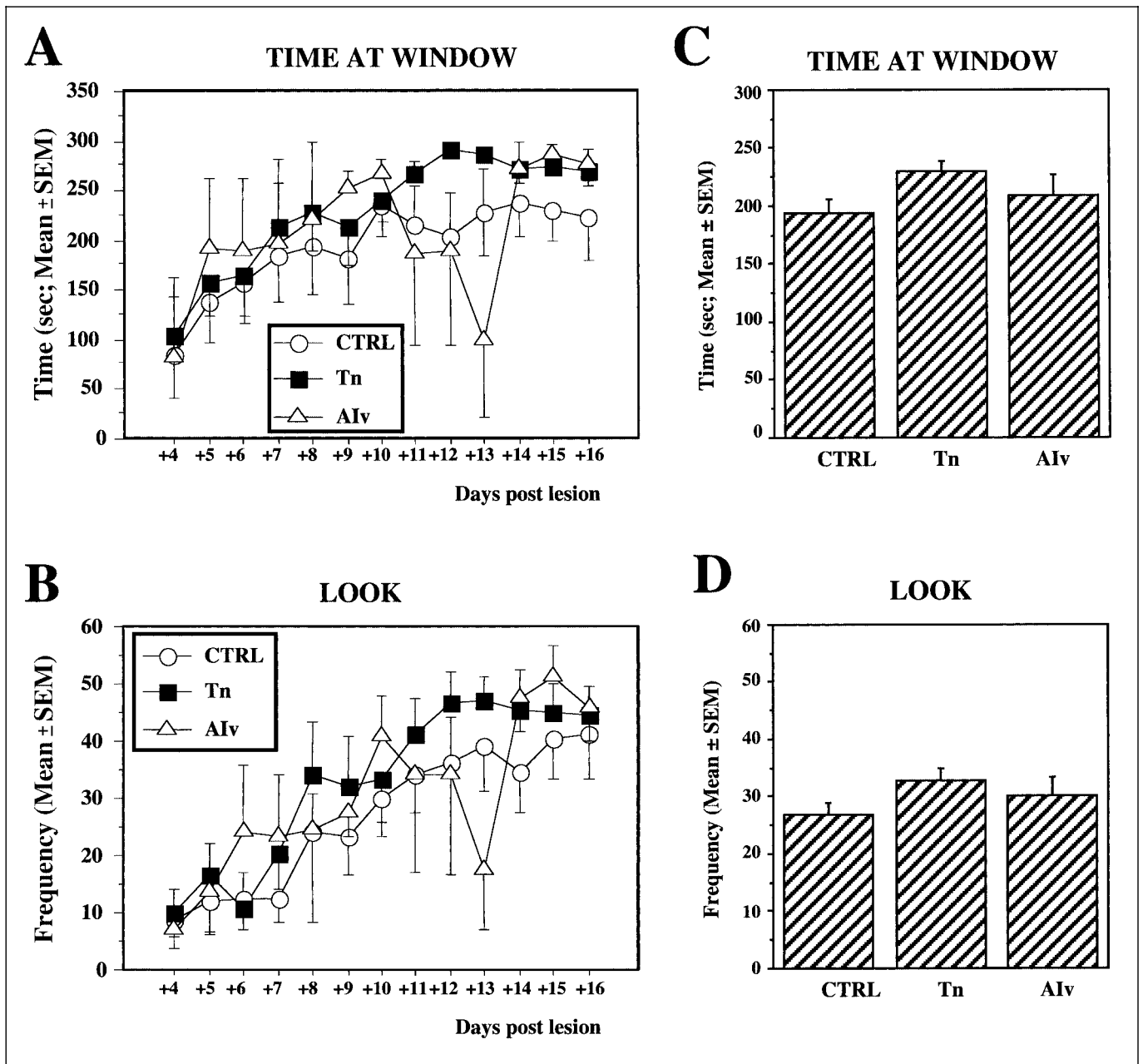
Fig. 8. Effects of lesions of the Tn or AIv on the acquisition of the learned social proximity response indicative of appetitive sexual behavior of male quail quantified by two behavioral measures: the time at window (**A**, **C**) and the frequency of looks (**B**, **D**). Data for control (CTRL) birds bearing sham lesions are also illustrated. Data shown in **A** and **B** represent the acquisition of the responses during the 16 tests that were performed after the stereotaxic surgery. Data shown in **C** and **D** represent the average responses in the 3 experimental groups during all these tests.

Appetitive Sexual Behavior

Social Proximity Response

Changes in expression of the two measures of the social proximity response (TIME and LOOK) in the three experimental groups are summarized in figure 8 (panels A, B)

as are the average values of these scores throughout the 13 tests (panels C, D).

It can easily be observed at the qualitative level that birds in all three groups efficiently learned the social proximity response and did so at a very similar rate. Accord-
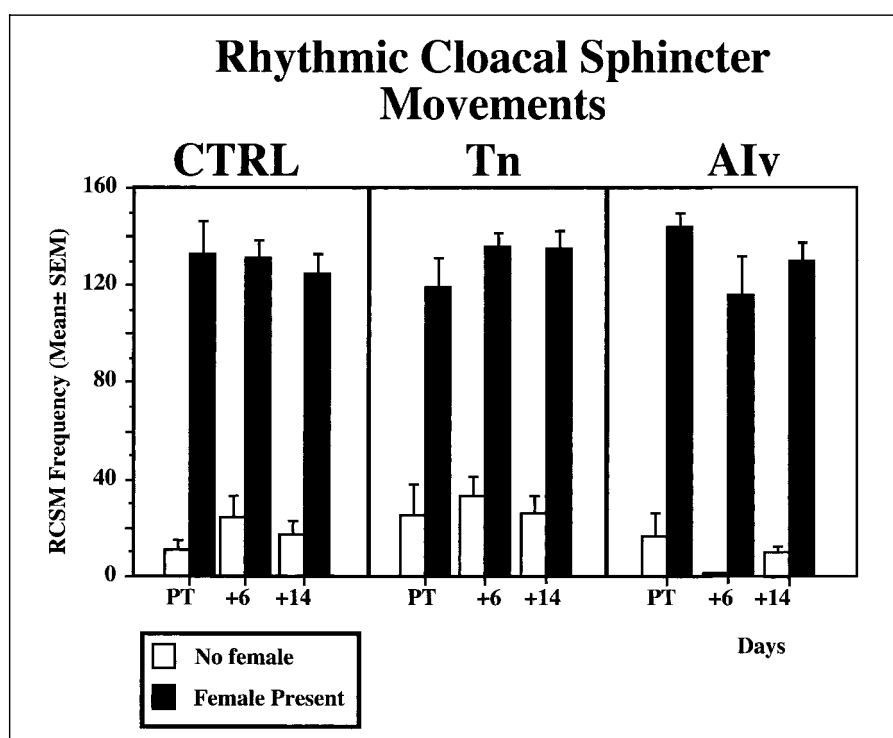
Fig. 9. Effects of lesions of the Tn or AIv on the number of rhythmic cloacal sphincter movements displayed in the absence or in the presence of a stimulus female. Data for control (CTRL) birds bearing sham lesions are also illustrated. The behavior was recorded before the lesions (Pretest, PT) as well as 6 and 14 days after lesions.

ingly, the two-way ANOVA of these data identified very significant behavioral changes between tests (TIME: $F_{12,204} = 7.469$, $p < 0.0001$; LOOK: $F_{12,204} = 15.193$, $p < 0.0001$) but no group difference (TIME: $F_{2,17} = 0.397$, $p = 0.6784$; LOOK: $F_{2,17} = 0.412$, $p = 0.6688$) and no interaction between these two factors (TIME: $F_{24,204} = 1.079$, $p = 0.3704$; LOOK: $F_{24,204} = 0.976$, $p = 0.4996$). The similarity of the behavior in the three groups is clearly illustrated by the average scores computed for the entire experiment (fig. 8C–D).

Rhythmic Cloacal Sphincter Movements

Similarly, the expression of RCSM in response to the visual presentation of a female was not affected by the lesions of the Tn or AIv (fig. 9). The number of RCSM produced in the presence or absence of a stimulus female was recorded once just before the surgery (lesions) and twice thereafter (days 6 and 14 post-lesion).

A general three-way ANOVA of these data (groups as independent factors, presence/absence of the female and successive tests as repeated factors) confirmed that the RCSM frequency is markedly and significantly increased when a female is visible (from 20.5 ± 2.8 to 129.8 ± 3.4, $F_{1,17} = 820.283$, $p < 0.0001$), but there was no effect of the two main factors and all primary or secondary interactions were non-significant ($p \geq 0.18$ in each case).

Consummatory Sexual Behavior

The frequencies of MA and CCM observed in the three experimental groups during the 13 behavior tests are illustrated in figure 10A, B together with the average frequencies throughout the entire experiment (fig. 10C, D). As in experiment 1, both MA and CCM were expressed more frequently in birds bearing a Tn lesions than in the two other groups, and these group differences were significant for both behavior patterns.

The two-way ANOVA of these data indeed confirmed the presence of significant group differences (MA: $F_{2,17} = 4.207$, $p = 0.0328$; CCM: $F_{2,17} = 5.865$, $p = 0.0116$). There was no change in time and no interaction of groups by time in the analysis of MA frequencies (time: $F_{12,204} = 1.394$, $p = 0.1708$; interaction: $F_{24,204} = 0.621$, $p = 0.9160$), but CCM frequencies increased significantly over time ($F_{12,204} = 2.313$, $p < 0.0087$). This increase was similar in the three experimental groups ($F_{24,204} = 0.476$, $p = 0.9829$). Post-hoc comparisons (Fisher PLSD test) indicated that birds with a bilateral lesion to the Tn expressed significantly more MA and CCM than the CTRL animals (MA: $p = 0.0156$; CCM: $p = 0.0044$) and more CCM than the AIv birds ($p = 0.0465$).
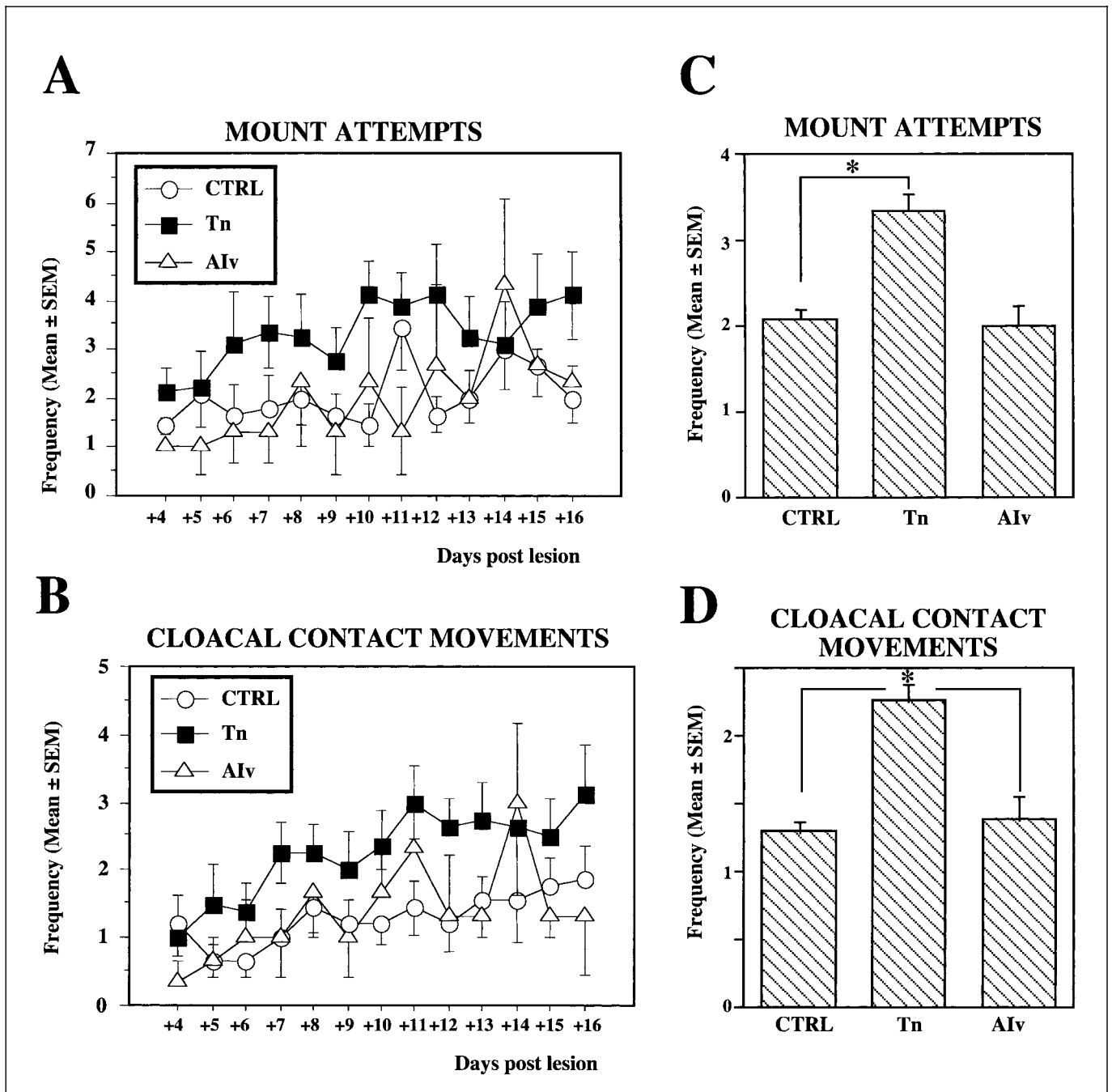
Fig. 10. Effects of lesions of the Tn or AIv on two measures (mount attempts (**A**, **C**) and cloacal contact movements (**B**, **D**)) of consummatory sexual behavior of male quail. Data for control (CTRL) birds bearing sham lesions are also illustrated. Data shown in **A** and **B** present the behavioral responses during the 16 tests that were performed after the lesions. Data shown in **C** and **D** represent the average responses in the 3 experimental groups during all these tests.
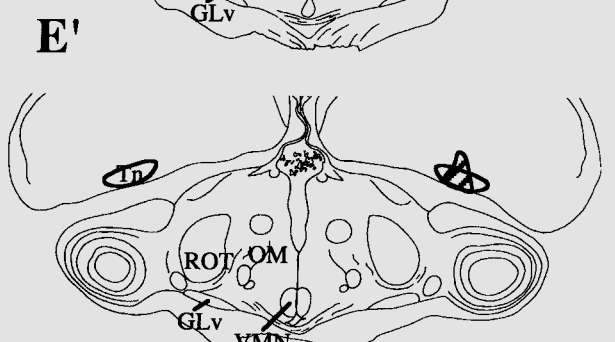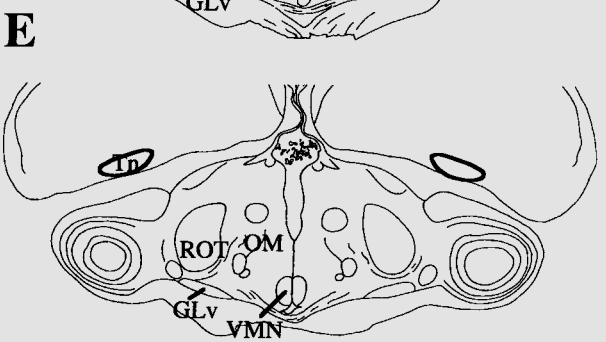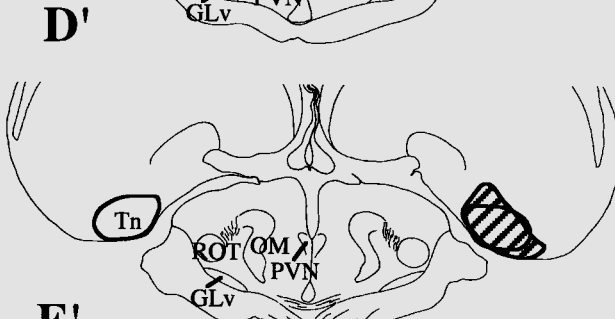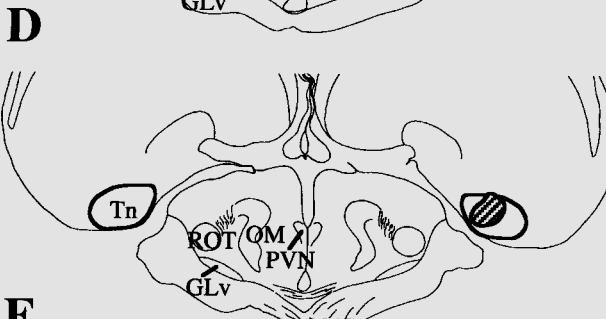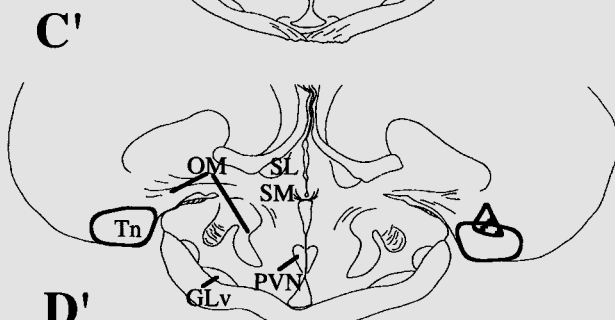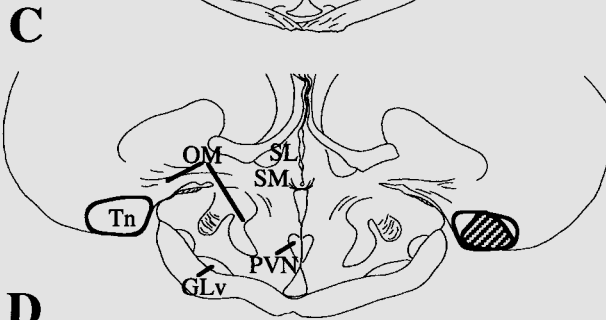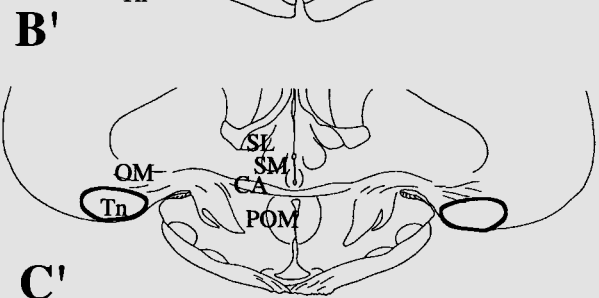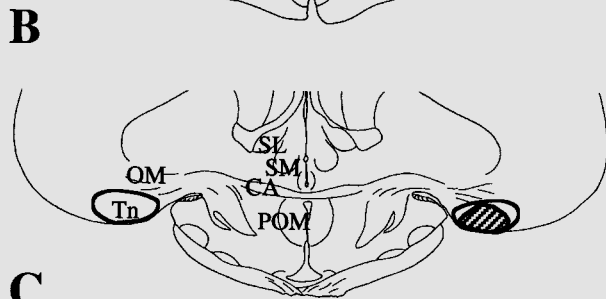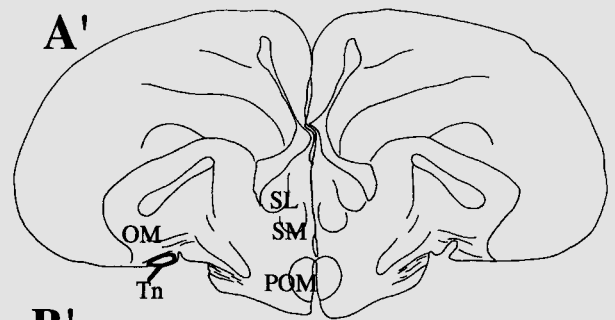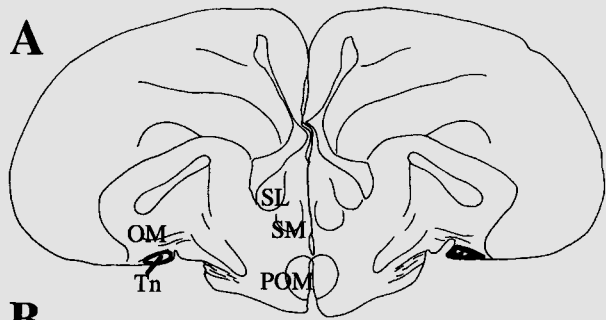
Discussion

In this study we found that lesions confined to the rostral part of the nucleus Tn, a discrete sub-region in the archistriatal complex of male quail, had no dramatic effect on two measures of appetitive male sexual responding, namely the RCSM and the learned social proximity response. Similarly, we found that lesions to an adjacent part of the archistriatum, AIv, also had no effect on these two measures of male appetitive sexual behavior. We did find that lesions to the rostral part of Tn facilitated two measures of male copulatory behavior namely MA and CCM in inexperienced birds and MA in experienced male quail. In contrast, lesions to AIv had no effect on our measures of male copulatory behavior. These findings raise several issues relevant to an understanding of the neuroendocrine control of male sexual behavior in quail and other species that we will discuss here.

In a previous study in male Japanese quail, bilateral electrolytic lesions directed at Tn produced results quite different from those observed in this study [Thompson et al., 1998]. Several measures indicative of appetitive male sexual responding, such as crowing in the absence of a female, the latency to approach a female and to neck grab a female (a first step in the copulation sequence), as well as the occurrence of RCSM in the presence of a female, were found to be reduced in birds subjected to bilateral Tn-lesions [Thompson et al., 1998]. The number of MA in the bilaterally Tn-lesioned birds as opposed to sham-operated controls was also found to be significantly reduced. In the present study, two measures of appetitive sexual behavior were not affected by the lesion manipulation, whereas measures of copulatory behavior such as MA were actually enhanced by the lesion. What might explain this apparent discrepancy in the results of these two studies? First, one could point to several methodological differences employed by the behavioral testing procedures. Various aspects of the stimulus conditions used to test

subjects were different in the two studies (size of chambers, distance of male subject from females, length of time of the studies, etc.). Also, the behavioral measures used were quite different in some cases. We used, for example, a learned social proximity response whereas Thompson et al. employed a somewhat different sexual conditioning procedure. However, in other cases the behavioral measures were quite similar between the two studies, such as the RCSM and the MA measures collected by both groups. It seems unlikely that these differences in behavioral procedures could be responsible for the very different results obtained in the two studies. The suite of behavioral measures used in both studies are broad indicators of sexual arousal characteristic of appetitive behaviors and copulatory behavior, and differences in testing parameters would not easily explain such very different results. A more likely explanation stems from the position of the lesions in the two studies. In the Thompson et al. study the Tn lesions were quite posterior and in most cases were associated with collateral damage in the surrounding archistriatum (fig. 11). In our study, the lesions of Tn were placed in the rostral part of the nucleus and did not extend beyond the borders of the nucleus in any case (fig. 11). The data from these two studies suggest that there are distinct sub-divisions of this nucleus that sub-serve different but related functions in the control of male sexual behavior.

Such divisions might be expected if Tn is indeed the avian homologue of the mammalian medial amygdala (Me). Tract-tracing studies in hamsters indicate that the anterior medial amygdala and posterior medial amygdala are differentially connected to other structures such as the bed nucleus of the stria terminalis (BST). Thus there are at least two parallel circuits of the so-called extended amygdala involved in different aspects of male sexual behavior [Wood and Newman, 1995b]. Wood and Newman [1995b] argue that the anterior medial amygdala is part of a chemosensory sub-circuit, whereas the posterior Me is part of a hormonally modulated sub-circuit. Subdivisons of Tn have yet to be identified in any avian species. Interestingly though, projections from the olfactory bulb have been identified in Tn in pigeons [Reiner and Karten, 1985]. It does appear from a careful examination of the figures in this study that the olfactory projections are more pronounced in the rostral part of the nucleus. Perhaps there are functional subdivisions in the quail Tn similar to those observed in the medial amygdala of hamsters. However, it is not necessarily the case that the rostral part of Tn corresponds to the rostral part of the Me, just that there are important functional differences between the rostral and caudal parts of the nucleus.

Fig. 11. Schematic representations of the Tn lesions produced in the present study (A-E; left) and in the previous study in quail of Thompson et al. (A′-E′; right). The boundaries of Tn are in all panels indicated by the continuous black line, the extent of the lesions is indicated by the hatched area. In each column, panels are organized in a rostral to caudal order. Abbreviations: CA: commissura anterior; GLv: nucleus geniculatus lateralis, pars ventralis; OM: tractus occipitomesencephalicus; POM: nucleus preopticus medialis; PVN: nucleus paraventricularis magnocellularis; ROT: nucleus rotundus; SL: nucleus septalis lateralis; SM: nucleus septalis medialis; Tn: nucleus taeniae; VMN: nucleus ventromedialis hypothalami. Magnification bar = 1 mm.

Based on the data presented in our study, it would seem that the rostral Tn is important in maintaining sexual satiety. Lesions to this area increase measures of copulatory behavior such as MA in experienced birds, and MA and CCM in inexperienced birds. Such a role for the medial amygdala has already been proposed in other species. Studies of the induction of the immediate early gene c-fos in male hamsters indicate that the caudal portion of the posterodorsal component of the medial amygdala along with its projection to the BST is particularly important for the onset of sexual satiety [Parfitt and Newman, 1998]. Lesions to the nucleus sphericus, a cell group in the posterior medial dorsal ventricular ridge in snakes and other reptiles that resembles the medial amygdala of mammals increases sexual behavior in garter snakes [Krohmer and Crews, 1987]. Perhaps the rostral Tn plays a similar role in regulating sexual satiety in quail as the medial amygdala and the nucleus sphericus do in hamsters and garter snakes, respectively.

If one combines the data from the current study with the study of Thompson et al. [1998] in quail and the study of Tn by Cheng et al. [1999] in female doves and male starlings, a coherent theory or circuitry organization can be proposed. The posterior Tn would be involved in sexual arousal and activation. Measures of both appetitive sexual responses and consummatory sexual responses were reduced in male quail that received lesions to this region [Thompson et al., 1998]. The anterior part of Tn appears to play a related role in mediating the onset of sexual satiety. In the study by Cheng et al. the behavioral analysis was not detailed but these authors did report an increase in nest-cooing behavior in female doves in the presence of males with lesions restricted to Tn. These authors attributed this effect to a reduction in fearfulness among the females making them more apt to exhibit such proceptive behaviors in response to the male. Alternatively, these birds might have exhibited an increase in nest-cooing because that part of a brain circuit regulating sexual satiety had been lesioned. Copulatory behavior per se was not measured in the Cheng et al. study. An examination of the lesion site in that study clearly indicates that the rostral Tn was damaged, although the lesion appears to extend into more posterior parts as well [see figure 15 in Cheng et al., 1999].

The mammalian amygdala is a complex structure that some have argued should not even be considered a single entity, given that some parts are more striatal in nature and other parts are more cortical [Swanson and Petrovich, 1988]. These authors argue that the term amygdala in mammals subsumes four distinct functional systems: accessory olfactory, main olfactory, autonomic and fronto-temporal cortical. These systems regulate a diverse range of functions including emotion, sexual motivation, fear and learned associations formed from these processes [Aggleton, 2000]. One of the challenges with investigating the Tn and the associated archistriatum in birds is that we still do not have a good theory about homologies between the archistriatum complex and the mammalian amygdalar complex. This makes interpreting the effects of the Tn lesions more difficult. For example, there is a substantial literature dating back to the 1960s indicating that lesions to various parts of the archistriatum reduces fearfulness in birds [e.g., Phillips, 1964; Martin et al., 1979]. As mentioned previously, the study of Tn lesions in doves suggested that some of the effects on sexual behavior could be due to a reduction in fearfulness [Cheng et al., 1999]. It is well known that amygdala lesions have similar effects in mammals, but these effects appear to be most pronounced when the central nucleus of the amygdala is lesioned or when the relevant sensory inputs to this nucleus via the lateral and basal lateral nucleus are damaged [Davis, 2000; LeDoux, 2000]. If we were confident that Tn is homologous to the central nucleus of the amygdala, we would be more likely to favor the hypothesis that the results observed in our study are not related to specific effects on sexual satiety, but rather are due to a reduction in fearfulness that results in an increase in copulatory rate. If one favors the hypothesis that Tn is similar to the medial amygdala then other interpretations of the data seem more likely.

There is increasing evidence that the medial amygdala is particularly involved in regulating male sexual behavior. Most of the detailed studies have been conducted in rodents where male sexual responses are heavily dependent on olfactory cues provided by females [Wood, 1997; Wood and Coolen, 1997; Coolen and Wood, 1999; Wood and Swann, 2000]. In quail, male responses to females are more dependent on visual and auditory cues [Balthazart and Ball, 1998]. Despite this fact, the Tn, a nucleus in birds that resembles the medial amygdala in many aspects, does also seem to play a role in regulating male sexual responses to females. As mentioned briefly in the introduction to this paper, several lines of evidence support the hypothesis that Tn is the avian equivalent of the mammalian medial amygdala. First, Tn is a medio-ventral sub-division of the archistriatum, a prominent sub-region of the avian telencephalon. Studies of the connectivity of the archistriatum in pigeons *(Columba livia)* led to the conclusion that medial and caudal portions of this structure are most apt to represent brain areas homolo-

gous to the mammalian amygdala [Zeier and Karten, 1971]. Tract-tracing studies conducted on ring doves and European starlings that focussed on Tn itself, rather than the surrounding archistriatum, found many similarities in both species between the connectivity of Tn and the amygdaloid complex in mammals, namely that there are sub-cortical sensory inputs and connections to the hippocampal complex [Cheng et al., 1999]. A variety of studies on sex steroid receptors indicate that the boundaries of Tn are clearly delineated by cells in this nucleus expressing either the protein or the mRNA for both androgens receptors (AR) and estrogen receptors (ER). For example, in Japanese quail autoradiographic studies employing [³H]-testosterone, [³H]dihydrotestosterone or [³H]17β-estradiol as the ligands all revealed a high density of cells binding these three hormones within Tn [Watson and Adkins-Regan, 1989]. Immunohistochemical studies of the AR or ER protein in quail produced comparable results [Balthazart et al., 1989, 1992, 1998a]. Recently, studies of the expression of mRNA for the β form of ER (ERβ) found that the mRNA for this hormone receptor type is also highly expressed in Tn of quail [Ball et al., 1999; Foidart et al., 1999]. Studies of AR, ERα and ERβ in other avian species besides quail have found similar patterns of high expression for the mRNA and/or protein of these important components of steroid hormone action in Tn [Arnold et al., 1976; Balthazart et al., 1992; Gahr et al., 1993; Ball et al., 1999; Bernard et al., 1999]. The medial amygdala in rats exhibits a similar pattern of expression for both of these three types of sex steroid receptors [Wood et al., 1992; Wood and Newman, 1995a; Kuiper et al., 1998]. However, a critical examination of the connectivity of this nucleus does not necessarily support the proposition that Tn is homologous to the mammalian Me. Tn does project to the hypothalamus and the preoptic region in quail and doves in a manner that is consistent with it being homologous to the Me [Thompson et al., 1998; Cheng et al., 1999]. However, in mammals the Me receives a major projection from the accessory olfactory bulb (a structure that is not present in birds), whereas the main olfactory bulb projects to the cortical nucleus of the amygdala [Swanson and Petrovich, 1988]. This has led some authors to doubt that Tn is homologous to the Me [Reiner and Karten, 1985]. Also relevant to our work here on the hormonal activation of sexual behavior, the distribution of the steroid metabolizing enzyme aromatase is more characteristic of the central nucleus of the amygdala than the Me [Naftolin et al., 2001]. Overall, the preponderance of evidence is consistent with the notion that Tn is the avian equivalent of at least certain parts of the mammalian amygdala, but making direct comparisons to distinct sub-regions of this nucleus in mammals should be done with caution.

The consequences of steroid action in the avian Tn are poorly understood but it must be mentioned that a recent study demonstrated that treatment of castrated male quail with testosterone increases the general metabolic activity in this nucleus as quantified by the uptake of 2-deoxyglucose [Balthazart et al., 2001]. As mentioned above, it is unclear whether the Tn of birds receives significant olfactory inputs, although it is suggested by tract-tracing studies [Reiner and Karten, 1985], but if so, then steroids might modulate the processing of this olfactory input even if it is greatly reduced compared to that observed in mammals. Such interactions between steroid hormone action and olfactory processing in the medial amygdala have been proposed to play a very important role in the regulation of male sexual behavior in hamsters [Wood and Swann, 2000]. In quail the modulation of visual and auditory cues provided by females might also be important although how such sensory information gets to Tn is still not well understood [Thompson et al., 1998]. There is some evidence that visual and auditory subcortical structures project to Tn in doves [Cheng et al., 1999]. Whether these projections represent the anatomical basis for an integration of these sensory and hormonal inputs in birds would require further investigation. Interestingly, the medial preoptic nucleus (POM) is bi-directionally connected to Tn [Balthazart and Absil, 1997] and lesions of the POM markedly reduce, but do not fully suppress, the effects of testosterone on the metabolic activity in Tn. Steroids could therefore affect Tn both directly through binding to receptors located in the nucleus and indirectly by controlling inputs from POM to Tn.

The functional study described in this paper, combined with previous studies on male quail [Thompson et al., 1998] and female ring doves [Cheng et al., 1999] indicate that Tn is important for male and female sexual responding. However, it is also becoming clear that the complexity of Tn in mediating different aspects of male sexual responding in quail will rival what has been described for the medial amygdala of hamsters. Both sexual arousal and sexual satiety are involved. What is needed now are more detailed anatomical studies combined with behavioral investigations to tease apart this complex circuit and identify the neurochemical and functional specialization of the different parts of this complex brain structure. These studies in quail will also contribute to the generation of a general model of the neurobiology of male sexual responding.

References

Adkins, E.K., and N.T. Adler (1972) Hormonal control of behavior in the Japanese quail. J. Comp. Physiol. Psychol., *81:* 27–36.

Adkins-Regan, E., V. Mansukhani, C. Seiwert, and R. Thompson (1994) Sexual differentiation of brain and behavior in the zebra finch: Critical periods for effects of early estrogen treatment. J. Neurobiol., *25:* 865–877.

Aggleton, J.P. (ed.) (2000) The Amygdala. Oxford University Press, Oxford, UK.

Arnold, A.P., F. Nottebohm, and D.W. Pfaff (1976) Hormone concentrating cells in vocal control areas of the brain of the zebra finch *(Poephila guttata)*. J.Comp.Neurol., *165:* 487–512.

Ball, G.F., D.J. Bernard, A. Foidart, B. Lakaye, and J. Balthazart (1999) Steroid sensitive sites in the avian brain: Does the distribution of the estrogen receptor alpha and beta types provide insight into their function? Brain Behav. Evol., *54:* 28–40.

Balthazart, J., and P. Absil (1997) Identification of catecholaminergic inputs to and outputs from aromatase-containing brain areas of the Japanese quail by tract tracing combined with tyrosine hydroxylase immunocytochemistry. J. Comp. Neurol., *382:* 401–428.

Balthazart, J., and G.F. Ball (1998) The Japanese quail as a model system for the investigation of steroid-catecholamine interactions mediating appetitive and consummatory aspects of male sexual behavior. Ann. Rev. Sex Res., *9:* 96–176.

Balthazart, J., and M. Schumacher (1984) Estradiol contributes to the postnatal demasculinization of female Japanese quail *(Coturnix coturnix japonica)*. Horm. Behav., *18:* 287–297.

Balthazart, J., P. Absil, M. Gérard, D. Appeltants, and G.F. Ball (1998b) Appetitive and consummatory male sexual behavior in Japanese quail are differentially regulated by subregions of the preoptic medial nucleus. J. Neurosci., *18:* 6512–6527.

Balthazart, J., C. Castagna, and G.F. Ball (1997) Aromatase inhibition blocks the activation and sexual differentiation of appetitive male sexual behavior in Japanese quail. Behav. Neurosci., *111:* 381–397.

Balthazart, J., A. Foidart, M. Houbart, G.S. Prins, and G.F. Ball (1998a) Distribution of androgen receptor-immunoreactive cells in the quail forebrain and their relationship with aromatase immunoreactivity. J. Neurobiol., *35:* 323–340.

Balthazart, J., A. Foidart, E.M. Wilson, and G.F. Ball (1992) Immunocytochemical localization of androgen receptors in the male songbird and quail brain. J. Comp. Neurol., *317:* 407–420.

Balthazart, J., M. Gahr, and C. Surlemont (1989) Distribution of estrogen receptors in the brain of the Japanese quail: an immunocytochemical study. Brain Res., *501:* 205–214.

Balthazart, J., J. Reid, P. Absil, A. Foidart, and G.F. Ball (1995) Appetitive as well as consummatory aspects of male sexual behavior in quail are activated by androgens and estrogens. Behav. Neurosci., *109:* 485–501.

Balthazart, J., A. Stamatakis, S. Bacola, P. Absil, and C.R. Dermon (2001) Effects of lesions of the medial preoptic nucleus on the testosterone-induced metabolic changes in specific brain areas in male quail. Neuroscience, *108:* 447–466.

Baum, M.J. (1995) Reassessing the role of medial preoptic area/anterior hypothalamic neurons in appetitive aspects of masculine sexual behavior. *In* The Pharmacology of Sexual Function and Dysfunction (ed. by J. Bancroft), Elsevier Science, Amsterdam, pp. 133–139.

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

Beach, F.A. (1956) Characteristics of masculine 'sex drive'. Nebraska Symposium on Motivation, *4:* 1–32.

Bernard, D.J., G.E. Bentley, J. Balthazart, F.W. Turek, and G.F. Ball (1999) Androgen receptor, estrogen receptor alpha, and estrogen receptor beta show distinct patterns of expression in forebrain song control nuclei of European starlings. Endocrinology, *140:* 4633–4643.

Castagna, C., G.F. Ball, and J. Balthazart (1997) Effects of dopamine agonists on appetitive and consummatory male sexual behavior in Japanese quail. Pharmacol. Biochem. Behav., *58:* 403–414.

Cheng, K.M., A.R. Hickman, and C.R. Nichols (1989a) Role of the proctodeal gland foam of male Japanese quail in natural copulations. The Auk, *106:* 279–285.

Cheng, K.M., R.F. McIntyre, and A.R. Hickman (1989b) Proctodeal gland foam enhances competitive fertilization in domestic Japanese quail. The Auk, *106:* 286–291.

Cheng, M.F., M. Chaiken, M. Zuo, and H. Miller (1999) Nucleus taenia of the amygdala of birds: Anatomical and functional studies in ring doves *(Streptopelia risoria)* and European starlings *(Sturnus vulgaris)*. Brain Behav. Evol., *53:* 243–270.

Coolen, L.M., and R.I. Wood (1999) Testosterone stimulation of the medial preoptic area and medial amygdala in the control of male hamster sexual behavior: redundancy without amplification. Behav. Brain Res., *98:* 143–153.

Crawford, L.L., K.S. Holloway, and M. Domjan (1993) The nature of sexual reinforcement. J. Exp. Anal. Behav., *60:* 55–66.

Davis, M. (2000) The role of the amygdala in conditioned and unconditioned fear and anxiety. *In* The Amygdala (ed. by J.P. Aggleton), Oxford University Press, Oxford, UK, pp. 213–287.

Delville, Y., J. Sulon, and J. Balthazart (1985) Hormonal correlates of gonadal regression and spontaneous recovery in Japanese quail exposed to short day-lengths. Arch. Int. Physiol. Biochem., *93:* 123–133.

Dominguez, J., J.V. Riolo, Z.J. Xu, and E.M. Hull (2001) Regulation by the medial amygdala of copulation and medial preoptic dopamine release. J. Neurosci., *21:* 349–355.

Domjan, M. (1987) Photoperiodic and endocrine control of social proximity behavior in male Japanese quail *(Coturnix coturnix japonica)*. Behav. Neurosci., *101:* 385–392.

Domjan, M., and S. Hall (1986a) Determinants of social proximity in Japanese quail *(Coturnix coturnix japonica)*: Male behavior. J. Comp. Psychol., *100:* 59–67.

Domjan, M., and S. Hall (1986b) Sexual dimorphism in the social proximity behavior of Japanese quail *(Coturnix coturnix japonica)*. J. Comp. Psychol., *100:* 68–71.

Domjan, M., and S. Nash (1988) Stimulus control of social behaviour in male Japanese quail. Anim. Behav., *36:* 1006–1015.

Domjan, M., R. Lyons, N. C. North, and J. Bruell (1986) Sexual pavlovian conditioned approach behavior in male Japanese quail *(Coturnix coturnix japonica)*. J. Comp. Psychol., *100:* 413–421.

Domjan, M.J. (1994) Formulation of a behavior system for sexual conditioning. Psychonom. Bull. Rev. *1:* 421–428.

Du, J.F., D.S. Lorrain, and E.M. Hull (1998) Castration decreases extracellular, but increases intracellular, dopamine in medial preoptic area of male rats. Brain Res., *782:* 11–17.

Everitt, B.J. (1990) Sexual motivation: A neural and behavioural analysis of the mechanisms underlying appetitive and copulatory responses in male rats. Neurosci. Biobehav. Rev., *14:* 217–232.

Everitt, B.J. (1995) Neuroendocrine mechanisms underlying appetitive and consummatory elements of masculine sexual behavior. *In* The Pharmacology of Sexual Function and Dysfunction (ed. by J. Bancroft), Elsevier, Amsterdam, pp. 15–31.

Foidart, A., B. Lakaye, T. Grisar, G.F. Ball, and J. Balthazart (1999) Estrogen receptor-beta in quail: Cloning, tissue expression and neuroanatomical distribution. J. Neurobiol., *40:* 327–342.

Follett, B.K., and S.L. Maung (1978) Rate of testicular maturation, in relation to gonadotrophin and testosterone levels, in quail exposed to various artificial potoperiods and to natural daylengths. J. Endocrinol., *78:* 267–280.

Gahr, M., H.-R. Güttinger, and D.E. Kroodsma (1993) Estrogen receptors in the avian brain: Survey reveals general distribution and forebrain areas unique to songbirds. J. Comp. Neurol., *327:* 112–122.

Hull, E.M. (1995) Dopaminergic influences on male rat sexual behavior. *In* Neurobiological Effects of Sex Steroid Hormones. (ed. by P. E. Micevych and R.P.J. Hammer), Cambridge University Press, Cambridge UK, pp. 234–253.

Hull, E.M., R.L. Meisel, and B.D. Sachs (2002) Male sexual behavior. *In* Hormones, Brain and Behavior (ed. by D.W. Pfaff), Academic Press, San Diego, CA.

Hull, E.M., J.F. Du, D.S. Lorrain, and L. Matuszewich (1997) Testosterone, preoptic dopamine, and copulation in male rats. Brain Res. Bull., *44:*327–333.

Hutchison, R.E. (1978) Hormonal differentiation of sexual behavior in Japanese quail. Horm. Behav., *11:* 363–387.

Krohmer, R.W., and D. Crews (1987) Facilitation of courtship behavior in the male red-sided garter snake *(Thamnophis sirtalis parietalis)* following lesions of the septum or nucleus sphericus. Physiol. Behav., *40:* 759–765.

Kuiper, G.G.J.M., P.J. Shughrue, I. Merchenthaler, and J.-Å. Gustafsson (1998) The estrogen receptor b subtype: a novel mediator of estrogen action in neuroendocrine systems. Front. Neuroendocrinol., *19:* 253–286.

LeDoux, J.E. (2000) The amygdala and emotion. *In* The Amygdala (ed. by J.P. Aggleton), Oxford University Press, Oxford, UK, pp. 289–310.

Martin, J.T., N. DeLanerolle, and R.E. Phillips (1979) Avian archistriatal control of fear-motivated behaviour and adrenocortical function. Behav. Processes, *4:* 283–293.

Martin, P., and P. Bateson (1986) Measuring Behaviour. An Introductory Guide, Cambridge University Press, Cambridge, UK.

Naftolin, F., T.L. Horvath, and J. Balthazart (2001) Estrogen synthetase (aromatase) immunohistochemistry reveals concordance between avian and rodent limbic systems and hypothalami. Proc. Soc. Exp. Biol. Med., *226:* 717–725.

Paredes, R.G., T. Tzschentke, and N. Nakach (1998) Lesions of the medial preoptic area anterior hypothalamus (MPOA/AH) modify partner preference in male rats. Brain Res., *813:* 1–8.

Parfitt, D.B., and S.W. Newman (1998) Fos-immunoreactivity within the extended amygdala is correlated with the onset of sexual satiety. Horm. Behav., *34:* 17–29.

Pfaus, J.G. (1996) Frank A. Beach Award – Homologies of animal and human sexual behaviors. Horm. Behav., *30:* 187–200.

Pfaus, J.G., T.E. Kippin, and S. Centeno (2001) Conditioning and sexual behavior: A review. Horm Behav., *40:* 291–321.

Phillips, R.E. (1964) Wildness in the mallardduck. Effects of brain lesions and stimulation on escape behavior and reproduction. J. Comp. Neurol., *122:* 139–155.

Reiner, A., and H.J. Karten (1985) Comparison of olfactory bulb projections in pigeons and turtles. Brain Behav. Evol., *27:* 11–27.

Sachs, B.D. (1967) Photoperiodic control of the cloacal gland of the Japanese quail. Science, *157:* 201–203.

Sachs, B.D. (1983) Potency and fertility: hormonal and mechanical causes and effects of penile actions in rats. *In* Hormones and Behaviour in Higher Vertebrates. (ed. by J. Balthazart, E. Pröve and R. Gilles), Springer-Verlag, Berlin, pp. 86–110.

Sachs, B.D. (1995) Context-sensitive variation in the regulation of erection. *In* The Pharmacology of Sexual Function and Dysfunction (ed. by J. Bancroft), Elsevier, Amsterdam, pp. 97–108.

Seiwert, C.M. (1994) The Neuromuscular System Controlling Foam Production in Japanese Quail: An Investigation of Structure and Function, Cornell University, Ithaca, NY.

Seiwert, C.M., and E. Adkins-Regan (1998) The foam production system of the male Japanese quail: Characterization of structure and function. Brain Behav. Evol., *52:* 61–80.

Swanson, L.W., and G.D. Petrovich (1988) What is the amygdala? Trends Neurosci, *21:* 323–331.

Thompson, R.R., J.L. Goodson, M.G. Ruscio, and E. Adkins-Regan (1998) Role of the archistriatal nucleus taeniae in the sexual behavior of male Japanese quail (*Coturnix japonica*): A comparison of function with the medial nucleus of the amygdala in mammals. Brain Behav. Evol., *51:* 215–229.

Timberlake, W., and K.M. Silva (1995) Appetitive behavior in ethology, psychology, and behavior systems. *In* Perspectives in Ethology, Volume 11: Behavioral Design. (ed. by N.S. Thompson), Plenum Press, New York, pp. 211–253.

Watson, J.T., and E. Adkins-Regan (1989) Neuroanatomical localization of sex steroid-concentrating cells in the Japanese quail *(Coturnix japonica)*: Autoradiography with [3H]-testosterone, [3H]-estradiol, and [3H]-dihydrotestosterone. Neuroendocrinology, *49:* 51–64.

Wood, R.I. (1997) Thinking about networks in the control of male hamster sexual behavior. Horm. Behav., *32:* 40–45.

Wood, R.I., R.K. Brabec, J.M. Swann, and S.W. Newman (1992) Androgen and estrogen concentrating neurons in chemosensory pathways of the male Syrian hamster brain. Brain Res., *596:* 89–98.

Wood, R.I., and L.M. Coolen (1997) Integration of chemosensory and hormonal cues is essential for sexual behaviour in the male Syrian hamster: Role of the medial amygdaloid nucleus. Neuroscience, *78:*1027–1035.

Wood, R.I., and S.W. Newman (1995a) Androgen and estrogen receptors coexist within individual neurons in the brain of the syrian hamster. Neuroendocrinology, *62:* 487–497.

Wood, R.I., and S.W. Newman (1995b) Hormonal influence on neurons of the mating behavior pathway in male hamsters. *In* Neurobiological Effects of Sex Steroid Hormones (ed. by P.E. Micevych and R.P.J. Hammer), Cambridge University Press, Cambridge, UK, pp. 3–39.

Wood, R.I., and J.M. Swann (2000) Neuronal integration of chemosensory and hormonal signals in the control of male sexual behavior. *In* Reproduction in Context (ed. by K. Wallen and J.E. Schneider), MIT Press, Cambridge, MA, pp. 423–444.

Zeier, H., and H.J. Karten (1971) The archistriatum of the pigeon: organization of afferent and efferent connections. Brain Res., *31:* 313–326.