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Subpallial and hypothalamic areas activated following sexual and agonistic encounters in male chickens

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ABSTRACT

Male sexual and agonistic behaviors are controlled by the common social behavior network, involving subpallial and hypothalamic brain areas. In order to understand how this common network generates different behavioral outcomes, induction of FOS protein was used to examine the patterns of neuronal activation in adult male chickens following interaction with a female or a male. Males were subjected to one of the following treatments: handling control, non-contact interaction with a female, contact interaction with a live female, a taxidermy female model or another male. The number of FOS-immunoreactive (FOS-ir) cells, and the area and immunostaining density of individual cells were quantified in the medial preoptic nucleus (POM), medial extended amygdala (nucleus taeniae of the amygdala, TnA, and dorsolateral and ventromedial subdivisions of the medial portion of the bed nucleus of stria terminalis, BSTM1 and BSTM2, respectively), lateral septum (SL), hypothalamic paraventricular nucleus (PVN), bed nucleus of the pallial commissure (NCPa) and ventrolateral thalamic nucleus (VLT). An increase in FOS-ir cells following appetitive sexual behavior was found in BSTM2 and NCPa. Copulation augmented FOS-ir in POM, SL, VLT, and PVN. Intermale interactions increased FOS-ir in all examined brain regions except the TnA and BSTM. Within the SL, copulatory and agonistic behavior activated spatially segregated cell groups. In the PVN, different social behaviors induced significant changes in the distribution of FOS-ir cell sizes suggesting activation of heterogeneous subpopulations of cells. Collectively, behavioral outcomes of male–female and male–male interactions are associated with a combination of common and site-specific patterns of neural activation.

1. Introduction

Mating behavior and aggression are two important components of social behavior that are indispensable for the survival of individuals and species. Mating and aggressive behaviors are not always unequivocally distinct and there is considerable overlap between sexual and aggressive displays. Numerous studies involving many vertebrate species, in particular birds and teleost fish, have reported that some male courtship and aggressive displays are virtually indistinguishable [1–5]. Involvement of aggression in precopulatory courtship behavior has been considered important for eliciting sexual excitement [6]. Classical ethologists interpreted these observations as activation of common motor patterns elicited by different combinations of conflicting motivations: sexual, attacking and fleeing motivations during courtship, and attacking and fleeing motivations during agonistic encounters [1,5]. Subsequent mechanistic studies have focused on neuroendocrine regulation of either sexual or aggressive behavior; however, no specific neural mechanism has been proposed to explain the observed interconnections between sexual and aggressive behavior.

An integrated neural mechanism is substantiated since mating and aggressive behaviors in males of many vertebrate species are associated with testicular and intracerebral synthesis of steroid hormones. Testosterone and its estrogen metabolites are preferentially accumulated by hypothalamic and subpallial neurons, which exert activational effects on male-typical behavior [7]. These steroid-sensitive neural pathways were proposed to comprise a unitary brain network regulating various aspects of social behavior in vertebrates [8,9]. In birds, efforts have been made to define patterns of neural activation induced by sexual and agonistic encounters in various brain areas which constitute the putative avian social behavior network [10–13]. Studies utilizing immediate early gene (IEG) transcripts and protein products resulted in important insights into behavioral functions of specific neuronal groups in birds. Comparative analysis of IEG patterns induced by mating and agonistic behaviors, however, is confounded since male–female and male–male encounters were studied in different avian species that differed in their social organization and mating systems. In order to identify cell groups important for the control of mating and agonistic behavior, the present study used FOS protein immunohistochemistry to examine changes in neural activation patterns within subpallial and hypothalamic brain areas of adult male

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chickens following interaction with a female or a male. We also attempted to differentiate between neural activations associated with courting (appetitive) and copulatory (consummatory) phases of mating behavior by providing a male with restricted access to a female placed behind a transparent barrier or by allowing full access to a female.

It has long been known that male chickens as well as some other gallinaceous and non-gallinaceous birds do not mate with females at random thereby exhibit preferential mating [14,15]. In the experimental paradigm used in this study as well as in many other studies dealing with sexual behavior, each male was presented with a different female. It is likely that different females could possess different qualities and behavioral idiosyncrasies, and therefore elicit different motivational and/or behavioral responses of the male. Hence variable activation of the brain can be expected. We addressed this issue by presenting the male with full access to a "standard" stimulus — a taxidermy mount of the adult female chicken soliciting copulation. We hypothesized that if the taxidermy female model is as powerful a behavioral releaser as the live female, both the taxidermy model and the live female will induce similar patterns of FOS protein in a male's brain structures but within-group variability of FOS-immunoreactive cell counts will be less prominent in males interacting with the taxidermy model.

2. Materials and methods

2.1. Subjects

Adult male and female broiler chickens *Gallus gallus* were used in this study. They were raised in same-sex floor pens. At the age of twenty weeks males were transferred to individual battery cages while females remained in large pens until thirty weeks of age when they were placed into smaller pens (1.75 × 1.84 m, 8 birds/pen) equipped with nest boxes. All pens were located in the same room with temperature set at 21 °C and the photoperiod was maintained at 16 h light and 8 h dark with lights on at 5 AM. Intensity of light was set at 20–40 lx. All birds received a commercial diet and were feed-restricted daily in compliance with a commercial poultry management guide for broiler breeders. Water was available *ad libitum*. Body weight of all birds was monitored on a weekly basis. All males received some sexual experience by placing them for several hours in pens with females, one male per pen. Each male was rotated to a different group of females at least two times. Each male was placed in a pen housing a group of eight females and his mating activity was scored for 20 min on two separate days. Only males who copulated at least once during each 20-min period were used in the experiment described below. All procedures and experimental protocols involving animals were approved by the University of Arkansas Institutional Animal Care and Use Committee.

2.2. Treatments

Selected males were randomly allocated to one of the following treatment groups ($n = 6$/group) and submitted to a 20-min long behavioral test: (1) males presented with an adult female placed in a transparent chamber (43 × 43 × 74 cm, length × width × height) in the middle of a test arena (male–female non-contact interaction, M–FN), (2) males presented with a taxidermy mount of an adult female in a crouching (receptive) position (male–taxidermy female interaction, M–FT), (3) males presented with an adult female (male–female contact interaction, M–FC), and (4) males presented with another randomly selected male (male–male interaction, M–M). All behavioral tests were performed in the same room divided into two parts by a screen with one-way mirror glass. The larger room portion contained two visually isolated pens (1.75 × 1.84 m), one of which was used for behavioral testing. The smaller part of the room accommodated an observer and the video recording equipment. The taxidermy model was cleaned after each test. At the end of observation periods, each male was returned to its home cage for 60 min before being administered an overdose of anesthetic and perfused with heparinized saline followed by a fixative. An additional group of males served as handling controls (CON). They were taken out of their cages, carried to the observation room but without entering it and then returned to their cages for 80 min before being anesthetized and perfused. Blood samples were taken from the brachial vein 60 min after completion of the behavioral testing. Timing of blood sampling was chosen to avoid any possible interference of sampling procedure with effects of social interactions on FOS expression. Plasma was separated and stored at −20 °C until assayed for testosterone. All treatments were conducted between 1 PM and 5 PM.

All behavioral tests were video-recorded using a color video camera (WV-CP480, Panasonic, Secaucus, NJ) connected via a digital video converter (model ADVC110, Canopus Corp., San Jose, CA) to a computer running The Observer XT 7.0 software (Noldus IT Inc., Leesburg, VA). After completion of all tests, behavior of males was scored from recorded video clips using The Observer XT software. Behavior was analyzed over the first 15 min of the observation period because the last 5 min of behavioral tests were found to be less eventful. The frequency and latency of mating and agonistic displays were determined. Brief descriptions of behavioral displays are given in Table 1.

2.3. Immunohistochemistry

Sixty min after completion of behavioral tests, each male was deeply anesthetized with sodium pentobarbital solution (40 mg/kg *i.v.*). They were perfused via carotid arteries with 200 ml heparinized phosphate

Table 1
Sexual and agonistic displays used to quantify behavior of males.

Sexual behavior	
Courtship behavior	
Waltzing	The male half-circles around the female with the outer side wing directed toward the ground.
Tidbitting	The male repeatedly pecks and/or scratches at the ground (litter).
Copulatory behavior	
Mounting attempt	The male puts one foot on the back of the female but does not proceed with full mounting.
Mounting	The male places both feet on the back of a crouching female.
Tailbending	The male depresses and repeatedly bends his tail downwards while standing on the female's back, apparently achieving cloacal contact; this normally indicates a successful copulation.
Agonistic behavior	
Offensive behavior	
Waltzing	The male half-circles around the male opponent with the outer side wing directed toward the ground; this display resembles courtship waltzing addressed to a female.
Threatening	The male stands in front of another male with its neck and head raised, hackle feather ruffled and wings slightly extended.
Chasing	The male runs after the other male.
Pecking	The male pecks the opponent's body or head.
Chest-fighting (chest bumps)	Males jump toward each other and collide in the air with their chests.
Leaping	The male jumps toward his opponent while the opponent flees.
Defensive behavior	
Avoidance	The male prevents the occurrence of the attack from the other male by running or walking away.
Hiding in the corner	The male lays on the ground facing the corner with his head lowered as a result of being defeated in the encounter.
Other behaviors	
Crowing	The male stretches up its neck and vocalizes loudly.
Wing flapping	The male flaps its wings vigorously.
Scratching the litter	While standing, the male scratches the litter or ground with one foot at a time.