Published in final edited form as:

Horm Behav. 2010 April ; 57(4-5): 515–522. doi:10.1016/j.yhbeh.2010.02.010.

Effects of social experience on subsequent sexual performance in naïve male Japanese quail (*Coturnix japonica*)

Charlotte A. Cornil^{1,2} and Gregory F. Ball¹

¹ Department of Psychological and Brain Sciences, Johns Hopkins University, Baltimore, USA

² GIGA Neurosciences, University of Liège, Liège, Belgium

Abstract

On their first sexual encounter, naïve male Japanese quail will attend to and approach a female; they sometimes mount but they do not always copulate. During the second encounter, most males successfully copulate. Although sexual experience facilitates subsequent sexual interactions, sensory cues provided by females, independent of any sexual encounter, may also enhance sexual performance. To investigate whether previous exposure to a conspecific affects subsequent sexual behavior, we allowed inexperienced males to observe an empty box, or a conspecific consisting of either an experienced female or male for 2.5 min/day on 7 days. Measures of appetitive sexual behavior were recorded during these tests. On day 8, subjects were allowed to copulate with a novel female for 5 min. On the following days, all subjects were repeatedly provided with visual access to a female and allowed to mate. In the pre-copulatory trials males initially exhibited a high frequency of appetitive responses that dissipated with repetition. Pre-copulatory experience also significantly affected motivation to mate with subjects exposed to females copulating more quickly than other subjects. Post-copulatory appetitive behavior also differed between groups: control subjects showed the highest behavioral frequency followed by males exposed to females and finally males exposed to males. These data indicate that pre-copulatory social experience profoundly influences subsequent sexual behavior and probably reproductive success. This experience effect is independent of any hormonal effect (such as one resulting from changes in secretion following different social interactions) given that the subjects were castrates chronically treated with testosterone.

Keywords

Japanese quail; Male sexual behavior; Sexual experience; Puberty; Reproductive success; Visual experience

Introduction

Sexual experience in males results in an increased efficiency in reproductive behaviors, as evidenced by improved copulatory abilities, decreased latencies to approach and mate, as well as enhanced responses to female-related stimuli (strengthened partner-preference; Domjan, et al., 1992; Pfaus, et al., 2001; Rosenblatt, 1965). Conditioning experiments with a variety of

Corresponding author: Charlotte A. Cornil, University of Liège, GIGA Neurosciences, Behavioral Neuroendocrinology Research Group, 1 avenue de l'hôpital (Bat. B36), Sart-Tilman, B-4000 Liège, Belgium. Charlotte.Cornil@ulg.ac.be.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

vertebrate species have demonstrated that pairing an arbitrary stimulus or a particular context with the opportunity to observe or to copulate with a female results in decreased latencies to copulate (Domjan, et al., 1986; Zamble, et al., 1985), increased semen volume and sperm release during ejaculation (Domjan, et al., 1998), increased number of fertilized eggs (Adkins-Regan and Mackillop, 2003; Mahometa and Domjan, 2005) and increased number of offspring (Hollis, et al., 1997; Matthews, et al., 2007). Even watching conspecifics of both sexes engaging in sexual or non-sexual behaviors provides information that can affect the occurrence of subsequent reproductive behaviors (Galef and White, 2000). Animals thus learn something from their social as well as mating environment and what is learnt may have a significant influence on their subsequent reproductive success. However, all these learning abilities that have been described in species as diverse as blue gouramis, quail and rats, and their functional consequences seem to depend on subjects being able to perform the copulatory sequence (since (1), when naïve males were used, copulation served as the unconditioned stimulus [US] and (2), in the case where the US consisted of a non-copulatory exposure to a female, males had to have had at least one previous copulatory experience). An important related question is thus whether individuals also learn something from their social environment before their first sexual encounter that can affect their future sexual behavior.

Japanese quail, like many other avian species, predominantly utilize visual and auditory cues to mediate social interactions (Balthazart and Ball, 1998; Lehrman, 1965; Mills, et al., 1997). On their first encounter, naïve male quail will usually attend to and approach the female. Most are able to grab her neck but they do not always succeed in completing copulation (about 60% of naïve males do achieve a full copulatory sequence on the first copulatory opportunity; personal observation). In the second encounter, most males successfully perform the sequence of male-typical copulatory behaviors characteristic for the species (i.e. neck grab, mount attempts, mount and cloacal contact movements; Hutchison, 1978) suggesting that the experience occurring during the first non-copulatory sexual encounters provide some information to naïve subjects that will improve their sexual performance during subsequent opportunities for sexual interactions.

The influence of pre-copulatory environmental or social cues on sexual behavior has rarely been investigated. In the present study, we sought to determine whether exposure to a female, prior to the very first copulation, confers a behavioral advantage over males pre-exposed to a male or simply handled as controls. Sexually inexperienced males were randomly assigned to three different groups that received (1) primarily visual exposure to a sexually experienced female, (2) a sexually experienced male or (3) an empty box (controls) during 2.5 min for 7 consecutive days (Phase 1). On subsequent days, all animals were tested for the appetitive and consummatory aspects of male sexual behavior (Phase 2). Appetitive behavior was assessed by measuring rhythmic cloacal sphincter movements (RCSM), a response displayed by sexually motivated males when they are presented with the view of a female (Seiwert, 1994; Seiwert and Adkins-Regan, 1998). Knowing that naïve male quail respond differentially to the visual presentation of males or females (Seiwert and Adkins-Regan, 1998), it is anticipated that naïve subjects will produce more cloacal contractions in response to the presentation of a female than the presentation of a male or of an empty cage in the pre-copulatory phase (Phase 1). If pre-copulatory exposure provides information preparing the male for future sexual encounters, males pre-exposed to a female should subsequently copulate at a higher rate and with a shorter latency than subjects from the two other groups. Similar to what has been described in other species, social interactions influence circulating concentrations of steroid hormones in quail (Cornil, et al., 2009; Delville, et al., 1984). To test whether the observed effects depend on gonadal steroid hormone changes following social interactions, subjects were castrated and chronically treated with testosterone capsules to restore sexual behavior.

Methods

Subjects

Twenty-one 8 week old adult male Japanese quail (*Coturnix japonica*) served as subjects. All subjects were experimentally and sexually naïve prior to experimental procedures. Adult and sexually experienced female (n = 22) and male (n = 7) Japanese quail served as stimuli. All animals were acquired from a local breeder. Experimental subjects were acquired at approximately 3 weeks of age and were housed as a group in large cages (91-cm long, 46-cm wide and 46-cm high), physically and visually separated from male and female stimuli. Stimulus males were housed in individual cages (25-cm long, 18-cm wide and 18-cm high), while female stimuli were housed in a single larger brooder as a group. All birds were housed in the same room and thus experienced to auditory cues produced by the other birds in the room. Throughout their life in the laboratory, birds were maintained on a photoperiod simulating long summer days (14L:10D). Food and water were available *ad libitum*. All experimental protocols were approved by and carried out under the guidelines laid down by the Johns Hopkins University Animal Care and Use Committee.

Surgical Procedures & hormonal treatments

Two or three days after their arrival, all subjects were deeply anesthetized (Secobarbital, 15 mg/kg) and both testes were removed through a unilateral incision below the last rib. Ten days later (between 5 and 6 weeks of age), subjects were implanted subcutaneously in the neck region with one testosterone filled Silastic™ capsule (Silclear™ Tubing, Degania Silicone Ltd., Degania Bet, 15130, Israel; 1.57 mm i.d.; 2.41 mm o.d.; length = 20 mm) after topical application of Lidocaine. Immediately after the capsule implantation, subjects were isolated in individual wire-mesh cages allowing visual access to their neighbors. Subjects were thus accustomed to the view of conspecific males. Throughout the experiment, subjects were periodically weighted to the nearest gram and the size of their cloacal gland, an androgen-dependent structure (Sachs, 1967), was measured with calipers (cloacal gland area = largest length × largest width in mm²). These data confirmed the efficacy of the testosterone replacement and the general health condition of the subjects.

Behavioral quantification

All behavioral manipulations were conducted in the same room. For each manipulation, subjects were transported from the aviary to the testing room in a bird carrier (box with 6 compartments designed for pigeons). They remained in the carrier until placed in the test arena.

Appetitive sexual behavior – the rhythmic cloacal sphincter movements (RCSM)

—Male quail possess a large sexually dimorphic, androgen-sensitive, external protuberance of the caudal lip of the cloaca, the cloacal or proctodeal gland (Sachs, 1967). This gland produces a stiff meringue-like foam that is transferred, along with semen, into the female's cloaca during copulation and are also deposited on top of the excreta during voiding (Seiwert and Adkins-Regan, 1998). This foam is thought to enhance the male fertilization success (Cheng, et al., 1989). Rhythmic cloacal sphincter movements (RCSM) occur independently of voiding or copulation and are greatly facilitated by the view of a female. These movements whip mucoproteins secreted by the gland into the foam that will be transferred to the female's cloaca during copulation. It has been shown previously that the frequency of RCSM immediately increases in gonadally intact, sexually active males when they are provided with visual access to a female (Absil, et al., 2002; Seiwert and Adkins-Regan, 1998). The quantification of the RCSM frequency in reaction to the visual presentation of a female is thus considered as a reliable measure of the male's appetitive sexual behavior (Cornil, et al., 2006).

The frequency of RSCSM was quantified by placing subjects in an aquarium (40-cm long $\times$ 20-cm wide $\times$ 25-cm high) located on a raised platform. A mirror was located under the aquarium at a 45° angle and provided the observer with an unobstructed view of the male's cloacal area. The aquarium was divided in two equal compartments defined by a plexi-glass (Fig. 1). The ceiling was made of plastic-board with holes allowing ventilation of the aquarium. At the beginning of each trial, an opaque board was placed along the plexi-glass partition to prevent the experimental and stimulus animals placed in each compartment from seeing each other. It was then removed to allow visual presentation of the stimulus bird or an empty aquarium. During trials, a piece of opaque board was attached to the exterior of the glass wall facing the experimenter to prevent the subject from being distracted by the presence of the observer. The experimental male was placed in one of the compartments and a stimulus egg-laying female or a sexually mature male was placed in the other compartment. The male had then visual access to the empty compartment, a female or a male although he could not physically interact with them. The RSCSM frequency was quantified for two periods of 2.5 min, first, in the absence of any stimulus in the other stimulus compartment (basal RSCSM) and then with one of the three experimental stimuli (socially-elicited RSCSM). It should be noticed that the present apparatus does not allow the isolation of visual from auditory cues emitted by the conspecific stimulus. However, birds rarely vocalize in these conditions and, when they do, stimulus vocalizations do not seem to elicit RSCSM in subjects. The stimulus is thus considered to be primarily visual.

Consummatory sexual behavior—The subject was introduced into a test arena with a floor covered with cedar chip bedding (60 $\times$ 40 $\times$ 50 cm) that contained a sexually mature female with which the male could freely interact for the duration of the test (5 min). The frequency and latency of first occurrence of the following behavior patterns were systematically recorded for the 5 min of interaction: strut, neck-grab (NG), mount attempt (MA), mount (M) and cloacal contact movements (CCM) (For a detailed description, see (Adkins and Adler, 1972; Hutchison, 1978). When a subject did not express a behavior within the cut-off period of 5 min, it was assigned a maximal latency of 300 seconds. Copulatory efficiency was evaluated by the copulatory efficiency ratio (expressed in %) obtained by dividing the total number of CCM by the total number of NG and then multiplying by 100. Using such index, an animal displaying as many CCM as NG scored a maximum of 100%. Lower scores illustrate that repeated neck grabs were necessary before they could complete the full copulatory sequence.

Experimental procedures

Birds were randomly assigned to 3 different groups. The experiment was conducted in two phases (Fig. 2). Phase 1 (Day 1 to 7) consisted of seven pre-copulatory trials conducted daily for 7 consecutive days in the glass aquarium where subjects received exposure through a glass partition to strictly one type of social stimulus. The members of the “Male” group were exposed to an unfamiliar and sexually experienced male ($n = 7$), while subjects belonging to the “Female” group were exposed to an unfamiliar and sexually experienced female ($n = 7$). Finally, the members of the control group were exposed to an empty box ($n = 7$). Within a given experimental group, the order of testing and the individual presented to each subject was different on each day. The rate of RSCSMs was first recorded during the pre-test period (2.5 min, Basal RSCSMs) and then during the presentation to a conspecific stimulus or to the empty box (2.5 min, Socially-elicited RSCSMs).

In phase 2, the effect of this pre-copulatory exposure on appetitive and consummatory sexual behaviors was tested. On day 8, all subjects were given an opportunity to copulate for 5 min with a sexually experienced female that they had not met before. On the following days, subjects were offered alternatively days with a copulatory test only (Fig. 2, *black blocks*) and

days with a RCSM test (Fig. 2, *white blocks*) with a female followed later (several hours) in the day by a copulatory test.

Statistical Analysis—Unless otherwise mentioned, all results were analyzed by mixed design two-way Analysis of Variance (ANOVA) with the behavioral treatment as the independent factor (three experimental groups) and the trials (one per day) as the repeated factor. Effects were considered significant based on a probability of $p \leq 0.05$. Planned comparisons were performed on the repeated factors and LSD pairwise comparisons were performed on the independent factor.

Results

Rhythmic cloacal sphincter movement (RCSM) frequency in sexually naïve animals

The repeated presentation of different social cues resulted in different patterns of cloacal responses (Fig. 3). As predicted, RCSM frequencies displayed in the absence of a stimulus (basal RCSM) were very low and relatively similar between groups (Fig. 3A). A mixed ANOVA with pre-copulatory experience as the independent factor and the 7 trials as the repeated measure showed no significant group effect on basal RCSM ($F_{2,18} = 2.335$, $p = 0.125$). In contrast, males exposed to a social stimulus exhibited a slight increase in basal RCSM frequency across trials as indicated by the trial effect and the interaction detected between groups and trials (Fig. 3B; $F_{6,108} = 3.184$, $p = 0.006$ and $F_{12,108} = 2.465$, $p = 0.007$, respectively).

Each group showed a different pattern of response to the visual presentation of a conspecific stimulus. In the “female” group, the first exposure to a female elicited the highest RCSM frequency in sexually naïve males. Then this frequency gradually decreased during subsequent presentations. As expected, males exposed to the empty box (“control” group) showed almost no cloacal response. The pattern of RCSM produced by naïve males exposed to sexually experienced males was variable. On the first trial, they produced only a few cloacal contractions. This number increased substantially in test 2 and 3 and finally dropped back to low frequencies for the rest of the trials. These observations are confirmed by the statistical analysis of socially-elicited RCSM that revealed significant group ($F_{2,18} = 5.464$, $p = 0.014$) and trial effects ($F_{6,108} = 4.450$, $p < 0.001$) as well as an interaction between the two factors ($F_{12,108} = 2.894$, $p = 0.002$). Pair-wise comparisons were performed to investigate the origin of the group effect: the “female” group was found to significantly differ from the two other groups. Additionally, planned comparisons revealed a linear trend across trials ($F_{1,18} = 10.849$, $p = 0.004$). Separate one-way ANOVAs were then performed on each group in order to study the evolution of the responses across trials. They indicated a significant effect of repetition in the “female” group ($F_{6,36} = 6.604$, $p < 0.001$) but not in the “male” group ($F_{6,36} = 0.990$, $p = 0.446$) and the “empty box” group ($F_{6,36} = 0.824$, $p = 0.559$). Planned comparisons revealed a linear trend across trials for female-stimulated RCSMs ($F_{1,6} = 7.800$, $p = 0.031$) but not in male-stimulated and RCSM produced in the empty box ($F_{1,6} < 0.915$, $p > 0.376$).

Together these data indicate that the response of sexually inexperienced male quail to the view of a male or a female is different. The view of a female elicits numerous contractions of the cloacal sphincter in preparation for mating but this response vanishes across trials probably as a result of a lack of reinforcement. On the other hand, the view of a male elicited an increasing number of cloacal contractions that gradually decreased with further pre-exposure. The discrepancy between the responses produced towards a male or a female thus disappeared with repetition.

Effects of pre-exposure to visual cues on first copulatory interaction

As illustrated in figure 4, pre-copulatory exposure to male or female conspecifics or to an empty box for 7 days resulted in different patterns of copulatory behavior during the first copulatory opportunity. All males who had been pre-exposed to females did copulate. On average, they exhibited higher behavioral frequencies, lower behavioral latencies and a higher copulatory efficiency ratio than males pre-exposed to males or to the empty box. For control males who had been pre-exposed to the empty box only, this test constituted their very first opportunity to see, approach and mate with a female. Most of them (6 out of 7), however, copulated. Their behavioral frequencies and latencies were intermediate between those observed in males pre-exposed to females or males. Interestingly, males pre-exposed to males hardly engaged in any sexual behavior at all as indicated by the very high latencies to copulate. Only 3 birds out of 7 in this group performed a complete copulatory sequence. These differences between groups did not reach statistical significance for CCM frequencies but there was a trend for mount frequencies ($F_{2,18} = 2.954$, $p > 0.078$). In contrast, latencies to mount attempt ($F_{2,18} = 6.980$, $p = 0.006$) and mount latencies ($F_{2,18} = 6.961$, $p = 0.006$) significantly differed between groups and effects of treatments on neck grab ($F_{2,18} = 3.238$, $p = 0.063$) and cloacal contact movement ($F_{2,18} = 3.091$, $p = 0.070$) latencies were close to significance. The copulatory efficiency ratio did not significantly differ between groups ($F_{2,18} = 1.778$; $p = 0.197$). Strut frequencies and latencies showed similar patterns in the three groups. Post-hoc analyses revealed that significant differences in MA and M latencies results from the longer latencies of subjects pre-exposed to males as compared to males pre-exposed to females or the empty box.

Effects of the first copulation on female-stimulated RCSM frequency

This first copulatory interaction also had an impact on subsequent RCSMs (Fig. 5). On day 9, all males were presented with a female in the RCSM aquarium. The basal and female-stimulated RCSMs significantly differed between groups ($F_{2,18} = 4.615$, $p = 0.024$ and $F_{2,18} = 4.146$, $p = 0.046$, respectively). The post-hoc analyses revealed that, in the absence of the female, males who had been pre-exposed to females displayed significantly more basal RCSMs than males who had been pre-exposed to males or to the empty box. In the presence of the female, subjects who had not been exposed to congeners during phase 1 ("empty box" group) and who saw and interacted with a female for the first time the day before produced more RCSMs than subjects of the other groups.

Visual presentation of male or female congeners results in long lasting effects on subsequent appetitive and consummatory aspects of male sexual behavior

The exposure to individuals of one sex only thus appears to have profound effects on the first sexual encounter. Are these effects long lasting or do they disappear after a few more sexual opportunities? In order to investigate this possibility, subjects were given daily copulatory opportunities preceded on every other day by visual presentation to a female during which RCSMs were recorded.

Frequencies of basal RCSM during tests 9 to 16 were relatively low and did not differ between groups (Group: $F_{2,18} = 1.897$, $p = 0.179$; Trial: $F_{3,54} = 5.88$, $p = 0.625$; Interaction: $F_{6,54} = 1.064$, $p = 0.396$; Fig. 6A). In the presence of the female, the group difference observed after the first copulation was maintained after repeated copulatory opportunities ($F_{2,18} = 9.621$, $p = 0.001$; Fig. 6B). Control subjects (Empty box) contracted their cloacal gland about twice as much as subjects previously exposed to a congener of either sex (phase 1). Post-hoc analyses indicated that these differences are significant. There was no trial effect and no interaction between the two factors (Trial: $F_{3,54} = 2.037$, $p = 0.120$; Interaction: $F_{6,54} = 1.162$, $p = 0.340$).

Mount attempt and cloacal contact movement frequencies also showed enduring differences between groups though these were more pronounced for cloacal contact movements (Fig. 7A–

B). A gradual rise in frequencies across trials was observed in most groups for MA and in all groups for CCM indicating an improvement of copulatory performance with training (MA: $F_{7,126} = 2.275$, $p = 0.032$; CCM: $F_{7,126} = 7.852$, $p < 0.001$). Significant group effects were detected (MA: $F_{2,18} = 4.076$, $p = 0.035$; CCM: $F_{2,18} = 4.391$, $p = 0.028$) and post-hoc analyses revealed that, overall, subjects pre-exposed to males displayed a lower sexual activity than subjects pre-exposed to females (MA: $p = 0.024$, CCM: $p = 0.020$) or controls (MA: $p = 0.024$, CCM: $p = 0.019$). No interaction was found between the two factors (visual experience and trials; MA: $F_{14,126} = 1.241$, $p = 0.255$; CCM: $F_{14,126} = 1.048$, $p = 0.411$). As illustrated in figure 8, the copulatory efficiency ratio also increased across trials ($F_{7,126} = 0.5964$, $p < 0.001$). However, no significant group effect ($F_{2,18} = 2.236$, $p = 0.136$) or interaction ($F_{14,126} = 0.5375$, $p = 0.9067$) was detected. The analysis of latencies of mount attempts and cloacal contact movements provided comparable results with reversed pattern (Fig. 7C–D). Latencies gradually decreased in most groups for MA and all groups for CCM after repeated mating opportunities (MA: $F_{7,126} = 15.046$, $p < 0.001$; CCM: $F_{7,126} = 8.457$, $p < 0.001$). Groups significantly differed for mount attempt latencies and the difference was marginally significant for cloacal contact movement latencies (MA: $F_{2,18} = 4.710$, $p = 0.023$; CCM: $F_{2,18} = 2.984$, $p = 0.076$). Post-hoc analyses revealed that subjects pre-exposed to males had longer latencies to engage in mating behavior than subjects pre-exposed to females (MA: $p = 0.007$) and tended to take longer than controls as well (MA: $p = 0.061$). An experience $\times$ trial interaction was found for MA latency but not CCM latency (MA: $F_{14,126} = 5.034$, $p < 0.001$; CCM: $F_{14,126} = 1.276$, $p = 0.231$).

Separate one-way ANOVAs were then performed in order to study the pattern of response change across trials of each group individually. They indicated a significant effect of repetition in the “empty box” group (with the exception of mount attempt frequencies; MA: $F_{7,42} = 1.639$, $p = 0.151$; Lat MA: $F_{7,42} = 3.462$, $p = 0.005$; CCM: $F_{7,42} = 4.875$, $p < 0.001$; Lat CCM: $F_{7,42} = 2.424$, $p = 0.035$) and in the “male” group (MA: $F_{7,42} = 5.527$, $p < 0.001$; Lat MA: $F_{7,42} = 11.932$, $p < 0.001$; CCM: $F_{7,42} = 5.892$, $p < 0.001$; Lat CCM: $F_{7,42} = 5.773$, $p < 0.001$) but not in the “female” group (MA: $F_{7,42} = 0.331$, $p = 0.935$; Lat MA: $F_{7,42} = 1.886$, $p = 0.096$; CCM: $F_{7,42} = 1.338$, $p = 0.257$; Lat CCM: $F_{7,42} = 1.200$, $p = 0.324$). This latter observation suggests that pre-exposure to females results in the development abilities for mount attempts that are similar to those of sexually experienced individuals and consequently do not require further improvement. Planned comparisons revealed that linear trends are associated with all these effects with the exception of mount attempt frequencies and latencies in controls (Empty box - MA: $F_{1,6} = 3.178$, $p = 0.125$; Lat MA: $F_{1,6} = 3.176$, $p = 0.110$; CCM: $F_{1,6} = 16.929$, $p = 0.006$; Lat CCM: $F_{1,6} = 6.276$, $p = 0.046$; Male - MA: $F_{1,6} = 9.953$, $p = 0.020$; Lat MA: $F_{1,6} = 13.378$, $p = 0.011$; CCM: $F_{1,6} = 26.624$, $p = 0.002$; Lat CCM: $F_{1,6} = 13.539$, $p = 0.010$).

In summary, these results indicate that groups significantly differed and that an overall progression was observed in control subjects and subjects pre-exposed to males. More importantly, it indicates that subjects pre-exposed to females did not show such a progression because their degree of performance on the first trials was close to values observed in experienced animals.

Finally, the comparison of groups on the very last trial day indicated that MA frequency still tended to differ between groups ($F_{2,18} = 2.848$, $p = 0.084$) and that the difference in CCM frequency persisted after repeated testing ($F_{2,18} = 3.573$, $p = 0.049$). However, groups no longer differed in terms of MA and CCM latencies ($F_{2,18} = 1.189$, $p = 0.327$; $F_{2,18} = 1.143$, $p = 0.341$).

Discussion

A host of studies conducted on a wide range of vertebrates have shown that reproductive success is influenced by the social and contextual environment in which males mate (Domjan

et al., 1998). However, most studies used mating as a reinforcer, so it is not known whether cues provided by the social environment also contribute to the development of male sexual behavior independently of any role they might play in the context of copulation. The present data indicate that pre-copulatory social experience does influence subsequent copulatory behavior. Interestingly, these data also suggests that the effect of such experience depends on the sex of the congeners the naïve males have been exposed to. Subjects were castrated and chronically treated with testosterone thus suggesting that the present effects cannot result from changes in gonadal steroid hormone secretions that might have resulted from the different social exposures. It must also be noted that the testosterone replacement treatment was initiated approximately at the time when the testes of the males would have started naturally secreting high levels of androgens. This treatment thus mimicked the physiological process of “puberty”.

The exposure of sexually naïve males to visual cues of females resulted in shorter latencies to initiate copulation and mate on their first copulatory opportunity and throughout the following copulatory sessions. Strikingly, the frequencies and latencies of mount attempts displayed by these males on their first copulatory opportunity were highly similar to those of sexually experienced animals (Fig 7: compare performances of female-exposed subjects on day 8 with the same measures in control subjects on the last day). Therefore, it appears that social experience acquired prior to the first actual copulatory interaction confers a reproductive advantage over other subjects, as individuals pre-exposed to a female appear to interact faster with the hen. Such an advantage may profoundly impact the outcome of subsequent copulatory opportunities/competitions. For example, Gutierrez and Domjan (1996) showed that the presentation of an arbitrary stimulus previously paired with access to the female provides an advantage in terms of competition for a mate as compared to a stimulus that was paired with the absence of the female (Gutierrez and Domjan, 1996). However, if previous non-copulatory exposure to females appears to facilitate the initiation of future sexual interactions, it does not seem to improve motor efficiency, at least based on the measure of the copulatory efficiency ratio. Although a general reduction of behavioral latencies might be interpreted as an increased efficiency to mate, the fact that there are no group difference in behavioral frequencies or in the copulatory efficiency index suggests that these reduced latencies might be more related to an increase in the motivation to mate than to enhanced motor skills.

As opposed to subjects that received visual access to a female in phase 1, subjects pre-exposed to males exhibited a reduced copulatory efficacy. Although no group difference was found in the copulatory efficiency index, these subjects displayed significantly fewer mount attempts and cloacal contact movements and showed longer latencies to engage in mating behavior than others on their first opportunity to mate (Fig. 4) and throughout all subsequent copulatory sessions (Fig. 7). Although training clearly reduced their latency to copulate to levels displayed by the other groups, their copulatory frequencies remained about half the frequency exhibited by controls and by males pre-exposed to females. In parallel, the frequency of RSCMs elicited by a female remained low without showing a clear tendency to improve with repeated mating opportunities in the copulatory chamber (Fig. 6). The maintenance of such reduced appetitive and consummatory performances of these males suggests that the somewhat aversive information conveyed by sexually experienced males to inexperienced subjects markedly and permanently alters the development of their sexual behavior. Ultimately, it is possible that such experiences will result in diminished chances to mate and could be viewed as a means for sexually experienced and dominant males to insure their reproductive success by limiting sexual activity of rivals and thus competition for mates. Alternatively, it is possible that the response of male pre-exposed subjects to a situation they perceive as aversive is to perform a limited number of successful sexual interactions. This hypothesis is supported by the fact that, although they scored less CCM, they reached a similar efficiency ratio as the other groups (comparison of Fig. 7 and Fig. 8).

Comparing the performances of controls to these of subjects previously exposed to females seems to contradict the idea that a substantial fraction of naïve males do not complete a full copulatory sequence on their first interaction with a female (see introduction). However, in a laboratory setting, the first copulatory test usually constitutes their first time being handled, transported and placed in a new environment. These new events constitute a stress that probably results, at least in part, in some behavioral inhibition. In the present experiment, control males were handled, transported and placed for 5 min in an empty aquarium on 7 occasions before their first sexual interaction. These manipulations certainly led to a reduction of the stress level associated to the novelty of the tests, which probably facilitated the initiation of copulation on their first encounter with a female. With previous social experience, such a habituation to the testing environment might thus constitute another factor contributing to the facilitation of copulatory behavior in sexually inexperienced males. If this is the case, naïve males that have been habituated to the testing conditions should copulate faster and more intensively than naïve subjects that have not been previously handled. This question should be formally tested in future experiments.

An additional interesting aspect of these data is that the exposure to a female prior to copulatory opportunity results in a reduction of female-elicited RSCM frequency that might be attributed to either a habituation to cues emanating from the female or a conditioned inhibition to the cues of the female and/or of the environmental context. Even in sexually experienced males repeated tests in the RSCM chamber that are not followed by mating opportunities progressively results in a reduced frequency of RSCM elicited by females (Cornil et al., 2006). Although both visual access to a hen and copulation support the classical conditioning of cloacal contractions, copulation itself seems to elicit a faster and more efficient learning of conditioned RSCMs (Taziaux, et al., 2008). Appetitive sexual behavior is thought to enable males to prepare for and thereby react more effectively to predictable mating opportunities (Pfaus, 1996; Timberlake and Silva, 1995). Therefore one might predict that subjects exhibiting a decline in their response to the visual presentation of a female would also display a lower rate of copulatory behavior when given the opportunity to mate. However, males exposed to a female prior to mating almost immediately engaged in sexual behavior on their first copulatory opportunity, while subjects of the two other groups were slower to mate or did not mate at all. A potential explanation for such dissociation between the appetitive and copulatory performance in animals pre-exposed to females lies in the fact that these two behaviors were assessed in two separate chambers so that the cues of the RSCM chamber probably came to signal the absence of the possibility to mate, while the arena where mating was assessed had no pre-existing value that might dissuade the male to approach the female and mate. It is possible that the competition of environmental cues signaling different mating opportunities also explain the persistence of low frequencies of RSCM across post-copulatory exposures to females in males that had been pre-exposed to females. Regardless of the cause of such dissociation between appetitive and consummatory behaviors, these observations further support the notion that pre-copulatory experience may profoundly impact the performance of subsequent behaviors.

Finally, one important physiological event promoting the development of sexual behavior is the rise of sex steroid hormone concentrations in the blood at the onset of sexual maturity (i.e., “puberty”) (Ottinger and Brinkley, 1979). Elevated circulating concentrations of testosterone resulting from gonadal maturation have enduring effects on brain morphology and activate neural pathways involved in the control of male sexual behavior (Balthazart, et al., 2004; Schulz and Sisk, 2006; Sisk and Foster, 2004). One way for sex steroids to modulate circuits regulating male sexual behavior is to alter the perception of social stimuli. For example, castrated males of a variety of species do not show a preference for estrus/receptive or anestrus/non-receptive females, but treatment with testosterone results in a distinct preference for estrus/receptive females (Wood and Swann, 2002). By changing the perceived significance of a

stimulus, the rise of sex steroid hormones at puberty thus constitutes a crucial step in the development of sexual behavior. Intriguingly, the role of the experience acquired when circulating sex hormones have reached their adult levels, but before they have actually had the opportunity to mate, has rarely been explored. Previous work primarily investigated the effect of prepubertal (Bischof and Rollenhagen, 1999) or postpubertal experience following copulation (See introduction) on male sexual behavior. The present study examined the effect on subsequent sexual behavior of social cues provided soon after sexual hormones have reached their adult concentrations. Because sexual encounters may result in acute changes of circulating concentrations of sex steroid hormones, we decided to conduct the present study on castrated males whose plasma concentrations of testosterone had been clamped to stable levels by implantation of capsules filled with testosterone. In order to mimic the physiological process of sexual maturation, testosterone replacement therapy was initiated approximately at the age at which the testes first start secreting high concentrations of androgens (Ottinger and Brinkley, 1979). Studies conducted in young rats and zebra finches revealed that the amount of time males spend looking at females as compared to males, family members or a neutral area gradually increases as they get older (Adkins-Regan and Leung, 2006; Eliasson and Meyerson, 1981). In rats, the amount of approach behavior towards females stabilizes at an age when sexual hormones have reached their adult concentrations (Ojeda and Urbanski, 1988). Interestingly, the magnitude of the preference for females appears to be altered by castration (Eliasson and Meyerson, 1981). In zebra finches, experimental manipulations of sex steroid hormones concentrations during the late juvenile development (prior to sexual maturation) resulted in a premature shift in the relative interest of males towards females as compared to family members (Adkins-Regan and Leung, 2006). The present data might thus provide another example of how stimuli that had no meaningful value in the absence of high hormonal concentrations acquire significance at puberty and, as a consequence, influence the reproductive success of an individual. Further experiments should however be performed to demonstrate conclusively whether steroid hormones are required for such learning or whether pre-pubertal males (or castrates) can also form an association between social cues and mating opportunities.

To achieve successful mating and ultimately pass on their genes, animals need to coordinate physiological and behavioral events related to reproduction with environmental cues that will predict the onset of conditions, such as an increase in food abundance, that are favorable for raising their offspring (Ball and Balthazart, 2002; Prendergast, et al., 2002). In particular, behavioral interactions between mates must be coordinated and their physiological state synchronized so that they can make the transitions from courtship and mating to incubation and the care of the young. These transitions and their endocrine control have been especially well characterized in birds (Lehrman, 1965; Wingfield, et al., 1994). Birds and other animals can glean information from their social and mating experiences that will influence subsequent sexual encounters and may profoundly impact their reproductive success. Most studies conducted to date were performed in sexually experienced animals or used arbitrary stimuli paired with mating opportunities. The present data demonstrate that, when sex hormones have reached adult circulating concentrations, animals also learn from their social environment before they actually have an opportunity to interact physically. The advantage conferred by such pre-mating learning may have profound consequences on the subsequent reproductive success of individuals. Although our knowledge about quail reproductive behavior in the wild is limited, reports suggest that hens form pairs with the male holding the territory in which they have settled (Moreau, 1951; Rodriguez-Teijeiro, et al., 2003; Wetherbee, 1961). The duration of the pair bond is short (3 to 9 days) and mate switching (defined as the breakage of a pair bond and formation of a new pair bond during the female fertile period) occurs frequently (in more than 50% of the cases) mainly after contests between newcomers and paired males. Hence, it is likely that both males and females mate with several partners during a breeding season. In these conditions, short latencies to initiate courtship behavior that may be achieved

by experienced juveniles might convey an advantage over inexperienced juveniles when they first get access to a female. In contrast, in the presence of one or several experienced males, known to be pugnacious in this species, it might be better to restrain from mating until females become available in the absence of a potential dangerous male rival. As already mentioned earlier, future experiments should be carried out to determine whether sex steroid hormones are required for such learning or whether social experience acts independently. Future studies should also investigate whether such a behavioral advantage conferred by “pubertal” sexual learning would be reflected in a higher number of offspring sired by males pre-exposed to females.

Acknowledgments

This work was supported by grant NIH/NIMH R01 MH50388 to GFB. CAC is Research Associate from the Fonds de la Recherche Scientifique (FRS-FNRS). We would like to thank Dr Kevin S. Holloway, Dr Jacques Balthazart and Isabelle Noirot as well as two anonymous reviewers for their thorough reading and the constructive comments they made on previous versions of this manuscript. We are also grateful to Jim Garmon and Philippe Humpers for their technical assistance.

References

- Abail P, Braquenier JB, Balthazart J, Ball GF. Effects of lesions of nucleus taeniae on appetitive and consummatory aspects of male sexual behavior in Japanese quail. *Brain Behav Evol* 2002;60(1):13–35. [PubMed: 12239468]
- Adkins EK, Adler NT. Hormonal control of behavior in the Japanese quail. *J Comp Physiol Psychol* 1972;81(1):27–36. [PubMed: 5074305]
- Adkins-Regan E, Leung CH. Sex steroids modulate changes in social and sexual preference during juvenile development in zebra finches. *Horm Behav* 2006;50:772–778. [PubMed: 16919276]
- Adkins-Regan E, MacKillop EA. Japanese quail (*Coturnix japonica*) inseminations are more likely to fertilize eggs in a context predicting mating opportunities. *Proc R Soc Lond B* 2003;270:1685–1689.
- Ball, GF.; Balthazart, J. Neuroendocrine mechanisms regulating reproductive cycles and reproductive behavior in birds. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. *Hormones, brain and behavior*. Vol. 2. Academic Press; San Diego, CA: 2002. p. 649–798.
- Balthazart J, Baillien M, Cornil CA, Ball GF. Preoptic aromatase modulates male sexual behavior: slow and fast mechanisms of action. *Physiology and Behavior* 2004;83:247–270. [PubMed: 15488543]
- Balthazart J, Ball GF. The Japanese quail as a model system for the investigation of steroid-catecholamine interactions mediating appetitive and consummatory aspects of male sexual behavior. *Annual Review of Sex Research* 1998;9:96–176.
- Bischof HJ, Rollenhagen A. Behavioural and neurophysiological aspects of sexual imprinting in zebra finches. *Behavioural Brain Research* 1999;98:267–276. [PubMed: 10683116]
- Cheng KM, McIntyre RF, Hickman AR. Proctodeal gland foam enhances competitive fertilization in domestic Japanese quail. *The Auk* 1989;106:286–291.
- Cornil CA, Stevenson TJ, Ball GF. Are rapid changes in gonadal testosterone release involved in the fast modulation of brain estrogen effects? *Gen Comp Endocrinol* 2009;163(3):298–305. [PubMed: 19416729]
- Cornil CA, Taziaux M, Baillien M, Ball GF, Balthazart J. Rapid effects of aromatase inhibition on male reproductive behaviors in Japanese quail. *Horm Behav* 2006;49(1):45–67. [PubMed: 15963995]
- Delville Y, Sulon J, Hendrick JC, Balthazart J. Effect of the presence of females on the pituitary-testicular activity in male Japanese quail (*Coturnix coturnix japonica*). *Gen Comp Endocrinol* 1984;55:295–305. [PubMed: 6479577]
- Domjan M, Akins C, Vandergriff DH. Increased responding to female stimuli as a result of sexual experience: tests of mechanisms of learning. *Quart J exp Psychol* 1992;45B:139–157.
- Domjan M, Blesbois E, Williams J. The adaptive significance of sexual conditioning: Pavlovian control of sperm release. *Psychological Science* 1998;9:411–415.

- Domjan M, Lyons R, Camille North N, Bruell J. Sexual pavlovian conditioned approach behavior in male Japanese quail (*Coturnix coturnix japonica*). *Journal of Comparative Psychology* 1986;100:413–421. [PubMed: 3802785]
- Eliasson M, Meyerson B. Development of sociosexual approach behavior in male laboratory rats. *Journal of Comparative and Physiological Psychology* 1981;95 (1):160–165.
- Galef BG, White DJ. Evidence of social effects on mate choice in vertebrates. *Behav Processes* 2000;51 (1–3):167–175. [PubMed: 11074319]
- Gutierrez G, Domjan M. Learning and male-male sexual competition in Japanese quail (*Coturnix japonica*). *J Comp Psychol* 1996;110(2):170–5. [PubMed: 8681530]
- Hollis KL, Pharr VL, Dumas MJ, Britton GB, Field J. Classical conditioning provides paternity advantage for territorial male blue gouramis (*Trichogaster trichopterus*). *Journal of Comparative Psychology* 1997;111(3):219–225.
- Hutchison RE. Hormonal differentiation of sexual behavior in Japanese quail. *Hormones and Behavior* 1978;11:363–387. [PubMed: 753700]
- Lehrman, D. Interaction between internal and external environments in the regulation of the reproductive cycle of the ring dove. In: Beach, FA., editor. *Sex and Behavior*. John Wiley and Sons; New York: 1965. p. 355–379.
- Mahometa MJ, Domjan M. Classical conditioning increases reproductive success in Japanese quail, *Coturnix japonica*. *Animal Behaviour* 2005;69:983–989.
- Matthews RN, Domjan M, Ramsey M, Crews D. Learning effects on sperm competition and reproductive fitness. *Psychol Sci* 2007;18(9):758–62. [PubMed: 17760768]
- Mills AD, Crawford LL, Domjan M, Faure JM. The behavior of the Japanese or domestic quail *Coturnix japonica*. *Neuroscience and Biobehavioral Reviews* 1997;21(3):261–281. [PubMed: 9168263]
- Moreau RE. The British status of the quail and some problems of its biology. *British birds* 1951;44(8): 257–276.
- Ojeda, SR.; Urbanski, HF. Puberty in the rat. In: Knobil, E.; Neill, JD., editors. *The Physiology of reproduction*. Vol. 2. Raven Press; New York: 1988. p. 1699–1737.
- Ottinger MA, Brinkley HJ. Testosterone and sex related physical characteristics during the maturation of the male Japanese quail (*coturnix coturnix japonica*). *Biol Reprod* 1979;20(4):905–9. [PubMed: 572248]
- Pfaus JG. Homologies of animal and human sexual behaviors. *Hormones and Behavior* 1996;30(3):187–200. [PubMed: 8918675]
- Pfaus JG, Kippin TE, Centeno S. Conditioning and sexual behavior: a review. *Hormones and Behavior* 2001;40:291–321. [PubMed: 11534994]
- Prendergast, BJ.; Nelson, RJ.; Zucker, I. Mammalian seasonal rhythms: Behavior and neuroendocrine substrates. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. *Hormones, brain and behavior*. Vol. 2. Academic Press; San Diego, CA: 2002. p. 93–156.
- Rodriguez-Teijeiro JD, Puigcerver M, Gallego S, Cordero PJ, Parkin DT. Pair bonding and multiple paternity in the polygamous common quail *Coturnix coturnix*. *Ethology* 2003;109(4):291–302.
- Rosenblatt, JS. Effects of experience on Sexual Behavior in Male Cats. In: Beach, FA., editor. *Sex & Behavior*. John Wiley & Sons, Inc; New York - London - Sydney: 1965. p. 416–439.
- Sachs BD. Photoperiodic control of the cloacal gland of the Japanese quail. *Science* 1967;157:201–203. [PubMed: 17806265]
- Schulz KM, Sisk CL. Pubertal hormones, the adolescent brain, and the maturation of social behaviors: Lessons from the Syrian hamster. *Mol Cell Endocrinol* 2006;254–255:120–6.
- Seiwert, CM. The neuromuscular system controlling foam production in Japanese quail: an investigation of structure and function. O. Cornell University; Ithaca, NY: 1994.
- Seiwert CM, Adkins-Regan E. The foam production system of the male Japanese quail: characterization of structure and function. *Brain Behav Evol* 1998;52:61–80. [PubMed: 9681161]
- Sisk CL, Foster DL. The neural basis of puberty and adolescence. *Nature Neuroscience* 2004;7(10):1040–1047.

- Taziaux M, Kahn A, Moore J 3rd, Balthazart J, Holloway KS. Enhanced neural activation in brain regions mediating sexual responses following exposure to a conditioned stimulus that predicts copulation. *Neuroscience* 2008;151(3):644–58. [PubMed: 18164139]
- Timberlake, W.; Silva, KM. Appetitive behavior in ethology, psychology, and behavior systems. In: Thompson, NS., editor. *Perspective in Ethology*. Plenum Press; New-York: 1995. p. 211-253.
- Wetherbee DK. Investigations in the life history of the common coturnix. *American Midland Naturalist* 1961;65(1):168–186.
- Wingfield, JC.; Whaling, CS.; Marler, P. Communication in vertebrate aggression and reproduction: the role of hormones. In: Knobil, E.; Neill, JD., editors. *The physiology of reproduction*. 2. Raven Press; New York: 1994. p. 303-341.
- Wood, RI.; Swann, JM. Neuronal Integration of Chemosensory and Hormonal Signals in the Control of Male Sexual Behavior. In: Wallen, K.; Schneider, JE., editors. *Reproduction in Context*. MIT press; cambridge: 2002. p. 423-444.
- Zamble E, Hadad GM, Mitchell JB, Cutmore TRH. Pavlovian conditioning of sexual arousal: first- and second-order effects. *Journal of Experimental Psychology* 1985;11(4):598–610. [PubMed: 4067513]

Figure 1. Schematic representation of the aquarium used to measure the rhythmic cloacal sphincter movement (RCSM) and expose the subjects to the view of the female
See methods for a description of the apparatus.

Figure 2. Timeline of the tests

Each block represents a test period of 5 min (T). Blocks on the horizontal line define tests carried out on different days, while blocks on the same vertical line indicate that two tests have been run on a same day. All subjects were sexually inexperienced prior to the first test. In phase 1 (*Striped blocks*), subjects received non-copulatory exposure to three different stimuli depending on the experimental group they belonged to (see methods) and the frequency of rhythmic cloacal sphincter movements (RCSM) was recorded. During phase 2, all subjects (regardless of the group they belonged to) were submitted to the same stimulus, an unfamiliar sexually experienced female. They were tested either for copulatory behavior (*black blocks*) or for RCSM (*white blocks*). When two tests were carried out on a same day, they were performed several hours apart and the RCSM test always preceded the copulatory test. The black arrow indicates the first copulatory opportunity.

Figure 3. Effect of pre-copulatory experience on the frequency of the rhythmic cloacal sphincter movements

Data were analyzed by mixed-design ANOVA with the non-copulatory experience (Exp.) as the independent factor and trials as the repeated factor. Pairwise comparisons (LSD) were performed to investigate the origin of the experience effects: * $p < 0.05$ Vs. female.

Figure 4. Effect of the pre-copulatory experience on the first copulatory interaction with a female Males pre-exposed to a female, a male or an empty box (controls) for 7 trials on 7 consecutive days were allowed to physically interact for 5 min with a female that they had never met before. Frequencies and latencies of copulatory behaviors (NG: neck grabs, MA: mount attempts; M: mounts; CCM: cloacal contact movements) performed during this test were recorded. The copulatory efficiency ratio (expressed in %) was obtained by dividing the total number of CCM by the total number of NG and then multiplying by 100. When a subject did not show a behavior, it was given the 300 sec maximum latency. Data were analyzed by one-way ANOVA with the non-copulatory experience as the independent factor. * $p < 0.05$ Vs. Male.

Figure 5. Effect of the first sexual encounter on rhythmic cloacal sphincter movements (RCSM) frequency in the absence (“without”, basal RCSM) or in the presence of a female (“with”)

Males pre-exposed to a female, a male or an empty box (controls) for 7 trials were allowed to see a female placed behind a plexi-glass on the day following their first sexual encounter with a female. RCSM frequencies were recorded for 2.5 min (duration of the visual presentation of the female). Data were analyzed by a one-way ANOVA with the visual experience as the independent factor. Post-hoc comparison (LSD): Δ $p < 0.05$ Vs. Male, * $p < 0.05$ Vs. Empty box.

Figure 6. Effect of pre-copulatory exposure and repeated copulatory opportunities on female-elicited rhythmic cloacal sphincter movements (RCSM) frequency

Males pre-exposed to a female, a male or an empty box (controls) received a daily opportunity to physically interact with a female. On every other day, they were tested for RCSM frequency to examine the effect of newly acquired sexual experience on appetitive behavior. Data were analyzed by a mixed ANOVA with pre-copulatory experience (Exp.) as the independent factor and trials as the repeated factor. Pairwise comparisons (LSD) were performed to investigate the origin of the experience effect: * $p < 0.05$ Vs. empty box.

Figure 7. Effect of pre-copulatory exposure and repeated copulatory opportunities on copulatory behavior

Males pre-exposed to a female, a male or an empty box (controls) received a daily opportunity to physically interact with a female. Behavioral frequencies and latencies were recorded during 5 min. When a subject did not show a behavior it was given the 300 sec maximum latency. Data were analyzed by a mixed ANOVA with pre-copulatory experience (Exp.) as the independent factor and trials as the repeated factor. Pairwise comparisons (LSD) were performed to investigate the origin of the experience effect: * $p < 0.05$ Vs. empty box, $\Delta p < 0.05$ Vs. female.

Figure 8. Effect of pre-copulatory exposure and repeated copulatory opportunities on copulatory efficiency

Males pre-exposed to a female, a male or an empty box (controls) received a daily opportunity to physically interact with a female. The copulatory efficiency ratio (expressed in %) was obtained by dividing the total number of CCM by the total number of NG and then multiplying by 100. Data were analyzed by a mixed ANOVA with pre-copulatory experience (Exp.) as the independent factor and trials as the repeated factor.