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Sexual arousal, is it for mammals only?

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Abstract

Sexual arousal has many dimensions and has consequently been defined in various ways. In humans sexual arousal can be assessed based in part on verbal communication. In male non-human mammalian species it has been argued that arousal can only be definitively inferred if the subject exhibits a penile erection in a sexual context. In non-mammalian species that lack an intromittent organ, as is the case for most avian species, the question of how to assess sexual arousal has not been thoroughly addressed. Based on studies performed in male Japanese quail, we argue that several behavioral or physiological characteristics provide suitable measures of sexual arousal in birds and probably also in other tetrapods. These indices include the performance of appetitive sexual behavior in anticipation of copulation (although anticipation and arousal are not the synonyms), the activation of specific brain areas as identified by the detection of the expression of immediate early genes (*fos*, *egr-1*) or by 2-deoxyguanosine quantitative autoradiography, and above all the release of dopamine in the medial preoptic areas as measured by in vivo dialysis. Based on these criteria, it is possible to assess in birds sexual arousal in its broadest sense but meeting the more restrictive definition of arousal proposed for mammals (erection in an explicit sexual context) is and will probably remain impossible in birds until refinement of in vivo imaging techniques such fMRI allow us to match in different species, with and without an intromittent organ, the brain areas that are activated in the presence of specific stimuli.

Keywords

Sexual behavior; Appetitive sexual behavior; Consummatory sexual behavior; Sexual learning; Sexual motivation; Dopamine; Japanese quail

1. Introduction: Sexual Arousal in Birds and Mammals

Arousal is a general psychological construct referring to an awake state in which one is ready to respond to a variety of stimuli. Sexual arousal is a specific instance of the aroused state in which an individual appears to be ready to respond to sexual stimuli with an appropriate behavioral response. Most studies of sexual arousal have focused on mammalian species (e.g., (Agmo, 2007; Ågmo et al., 2004; Bancroft, 2005; Pfaff and Agmo, 2010; Pfau et al., 2003; Sachs, 2007)). This review concerns whether the concept of sexual arousal can be applied to avian species. We have been studying the neuroendocrine control of sexual behavior and sexual motivation in Japanese quail (*Coturnix japonica*) for many years so the review will focus on examples based on studies of this species though

other species will be discussed when relevant data are available. We will begin with a general consideration of sexual arousal and the challenge faced when applying it to avian species.

Sexual arousal has many dimensions (see (Sachs, 2007) for a review). Some authors have defined it quite generally as a “state motivated toward the experience of sexual pleasure” (Bancroft, 2005)(p. 411) so that arousal encompasses phenomenon such as the cognitive processing of sexual stimuli, incentive motivation as well as genital reflexes. As noted previously (Bancroft, 2005), like most authors focused on mammals (humans in his case), especially males, so the genital reflex could be measured by studying penile erections. Others have also championed this response as the best measure especially when trying to ascertain sexual arousal in non-human mammals (Sachs, 2007). Sachs (2007) in particular has argued that an erect penis is the *sine qua non* for ascertaining sexual arousal but even in this case the mere appearance of an erection does not necessarily signal sexual arousal. The erection must be observed in the appropriate context to be sure it is an indicator of sexual arousal. Nocturnal erections for example do not constitute sexual arousal. According to Sachs (2007) then for sexual arousal to be a useful scientific concept it should be defined with precision and involve reliable observable measures so one must have a situation with both an unambiguous sexual context and an observed erection to be confident that one is assessing sexual arousal *per se*. This definition has obvious utility but it raises major issues when one is trying to generalize to non-mammalian species such as birds. Sachs (2007) noted that birds might be amenable to his definition since several species have an intermittent organ (Ball and Balthazart, 2002) or they exhibit cloacal movements in a manner that suggests sexual arousal. We will critically evaluate how one can assess the state of sexual arousal in birds. Our general view is that arousal is a state mediated by the autonomic nervous system that comes about when the animal is sexually motivated. Arousal based on this view is then a precursor for engaging in the sexual behavior sequence that we would argue involves appetitive and consummatory components. Thus if an animal displays appetitive sexual behavior in anticipation of copulation, it must be sexually motivated and potentially also sexually aroused. Analysis of these behaviors is thus likely to provide information on the arousal state of the experimental subjects. We will consider such behaviors in the next section of this review.

2. Measures of spontaneous behaviors associated with copulation

Behaviors that precede, in a reliable manner, copulatory behavior and whose intensity or frequency is variable could thus be considered as indicators of sexual motivation and as a consequence of sexual arousal. In birds, several types of behaviors potentially fulfill these criteria such as singing in songbirds, various pre-copulatory displays in different species (e.g., social displays in ducks, lekking behavior in grouse and associated species, even nest building in many passerine species,...).

It is problematic though for most of these behaviors to try to link them to sexual motivation or arousal. Pre-copulatory displays are indeed statistically associated with the performance of the copulatory act but this association is far from perfect. Some of these displays are exhibited in an aggressive as well as a sexual context. Social displays in ducks, for example, almost always precede copulation but they can also occur in unisexual groups of males where they reflect male-male competition. These social displays are also exhibited starting several months before copulations occur: in the northern temperate zone males engage in social displays from October to May whereas copulations with females are observed only between February and April (Balthazart and Hendrick, 1976; Raitasuo, 1964).

In Japanese quail (*Coturnix japonica*) whose sexual behavior has been intensively studied in the laboratory, one vocal behavior, crowing and one visual display, strutting could potentially be considered as indicators of sexual motivation and arousal. For example, in the absence of a female, sexually motivated male quail produce, under both laboratory and field conditions, a loud two- to three-syllable vocalization called crowing. This call was experimentally shown to attract females (Goodson and Adkins-Regan, 1999). Once the male is in the presence of a female the frequency of the crow vocalization declines. This observation confirms its role as a phonotactic call (Potash, 1974; Wetherbee, 1961). Crowing is thus a candidate to provide an easily discernible and sensitive measure of sexual motivation. This idea is further supported by the fact that the occurrence and frequency of these calls is markedly enhanced by testosterone as is the case for sexual behavior *sensu stricto* (Adkins, 1977; Adkins and Pniewski, 1978). Crowing frequency is, however, highly variable among males even in controlled laboratory conditions. It is also influenced by a variety of external stimuli that are in many cases not specifically relevant to sexual behavior. Its reliability as an indicator of motivation is thus fairly weak.

The precopulatory display called strutting has the same sort of problems when one wants to consider it as an indicator of sexual arousal. During this display, the male walks on its tiptoes around the female with its neck horizontally extended and its wing on the side closest to the female lowered. The behavior is also activated by testosterone and 5 α -reduced androgens (Adkins and Pniewski, 1978; Balthazart et al., 1983) but it occurs before as well as after copulation and its frequency exhibits a high degree of variability among males. This behavior also is thus probably not a very suitable indicator of sexual motivation or arousal.

3. Laboratory measures of the anticipation of copulation in quail

3.1. The Learned Social proximity response

Because the identification of spontaneous behaviors that could provide reliable measures of sexual motivation and arousal has been problematic in quail, attention was paid to other potential behavioral or physiological indicators. During studies on sexual conditioning (Domjan, 1998; Domjan and Hollis, 1988), Michael Domjan and his colleagues at the University of Texas in Austin identified a form of associative learning that they named the learned social proximity response (Domjan and Hall, 1986a; Domjan and Hall, 1986b; Domjan et al., 1986).

The procedure is based on the fact that when males are allowed to copulate with females in an arena, they learn to stand in front of a narrow window that provides a view of a female. The response involves a remarkable change in a male's behavior: males who originally paid no attention to the female behind the window will start spending the majority of their time (80–90% up to 14 hours a day) in front of the window staring at the female. This remarkable change in their behavior occurs after a single copulation (Domjan et al., 1986).

This response shares features that are normally associated with both classical conditioning or instrumental conditioning ((Domjan and Hollis, 1988) see (Balthazart and Ball, 1998) for additional discussion) and has thus been labeled as a form of associative learning acquired by males after they have been allowed to copulate with females (see (Domjan et al., 1992) for discussion).

We developed a faster version of this test in which the proximity response is quantified only during a 5 min period of presentation of the female after the male has been habituated to the test chamber. This entire test can be completed in 25 min so that a large number of subjects can be examined each day (Balthazart et al., 1995) as opposed to the procedure used by

Domjan and collaborators in which birds were tested continuously during the entire day (Domjan and Hall, 1986a; Domjan and Hall, 1986b).

Behavioral observations are carried out in an arena containing two compartments (see Fig. 1). The experimental male is tested by introducing him into the larger compartment (90×90 cm) that is separated by a sliding door from an adjacent smaller cage (20×26 cm) where the stimulus female is located.

A small “window” (vertical slit: 1 cm wide by 15 cm high) is located in the middle of the sliding door and provides the male with a limited visual access to the female. This window can be closed via an opaque swinging plywood panel (not shown in the figure) attached by a hinge just above the door. Both the door and the swinging panel can be manipulated from a remote location via a string and pulley system. One square area (30×30 cm) located in front of the door is mounted on springs and micro-switches and defines the test area for bird position. When the window is open, the male can see the female through the window only if he stands in this area in front of the window.

Each behavioral test lasts a total of 25 min. After 5 min of habituation in the large compartment of arena, the position of the male is recorded for 5 min with the window separating the two compartments closed (pretest). The window is then opened and the position of the male is recorded again for a 5 min period (test). During this period, the observer also records every 5 sec. whether the experimental subject is actually looking through the window or not. This point sampling observation provides a score for looking behavior ranging from 0 to 60. These data (time in the square in front of the window and the look score) constitute the measures of appetitive sexual behavior. The door between the two compartments is then lifted and the male and female are allowed to freely interact for another 5 min during which time the frequency of consummatory sexual behaviors, including mount attempts and cloacal contact movements are recorded by the observer. The female is then removed and the male spends 5 more min in the test arena before being returned to his home cage.

This learned response is relatively easy to obtain under laboratory conditions and involves aspects of the learning process that normally occurs in male quail when they engage in sexual behavior. The procedure appears to be a good indicator of appetitive sexual behavior in that the male seems clearly to be engaging in this behavior in anticipation of copulatory behavior itself. Male quail when isolated in a large arena or in nature will first pursue a generalized search for females and then when a female is localized to a particular place they exhibit a more focal search. Such variable searching behavior is characteristic of an appetitive behavioral response. The learned social proximity response in male quail appears to be a good example of a focal sexual search and thereby provides a useful measure of appetitive sexual behavior and presumably of sexual motivation that one can contrast with the stereotyped sequence of the neck grab, mounting and cloacal contact movements characteristic of the consummatory sexual response.

Control by stimuli originating from the female—When repeatedly tested with this procedure, males progressively spend an increasing amount of time (up to 95% of the 5 min period) in the area in front of the window. However, as noted above, this learned social proximity response only develops if the birds are permitted to copulate with the female in the arena. If the door between the two compartments is not opened, the response is never acquired. Similarly, if a given male is allowed to interact with a female, but fails to copulate during the first few tests, the acquisition of the proximity response is delayed until the first test when copulation will take place (Fig. 2). These observations clearly indicate the close

link between copulatory behavior and the acquisition of the proximity response (Balthazart et al., 1995).

Note, however, that once this response has been acquired, it no longer needs to be reinforced by copulating with a female. In the experiment described in figure 2, two males who had fully acquired the proximity response were tested repeatedly over a period of five weeks during which access to the female was no longer permitted: the male could see the female through the window but was no longer allowed to copulate with her. As can be seen this had nearly no impact on his behavior: the male continued to spend most of the 5 min of test watching the female in front of the window.

Quail, like other birds, predominantly use visual and auditory stimuli in their social life while olfactory or tactile sensations only play a minor role, if any (see (Hinde, 1965; Lehrman, 1965) for reviews). Accordingly, Domjan and Nash demonstrated that static visual cues, as obtained from a taxidermically prepared model, are sufficient to induce social proximity behavior (and suppress crowing) in male quail in the absence of behavioral, auditory, and olfactory cues (Domjan and Nash, 1988). The behavior induced by the female model was primarily elicited by visual cues from the head and neck region that are sexually dimorphic in quail (Domjan and Nash, 1988). Additional stimuli, especially vocalizations and behavioral cues such as copulation solicitation, will enhance the response and probably play a role in the natural situation but do not appear mandatory.

Endocrine control—The rapid procedure of quantification of the learned social proximity described above was used in a suite of experiments designed to investigate the endocrine controls of appetitive sexual behavior. It was demonstrated that this response does not develop in castrated males (Balthazart et al., 1995). However these birds also did not copulate with the female when they were given the opportunity to do so. The failure to develop the social proximity response could therefore relate to this lack of reinforcement by copulation (see section before).

Once established the response remains, however, stable almost indefinitely in the absence of reinforcement that results from copulation with the female. This stability therefore provides an opportunity to test the endocrine dependence of this response. We showed indeed that if castrated males learn the proximity response under the influence of this exogenous testosterone supplied in the form of subcutaneous Silastic™ implants, the removal of these implants rapidly leads to a decreased response (Balthazart et al., 1995)

Another series of experiments also tested the steroid specificity of the social proximity response and demonstrated that it is activated, like copulatory behavior, by estrogens derived from testosterone by aromatization. This was first demonstrated by the fact that castrated birds do acquire the proximity response if they are systemically treated with either the endogenous estrogen, estradiol-17 β or with a synthetic estrogen, such as diethylstilbestrol (DES). These compounds mimic almost perfectly the effects of exogenous testosterone when administered to castrated birds (Balthazart et al., 1995).

Furthermore, once the proximity response had been acquired, its expression can be blocked by daily injections of the antiestrogen, tamoxifen (Balthazart et al., 1995) or of the aromatase inhibitor, Vorozole™ (Balthazart et al., 1997a). During the period when these injections were administered, most tests of appetitive sexual behavior were performed under conditions of extinction which means that the door providing physical access to the female was no longer opened and free access to the female (door opens) was no longer provided. The effects of the inhibition of estrogen production (aromatase inhibitor) or action

(tamoxifen) could in this way be quantified while the birds were not exposed to the negative punishment possibly associated with their inability to copulate with the female.

In conclusion, these experiments demonstrate that the social proximity response, which is used as a measure of appetitive male sexual behavior in quail, is activated, as is the case for consummatory sexual behavior, by testosterone acting through its estrogenic metabolites. The endocrine specificity of these two aspects of behavior is therefore similar, if not identical.

Underlying brain mechanisms—Converging evidence derived from lesions studies, stereotaxic implantation of testosterone or its behaviorally active metabolites in the brain as well as from the quantification of protein product of immediate early genes after expression of sexual behavior indicates that the sexually dimorphic medial preoptic nucleus (POM, see (Panzica et al., 1996)) plays a critical role in the control of male copulatory behavior in quail (Balthazart and Ball, 2007; Panzica et al., 1996). A few studies investigated in parallel whether similar or different brain areas are implicated in the control of the learned social proximity response. Because previous experiments had demonstrated that testosterone aromatization is a limiting factor for the activation of this response, we focused our attention on brain areas that contain dense populations of neurons expressing the enzyme aromatase, namely the POM and the medial part of the bed nucleus of the stria terminalis, BSTM.

Castrated males treated with testosterone that had previously acquired the proximity response were submitted to bilateral electrolytic lesions aimed at the POM or BSTM (Balthazart et al., 1998). Lesions aimed at the BSTM moderately decreased consummatory sexual behavior but had no effect on the proximity response and will therefore not be discussed further. In contrast, and as expected based on previous results, lesions destroying a significant portion of the POM abolished consummatory sexual behavior but, interestingly, they also significantly decreased the expression of the social proximity response. Interestingly however, these lesions did not completely suppress the proximity response and there was a large degree of variability in these effects. To identify the origin of this variability, lesions were then reconstructed in histological sections of the brain of these birds based on the damage that had been made to the dense group of aromatase-immunoreactive neurons that accurately defines the entire POM throughout its rostral to caudal axis. An anatomical dissociation between brain sites affecting copulatory behavior and the social proximity response was detected in this analysis. Lesion to the portion of the POM just rostral to the anterior commissure resulted in the complete inhibition of consummatory sexual behavior while expression of the proximity response was not affected by these lesions but inhibited by the destruction of more rostral portions of this nucleus (Balthazart et al., 1998). A parallel study demonstrated that stereotaxic implantation of testosterone in the POM but not in adjacent parts of the preoptic area increases the expression of the social proximity response in addition while also activating copulatory behavior (Riters et al., 1998).

Overall, these experiments involving lesions of and testosterone implantation in the POM support the notion that this nucleus plays an important role in the control of both appetitive and consummatory male sexual behavior. Some fine anatomical differences may, however, distinguish sub-regions of the nucleus that relate more specifically to these two aspects of the behavior.

3.2. The rhythmic contraction of the cloacal gland muscles

One potential problem with the use of the social proximity response as a measure of appetitive sexual behavior is that the response is learned after being reinforced by the performance of copulatory behavior. Consequently, this linkage prevents the assessment of

appetitive male sexual behavior in subjects that fail to copulate. It is thus impossible to discern whether experimental manipulations that inhibit copulation also affect the appetitive sexual behavior *per se* or only the ability to learn a new response.

A second behavioral procedure that does not suffer from this problem allows a measure in quail of appetitive sexual behavior and thus provides insight into their potential arousal. It is based on studies conducted by Seiwert, Thompson, Adkins-Regan and colleagues at Cornell University showing that rhythmic contractions of the cloacal gland and cloacal sphincter muscles (RCSM) are greatly facilitated in males when they are exposed to a female (Seiwert and Adkins-Regan, 1998; Thompson et al., 1998). The cloacal or proctodeal gland is an androgen-sensitive structure located on the caudal lip of the cloaca that is characteristic of the *Coturnix* family. It produces a meringue-like foam by rhythmic movements of a sexually dimorphic striated cloacal sphincter muscle that is interdigitated with the proctodeal gland (Seiwert, 1994; Seiwert and Adkins-Regan, 1998). This foam is transferred to females during copulation and enhances the probability that the sperm will fertilize the egg (Seiwert and Adkins-Regan, 1998). These contractions 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.

Control by stimuli originating from the female—These RCSM are greatly facilitated in males, including sexually naïve males, when they are presented with a female as compared to when it is a male (Seiwert, 1994; Seiwert and Adkins-Regan, 1998; Thompson et al., 1998).

Although naïve males can produce this response, the frequency of these contractions is modulated by the past experience of the birds. We observed for example that the RCSM frequency decreases progressively during successive tests in which the male is never provided access with the female, presumably due to a lack of sexual reinforcement (Cornil et al., 2006). This frequency is also drastically different if males have been previously exposed visually to a male or to a female (Cornil and Ball, 2010).

It is also notable that the production of RCSM can be conditioned to an arbitrary stimulus. Males that are repeatedly exposed to a neutral CS (terry-cloth covered foam block with an 8×2 cm pillar at one long end, vaguely reminiscent of a squatting female) paired with copulatory access to a female progressively learn to express RCSM at the simple view of the CS (Cornil et al., 2004; Holloway et al., 2005).

Endocrine control—Besides their link to the presence of a female, RCSM are connected to the expression of male sexual behavior in multiple ways. Their expression first depends markedly on the presence of high circulating concentrations of testosterone. Castration markedly reduces this behavior that can be easily restored by a treatment with exogenous androgens (Balthazart et al., 1998). These 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., 1998). Interestingly the RCSM expressed in response to a conditioned stimulus are also androgen dependent (Cornil et al., 2004; Holloway et al., 2005).

Like copulatory behavior *sensu stricto*, the expression of RCSM in response to the normal biological stimulus (a female) or to a conditioned stimulus, critically depend on the aromatization of testosterone into an estrogen. If castrated males treated with exogenous testosterone that express RCSM at high frequencies when exposed to a female are treated with the aromatase inhibitor Vorozole™, there will be a progressive decrease in RCSM

frequency over the course of the experiment so that after 3–4 weeks their frequency will be similar to the frequency observed in castrates that receive no testosterone supplementation (Taziaux et al., 2004). Similarly, RCSM expressed in response to a conditioned arbitrary stimulus are inhibited by treatment with an aromatase inhibitor (Cornil et al., 2004).

Underlying brain mechanisms—Additionally, the expression of RCSM is, like the expression of copulatory behavior, controlled by the POM. Lesions destroying a significant portion of this nucleus almost completely abolish the production of RCSM in response to the presentation of a female (Balthazart et al 1998). This is observed in castrated birds treated with exogenous testosterone so that the decrease in behavior cannot possibly result from an inhibition of testosterone secretion by the testes. Conversely, the cloacal sphincter contractions are enhanced in castrated birds that received a stereotaxic implant of testosterone in the POM (Riters et al 1998) even if this specific effect was not fully significant in this experiment because it was observed in only a fraction of the experimental subjects.

4. Changes in brain (neurochemical) activity

In mammals, changes in brain activity have also been linked to performance of male sexual behavior and more specifically to the behavioral condition that immediately precedes this behavior that must be characteristic of the state of sexual arousal. In a few limited cases, similar information is available for one avian species, the Japanese quail, thus indicating that similar neuronal changes occur in this species as in rodents in anticipation of copulation.

4.1. The Release of dopamine in the preoptic area

In rats, exposure to an estrus female enhances the concentration of extracellular dopamine (DA) in the medial preoptic area (mPOA) as detected by *in vivo* dialysis coupled with electrochemical detection and quantification (Hull et al., 1995). This increase in preoptic DA seems to be required for the successful performance of copulation as suggested by the observation that males exhibiting a substantial rise in extracellular DA concentration in response to the female will subsequently copulate whereas subjects that do not show this rise will not (Hull et al., 1995). The increased extracellular DA concentration might thus be viewed as a signal of sexual arousal and motivation.

In quail as in rats, pharmacological studies involving the administration of a variety of dopamine receptor agonists and antagonists indicate that dopamine plays a key role in the control of copulation (in rat: for review see: (Hull, 1995; Hull and Rodriguez-Manzo, 2009; Hull et al., 2005); quail: (Absil et al., 1994; Balthazart et al., 1997b; Castagna et al., 1997; Kleitz-Nelson et al., 2010a)). Detailed studies in quail also suggested that the performance of appetitive sexual behavior as measured by the social proximity response or by the RCSM frequency is similarly controlled by DA (Balthazart et al., 1997b; Castagna et al., 1997; Kleitz-Nelson et al., 2010a). These data therefore prompted us to investigate the potential changes in DA release in the preoptic area of quail exposed to a female and, in some case, allowed to freely copulate with her.

During a first study, a dialysis probe was inserted into the brain of male quail approximately 2 mm above the mPOA and DA was assayed in samples collected every 6 min before and while a male was exposed to a sexually mature female and allowed to copulate with her (Fig. 3A).

Three baseline samples were first collected and then a female was introduced into the chamber containing the male and six additional samples were collected. The female was then removed and another six samples were collected. The small size of the quail brain

prevented collection of samples more frequently than every 6 min and since copulatory behavior in quail often occurs very rapidly when the female becomes available (sometimes with a few sec in experienced males), it was impossible to obtain assays of DA concentrations in males exposed to the female before the actual copulation took place. As can be seen in figure 3A, the introduction of a female did result in a marked increase in extracellular DA presumably related to an increased release from dopaminergic terminals in the area which returned to baseline after removal of the female (Kleitz-Nelson et al., 2010c). Quite interestingly, a few subjects did not copulate during this behavioral test and these subjects showed no increase in extracellular DA concentration, thus linking this increase quite specifically to sexual behavior and the female. It is also useful to note that because copulation occurs rapidly in quail, the birds did not copulate during all six collection periods when the female was present (Kleitz-Nelson et al., 2010c). A separate analysis of data of birds that copulated at least once comparing the six min period in which birds did or did not copulate revealed no difference in DA concentration suggesting that the increase in DA was related to the presence of the female rather than to the performance of copulation *per se*. Extracellular DA concentration was high during the entire period when the female was present irrespective of whether the male did or did not copulate.

Because the temporal resolution of the method did not allow us to measure potential changes in DA that would result from only viewing the female (in anticipation of copulation?), a second experiment was performed with a similar design but in this case the male was visually exposed to the female behind a glass partition during three sample collection periods (total 18 min) and did not have the opportunity to copulate with her during that time but he performed the RSCM at high rates. The partition was then removed and the male and female could interact freely during another 18 min while 3 additional samples were collected. The female was then removed and 3 final post-test samples were collected (see Fig. 3B).

A significant increase in extracellular DA was observed here when males were visually exposed to a female but could not interact with her (Kleitz-Nelson et al., 2010b). This increase was only observed in birds that did subsequently copulate when the female was released. Non-copulators showed no DA increase in response to the view of the female. In copulators, no statistically significant further increase in DA was observed when the barrier between the male and the female was removed.

These data therefore clearly indicate that the extracellular DA concentration in the mPOA is an accurate predictor of the subsequent occurrence of copulatory behavior and thus likely represents a brain indicator of arousal in male quail. Our findings concerning dopamine release in male quail during sexual behavior is remarkably similar to what has been reported for rats (Blackburn et al., 1992; Du et al., 1998; Hull and Dominguez, 2006; Hull et al., 1995; Hull et al., 1999; Putnam et al., 2001). These findings do suggest that in quail as in rats sexual stimuli release dopamine to coordinate sexual behavior with autonomic responses associated with sexual behavior. These autonomic responses in rats (such as penile erections) are generally considered to be measures of sexual arousal. Thus the link between dopamine and sexual behavior in male quail is entirely consistent with it being a measure of sexual arousal.

4.2 Immediate early genes

The immunohistochemical quantification of the protein products of immediate early genes such as *c-fos* or *egr-1* (also called *zenk* in birds, (Mello et al., 1992)) has been extensively used to identify brain areas that are activated during sexual behavior. This activation can obviously be associated with the perception of stimuli originating from the female or with

the performance of motor acts related to copulation. It may, however, also reflect changes in the internal state of the animal including its sexual motivation or arousal.

In quail like in rodents, performance of copulatory behavior enhances the expression of *c-fos* in a variety of brain regions that are sometimes referred to as the social behavior network in the brain, including the mPOA, ventromedial nucleus of the hypothalamus, lateral septum and bed nucleus of the stria terminalis, BSTM (Goodson, 2005; Newman, 1999). In quail specifically, an increase in *c-fos* expression has been reported in the POM and BSTM following interactions with a female ranging in duration between 2 and 90 min (Charlier et al., 2005; Meddle et al., 1997).

It has also been demonstrated that the induction of *c-fos* expression in the mPOA and BSTM relates at least in part to the perception of stimuli originating from the female. Visual and acoustic cues are clearly the primary signals orienting sexual interactions in male and female quail (Domjan and Hall, 1986a; Domjan and Nash, 1988) but, somewhat unexpectedly, olfactory signals also seem to play a role as they do in mammals (Balthazart and Taziaux, 2009). Therefore we investigated the sexual behavior of male quail after their nares had been mechanically occluded (blocked with a layer of rapid-drying dental cement) so that they could no longer detect most olfactory stimuli. Another group of subjects had their cloacal region anesthetized before the copulatory test so that they could no longer perceive stimuli originating from this region during copulation. As illustrated in figure 4, these experimental manipulations, alone or in combination, did not significantly affect the occurrence of sexual behaviors (similar frequencies of mount attempts and cloacal contact movements as in the group presented with females after control manipulations). However, a significant decrease in the number of FOS-immunoreactive cells was detected in the POM and BSTM of birds that copulated while they were deprived of olfaction and/or from somato-sensory feedback from the cloacal region (Taziaux et al., 2008b).

These data therefore indicate that the neural activation (*c-fos* induction) observed during copulation in male quail primarily results from the perception of olfactory stimuli originating from the female and from cloacal feedback or reflects the central activation of integration neurons affected by these stimuli in preparation for copulation. This *c-fos* activation cannot reflect in a major manner the premotor outputs related to copulation since *c-fos* expression was decreased whereas frequencies of copulatory behaviors remained essentially the same. Why these sensory deprivations did not affect behavioral expression remains somewhat unclear at present but may relate to the fact that the experimental birds were not sexually naïve in these experiments and were therefore able to rely on a variety of stimuli (visual, acoustic) to control their behavior (see (Taziaux et al., 2008b) for additional discussion of this observation that does not related in a direct manner to the present topic)

During more recent experiments, we also wondered whether the performance of copulation was actually required to induce this *c-fos* expression or whether visual access to the female by itself would be sufficient. To answer this question, four groups of castrated males treated with testosterone were thus exposed for 5 min to one of four experimental conditions: a) free interaction with a female resulting in the expression of active copulatory behavior (copulation group group), b) visual presentation to a female associated with the expression of RSCM (male RSCM group), c) visual presentation to a male resulting in a lower frequency of RSCM (ASB-M group), and finally d) handling as a control manipulation (Taziaux et al., 2006). Brains were collected 90 min after the start of these manipulations and processed by immunohistochemistry for the FOS protein.

In males that had copulated with a female as compared to the control birds, an increase in *c-fos* expression was observed throughout the POM, the medial part of bed nucleus striae

terminalis (BSTM), the lateral septum and the ventro-lateral thalamus (VLT), a brain area related to visual processing (Taziaux et al., 2006). Quite interestingly, *c-fos* expression was also increased in some of these brain areas of birds that had only seen the females and produced RSCM in response to this stimulus but had not had an opportunity to copulate with her. These brain areas included the POM, although in a portion more rostral than in birds that had copulated, and the VLT. A numerical increase was observed in the BSTM but it did not reach statistical significance (Taziaux et al., 2006). These increases were specific to the female stimulus because males that had been visually exposed to another male displayed a different pattern of brain activation (increase in lateral septum but not in POM, BSTM nor VLT).

These data support the notion that while there is an overlap among some brain areas there is also a partial anatomical dissociation between structures involved in the control of appetitive and consummatory aspects of male sexual behavior in quail. More relevant to the present topic, the data demonstrate the existence of an activation of specific brain regions in male that are visually exposed to a female and would therefore be expected to exhibit some form of sexual arousal. To our knowledge, the specificity of a connection between arousal *per se* and *c-fos* expression in the brain has not been demonstrated.

A recent conditioning experiment, however, does suggest the existence of such a link. As mentioned already, quail are able to associate an arbitrary conditioned stimulus (CS) with the access to copulation. It was demonstrated that such an association enhances the activation of brain areas during expression of sexual behavior. The expression of *c-fos* was indeed more intense (as assessed by a greater number of labeled cells) in the POM and BSTM of birds that had been sequentially exposed to the CS paired to the female than in birds exposed either to the CS or to the female alone (Taziaux et al., 2008a). This differential brain activation could represent at least a component of the mechanism mediating the improved copulatory efficiency associated with the presentation of a CS before sexual interactions and, more critically for the present review, could reflect the increased sexual arousal in birds exposed to the conditioned stimulus.

4.3. Deoxyglucose autoradiography

The quantification of 2-deoxyglucose (2DG) incorporation represents an attractive alternative to the study of immediate early genes for identifying brain regions that are active during the performance of a specific task. Neuronal activity is indeed reflected in glucose utilization since function-related energy requirements in the brain are almost exclusively met from the oxidative catabolism of glucose supplied by brain blood flow. If it is injected into an animal just before performance of behavioral task, radioactive 2DG will be trapped and accumulate in brain cells showing an increased metabolism and thus glucose utilization during this task. This accumulation of radioactivity can subsequently be quantified in anatomically discrete structures by *in vitro* quantitative autoradiography.

In one experiment, we compared by this method the brain activation resulting from the free interaction (including copulation) with a female for a period of 45 min with the activation resulting from the visual interaction only of the male with the female for the same duration (Dermon et al., 1999). Males used in this experiment had acquired during previous training sessions the social proximity response so that they actually spend the majority of the test session watching the female through the small window separating the two chambers of the test cage (see section 3.1 before).

A complex pattern of activation and inhibition of anatomically discrete brain regions was observed in these two groups of birds as compared to control subjects that were placed in the same two compartment testing cage but had not previously acquired the social proximity

response so that they spend no time observing the female located in the adjacent compartment (window between the compartment was open but door was closed so that physical interactions were not possible).

In both experimental groups (visual and copulatory interactions) analyses of the autoradiograms revealed significant increases by comparison with the control group in the deoxyglucose incorporation in the nucleus mesencephalicus lateralis, pars dorsalis and in the nucleus lemnisci lateralis. In addition, an increase in the deoxyglucose incorporation was specifically observed in the paleostriatum primitivum (now called globus pallidus; (Reiner et al., 2004)), rostral preoptic area, nucleus intercollicularis, nucleus interpeduncularis and third nerve but a decrease was observed in the dorsomedial part of the hippocampus and in the nucleus nervi oculomotori in birds that had been allowed to copulate by comparison with controls. In contrast, after visual exposure to the female, significant increases in deoxyglucose incorporation were observed in two telencephalic areas, the intermediate hyperstriatum ventrale and neostriatum caudolaterale by comparison with the controls, but decreases were detected in the stratum griseum et fibrosum superficiale of optic tectum by comparison with the consummatory behavior group (see Fig. 5).

Some of the changes observed in the two groups were thus similar while others were discordant. It is likely that some of the changes observed here should relate to the sexual arousal associated with the view of the female but additional specific experiments should obviously be performed to interpret the mechanisms leading to these anatomically discrete changes in brain metabolism.

5. Discussion

The goal of this review is to consider how one might assess sexual arousal in birds. As noted in the introduction to this paper, sexual arousal has generally been discussed exclusively in mammalian species. Some definitions of sexual arousal based on what has been observed in mammals (in particular in relation to penile erections) are so specific that one can wonder whether the concept could be applied to avian species at all. We have therefore summarized the neuroendocrine control of male sexual behavior in Japanese quail and how behavioral and neural components of these processes might serve as indicators of sexual arousal. When considering what sort of behavioral measures might be viewed as good indicators of sexual arousal in non-human animals, it is useful to consider why certain measures have been advocated as being the best measures in mammals. As noted previously, nearly all authors (e.g., (Bancroft, 2005; Pfau et al., 2010; Sachs, 2007)) advocate that penile erection be considered as at least a component of any measure of sexual arousal in male mammals. Why is this the case? The penis is a specialized structure that is involved in gamete transfer and micturation. It must be erect for gamete transfer to occur though it can also become erect for other reasons. Therefore Sachs argued that penile erections in a clear sexual context served as a definitive measure of sexual arousal in non-human mammals (Sachs, 2007). Bancroft agrees that this is a very useful measure in men but thinks that other aspects of sexual arousal such as the information processing of sexually relevant stimuli are also important (Bancroft, 2005). If one accepts this line of reasoning then behavioral measures involving specialized structures or responses that clearly lead to specifically to copulation are candidates to serve as good measures of sexual arousal when (but only when) they are detected in a clear sexual context.

What about the behavioral measures of appetitive sexual behavior in quail? Can these serve as legitimate measures of sexual arousal? The learned social proximity response is tied to the presence of a female but not a male and is clearly linked to a sexual context because a male who has not copulated in the arena where he sees the female will not exhibit the

response. The RSCM is even more similar to non-contact erections in that this behavior has a clear functional significance because it promotes the production of foam that enhances male fertilization success (Seiwert and Adkins-Regan, 1998). The cloaca like the penis is involved in elimination as well as in gamete transfer. There is not, however, a clear difference between movements involved in elimination and those involved in gamete transfer as is the case for the penis. As has already been reviewed the RSCM can be affected by stimuli in such a way that changes in its frequency are not always clearly linked to the occurrence of copulation behavior. However, we would argue that when its frequency is increased in a clear sexual context it can indeed serve as a good measure of sexual arousal when one employs similar criteria as are employed to support non-contact erections.

The recent findings by Kleitz-Nelson et al. (Kleitz-Nelson et al., 2010b; Kleitz-Nelson et al., 2010c) on dopamine release provide compelling support for the notion that these pre-copulatory appetitive behaviors in quail are in some cases linked specifically to occurrence of copulatory. As reviewed previously, when male quail are presented with a female they will respond with an increase in dopamine only if they will subsequently go on to copulate (Kleitz-Nelson et al., 2010c). This is similar to what has been reported in rats (Hull et al., 1995). Thus perhaps the final arbitrator as to whether our behavioral measures are indicators of true sexual arousal would be to have a read-out of dopamine release in the POA. This is at present no reliable external indicator of this event but the fact that dopamine is correlated to some degree with these appetitive behavioral measures in quail gives one confidence that these behavioral measures are an indicator of sexual arousal. These findings also suggest that the role of preoptic area dopamine in facilitating sexual arousal and being causally linked to the onset of male copulatory behavior has been highly conserved among vertebrate taxa.

One final observation is that this review has focused on males. Female birds do exhibit solicitations displays that are hormone dependent and are good predictors of their future probability of engaging in copulation (Searcy and Marler, 1981). There is also evidence that such solicitation displays are dependent on the action of estrogen in the ventromedial nucleus of the hypothalamus as is the case in rats as well (Gibson and Cheng, 1979). It is therefore quite probable that appetitive sexual behaviors in female birds can also serve as good indicators of sexual arousal. However, mechanistic studies of these behaviors that would provide more convincing evidence of this have yet to be performed. For now though, we are confident that the concept of sexual arousal can be usefully applied to male birds based on the appetitive behavioral measures reviewed in this paper. Another interesting dimension to pursue in the future would be to investigate birds that do have intromittent organs such as ducks (i.e. members of the family anatidae; (Brennan et al.)).

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Figure 1. Schematic representation of the two-compartment cage used to measure the learned social proximity response (A) or to quantify the frequency of rhythmic contraction of the cloacal gland muscles (B). See text for description of experimental procedures. The mirror under the cage in B allows visualization of the cloacal gland of the subjects.

Figure 2.

Acquisition and maintenance of the social proximity response in two adult sexually mature male quail. Birds were first subjected to 9 acquisition tests over a 14 day-period during which they acquired the response: time spent in front of the window increased to 80–90% of the test time (5 min or 300 sec). Note that the time spent at the window only increased after the male had started copulating with the females (indicated by upward-pointing arrows, Test 1 for male 60, Test 4 for male 16). Once established the response is not affected by two weeks of interruption in testing (tests 9 and 10 between lines A and B) nor by repeated testing during which access to the female is no longer provided (tests 12 to 35 after line B). Redrawn from data in (Balthazart et al., 1995).

Figure 3.

Changes in extracellular dopamine in the medial preoptic area as observed by *in vivo* dialysis and HPLC coupled with electrochemical detection in adult male quail that were allowed to interact and copulate freely with a female (A) or were first allowed to see the female and express rhythmic contractions of the sphincter muscles (RCSM) before they could actually copulate with the female (B). Control birds (non-copulators) were placed in the same experimental chamber but not exposed to a female. Successive samples were collected every 6 min. Three samples were collected before any manipulation (Pre-test) and 3 or 6 samples after the male was exposed to the female (Post-test). Redrawn from data in (Kleitz-Nelson et al., 2010c) and (Kleitz-Nelson et al., 2010b)

Figure 4.

Effects of manipulations of sensory inputs on sexual behavior and on the induction of *c-fos* expression in the brain of male quail. Males in this experiment were either kept in their home cage (A; Ctrl) or allowed to copulate with a female (B-E) after their cloacal gland area had been anesthetized with a local spray of xylocaine (GCA-x; C) or their nostrils had been blocked with dental cement (Anosmia; D) or they had received both manipulations (Both; E) or they had received control manipulations (spray of saline on the cloacal gland and dental cement applied next to the nostril (Sex; B). These manipulations did not affect the frequencies of sexual behavior (mount attempts and cloacal contact movements, top two graphs) in a statistically significant manner. The numbers of cells expressing FOS, the protein product of the *c-fos* gene was, however, decreased by the experimental manipulations in the caudal part of the medial preoptic area (mPOA) and/or in the rostral part of the bed nucleus of the stria terminalis (BSTM; two bottom graphs). High magnification photomicrographs of representative sections in the mPOA in the five experimental groups (A through E) are shown on the right side of the figure. Magnification bar = 100 μ m. Redrawn from data in (Taziaux et al., 2008b).

Figure 5. Effects of the performance of appetitive sexual behavior (ASB; quantified here by the learned social proximity response) or of consummatory sexual behavior (CSB) on brain metabolic activity as assessed by the accumulation of radioactive 2 deoxyglucose (2-DG). The top panel shows color-coded autoradiograms illustrating 2-DG incorporation in a control bird (Ctrl) and a subject of the ASB and of the CSB groups at the level of nucleus intercollicularis of the mesencephalon. Scale bar= 1 mm. The bottom panel summarizes the statistically significant changes in 2-DG accumulation (increases= up arrow; decreases= down arrow) that were observed by comparison with the control.

Abbreviations: Cb-Mol cerebellum-molecular layer, Hp-DM hippocampus- dorsomedial part, ICo nucleus intercollicularis, IMHV intermediate hyperstriatum ventrale (now renamed: intermediate and ventral hyperpallium see (Reiner et al., 2004)), IP nucleus interpeduncularis, LL nucleus lemnisci lateralis, MLd nucleus mesencephalicus lateralis pars dorsalis, Ndc neostriatum caudolaterale (now renamed: caudolateral nidopallium), NIII nervus oculomotorius, OM nucleus nervi oculomotori, POA preoptic area, PP paleostriatum primitivum (now renamed: globus pallidus), SGFS stratum griseum et fibrosum superficiale, TO tuberculum olfactorium, TeO optic tectum. Redrawn from data in (Dermon et al., 1999).

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