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Dopamine Release in the Medial Preoptic Area is Related to Hormonal Action and Sexual Motivation

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Abstract

In order to help elucidate how general the role of dopamine (DA) release in the medial preoptic area (mPOA) is for the activation of male sexual behavior in vertebrates, we recently developed an *in vivo* microdialysis procedure in the mPOA of Japanese quail. Using these techniques in the present experiment, the temporal pattern of DA release in relation to the pre-copulatory exposure to a female and to the expression of both appetitive and consummatory aspects of male sexual behavior was investigated. Extracellular samples from the mPOA of adult sexually-experienced male quail were collected every six minutes before, while viewing, while in physical contact with, and after exposure to a female. In the absence of a pre-copulatory rise in DA, males failed to copulate when the barrier separating them from the female was removed. In contrast, males that showed a substantial increase in mPOA DA during pre-copulatory interactions behind the barrier, copulated with females after its removal. However, there was no difference in DA during periods when the quail were copulating as compared to when the female was present but the males were not copulating. In addition, we show that pre-copulatory DA predicts future DA levels and copulatory behavior frequency. Furthermore, the size of the cloacal gland, an accurate indicator of testosterone action, is positively correlated with pre-copulatory DA. Taken together, these results provide further support for the hypothesis that DA action in the mPOA is specifically linked to sexual motivation as compared to copulatory behavior *per se*.

Keywords

birds; *in vivo* microdialysis; testosterone; male sexual behavior; appetitive behavior

Introduction

Dopamine (DA) facilitates male sexual behavior in rats and other mammals partly through its action in the medial preoptic area (mPOA) which is hypothesized to focus the male's motivation on sexually relevant stimuli, coordinate genital reflexes necessary for erection and ejaculation, and enhance male-typical motor patterns of copulation (for reviews, see Hull et al., 1999; Hull, Wood, & McKenna, 2006). Japanese quail (*Coturnix japonica*) have also served as a useful model species to investigate the dopaminergic regulation of male

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sexual behavior (for reviews, see Ball & Balthazart, 2010; Balthazart & Ball, 1998a). In support of this conclusion, we recently showed in quail via central intracerebroventricular injections of D1 and D2 DA receptor agonists and antagonists, that when these compounds are administered into the ventricles and can therefore potentially act throughout the brain, D1 receptors primarily facilitate while D2 receptors primarily inhibit male sexual behavior (Kleitz-Nelson, Cornil, Balthazart, & Ball, 2010). We also found that the microinjection directly into the mPOA of a D1 or a D2 antagonist in quail decreased the frequency of both appetitive and consummatory aspects of male sexual behavior and increased the latency to initiate copulation (Kleitz-Nelson, Cornil et al., 2010). A general implication of these findings is that the specific role played by DA action in the mPOA in the control of male-typical sexual behavior might be distinct from its actions elsewhere in the brain and periphery. Further, the results from this study showed that when dopaminergic antagonists are microinjected into the mPOA, male sexual behavior is inhibited, suggesting that there is an endogenous *in vivo* DA activity in the mPOA, since in its absence, there would be no dopaminergic activity to be blocked by the two receptor-selective antagonists (Kleitz-Nelson, Cornil et al., 2010). Thus, one critical question that remains to be answered concerns the temporal pattern of DA release in the mPOA during behavioral interactions with a female.

In rats, it has been shown that the presence of a female enhances DA release in the mPOA in a testosterone-dependent fashion (Hull, Du, Lorrain, & Matuszewich, 1995) and this release is critical for the activation of copulatory behavior. Until recently (Kleitz-Nelson, Dominguez, Cornil, & Ball, 2010), investigations of DA release in relation to male sexual behavior in the mPOA have been limited to rodents. Because quail do not exhibit erections, the detection of a change in the release of DA in the mPOA cannot be attributed solely to a change in arousal that might facilitate penile erections, but rather such changes can be more readily tied to sexual motivation and performance. Therefore, we developed an *in vivo* microdialysis procedure in the mPOA of Japanese quail (the only species other than rodents which this has been done) and showed that it is not solely the presence of a female that drives DA release, but how the male responds to her (Kleitz-Nelson, Dominguez et al., 2010). In the present experiment, the temporal pattern of this release in relation to the pre-copulatory exposure to a female and to the expression of both appetitive and consummatory aspects of male sexual behavior was investigated. We also correlated these measurements of DA release in the presence of a female in relation to testosterone action, as assessed by the size of the cloacal gland, a structure whose size is highly dependent in a positive direction on testosterone action (Sachs, 1967).

Materials and Methods

Subjects

This study was conducted with Japanese quail (*Coturnix japonica*). A total of 18 adult males and 10 females (who served as stimulus animals) were obtained from a breeder (Sherwood Game Farm, Racine, WI) and were experimentally and sexually naive prior to procedures they experienced in this study. Throughout their life at the breeding colony and in the laboratory, birds were individually housed and exposed to a photoperiod simulating long days (14h light and 10h dark per day), with food and water available *ad libitum*. Throughout the experiment, birds were periodically weighed to the nearest gram and the size of their cloacal gland, an androgen-dependent structure (Delville, Sulon, & Balthazart, 1985; Sachs, 1967) was measured with digital calipers (cloacal gland area = largest length $\times$ largest width in mm²). The cloacal gland size is highly correlated in a positive direction with plasma androgen levels in quail (Sachs, 1967). It is one of the most accurate peripheral structures that can be used as a bioassay to assess plasma androgen concentrations as experienced over time that has been identified in any species (Ball & Balthazart, 2010). Thus, the

measurement of cloacal gland size provided an indicator of testosterone action that is integrated over the recent past (Ball & Balthazart, 2010).

All birds received 5 pre-test trials for copulatory behavior after the cannula implantation surgery (see below) to ensure that they were all able to copulate before the microdialysis experiment. In quail, the copulatory sequence is as follows: neck-grab (NG), mount attempt (MA), mount (M) and finally culminating in cloacal contact movements (CCM) (for a detailed description, see Adkins & Adler, 1972; Hutchison, 1978). All birds exhibited at least one CCM during a minimum of 3 of the 5 pre-tests and they all copulated the day prior to the microdialysis experiment. Appetitive sexual behavior (ASB) in quail can be assessed by the measure of the rhythmic cloacal sphincter movements (RCSM). Gonadally intact males increase the rate of these movements when they are provided with only visual access to a female; the same cannot be said with castrated males or females (Cornil, Holloway, Taziaux, & Balthazart, 2004; Seiwert & Adkins-Regan, 1998; Thompson, Goodson, Ruscio, & Adkins-Regan, 1998). Thus, RCSM produced under these conditions in which a male is viewing a female in anticipation of copulation provides a measure of male ASB in quail.

Stereotaxic surgery

All male quail were deeply anesthetized with isoflurane gas anesthetic (IsoSol isoflurane from Vedco, Inc, St. Joseph, MO; Isotec 4 anesthesia machine from Surgivet, Inc., Waukesha, WI USA) and placed in a stereotaxic apparatus (David Kopf instruments, Tujunga, CA, USA) with the pigeon-head holder placed at a 45° angle below the horizontal axis of the stereotaxic assembly. The skull was drilled at the level of the inter-parietal suture. The guide cannulae (MAB 4.15.IC; SciPro Inc., Sanborn, NY) were inserted into the brain to end 2mm above the mPOA (AP+1.8mm, ML+0.3mm, DV+2.8mm) and fixed to the skull with dental cement. The birds were kept in a warm environment until they fully recovered and Metacam® (meloxicam; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA) was administered for three days following the surgery to reduce pain and inflammation.

Microdialysis and behavior

Concentric microdialysis probes were purchased from SciPro Inc. (MAB 4.15.2.PES). The dialysis membrane had an outer diameter of 0.2 mm, an inner diameter of 0.18 mm, an active dialyzing length of 2mm, and 6 kDa cutoff. Dulbecco's PBS (in mM: 138 NaCl, 2.7 KCl, 0.5 MgCl₂, 1.5 KH₂PO₄, and 1.2 CaCl₂, pH 6.8, filtered and degassed before use; Sigma, St. Louis, MO) was perfused with a KD Scientific (model KDS220) infusion pump, using a 1 ml gas-tight Hamilton syringe. We previously reported the adequate flow rate for dialysate collection of 0.5µl/min (Kleitz-Nelson, Dominguez et al., 2010) so this flow rate was used again and the samples were collected every 6 min thus yielding 3µl of dialysate per sample.

Behavioral tests

Copulatory behavior was first assessed in a separate "practice" chamber so that the birds never had copulatory experience in the microdialysis chamber. All birds exhibited copulatory behavior the day prior to testing. Also on the day prior to testing, the birds were placed in the chamber without a female for one hour to allow habituation to it. The chamber sat on a raised platform with a mirror placed under it at a 45° angle and provided the observer with an unobstructed view of the male's cloacal area. At the beginning of each test, the chamber was divided in two by an opaque sliding panel and a glass partition.

On the day of testing, the area of the cloacal gland for each male was measured with digital calipers (cloacal gland area = largest length × largest width in mm²) and later used as a measure of testosterone action (Delville, Hendrick, Sulon, & Balthazart, 1984; Sachs, 1967).

Next, the microdialysis probe was implanted in the experimental male and placed in one side of the chamber and a stimulus egg-laying female on the other side. The probe was then attached to the perfusion line and approximately six hours later, three baseline (BL) samples were collected. The sliding opaque panel was then removed so that the male and female were only separated by the glass barrier; the male had visual access to the female although he was not able to physically interact with her. The frequency of female-elicited RSCM was quantified by direct observation for an additional three samples (RSCM). Then, the glass panel was removed and the birds were able to interact physically. During this period, three additional samples were collected (FEMALE period) and the frequency of the consummatory behavior of the birds was recorded. Because an entire copulatory sequence can occur in 4 seconds (Hutchison, 1978) and the quail were not engaging in copulatory behavior during the entire 18 minutes the female was present, there were some FEMALE samples collected that contained dialysate from when the birds were copulating (COP), and other samples in which the birds were not copulating (NOCOP). After the last sample was collected, the female was removed and three final samples were collected (POST). The following samples were assayed using high performance liquid chromatography with electrochemical detection (HPLC-EC) by an experimenter blind to the testing conditions: three BL; three RSCM; three FEMALE (samples that included COP and NOCOP); and three POST.

At the conclusion of the experiment, cannula placements were verified histologically. Animals were deeply anesthetized with isoflurane gas anesthetic and immediately euthanized by rapid decapitation. Their brains were then removed, frozen, and sectioned (40 μ m) using a cryostat. Sections including the mPOA were mounted on slides and examined for cannula placement. No lesions of the mPOA were discovered, however four birds had cannula placement in adjacent structures outside the mPOA and were thus removed from analysis. All birds were housed, manipulated, and euthanized by using procedures approved by the IACUC at Johns Hopkins University.

Chromatography

The LC Packings (San Francisco, CA) chromatographic system consisted of an Accurate microflow processor and pulse damper, a Valco injector with a 500nl sample loop, and an Antec microelectrochemical detector, equipped with a microflow cell (11nl cell volume), with a glassy carbon working electrode and a Ag/AgCl reference electrode. The analytical column was an LC Packings Fusica reversed-phase capillary column (300 μ m inner diameter, 5cm long, packed with 3 μ m C-18 particles). The working electrode was maintained at an applied potential of +0.8 V relative to the reference electrode. A Gilson Medical Electronics (Middleton, WI) pump (model 307) delivered mobile phase through the system at 0.5ml/min; however, the Accurate microflow processor split the flow, so that flow through the analytical column was $\sim$ 7 μ l/min. The mobile phase consisted of 32mM citric acid, 54.3mM sodium acetate, 0.074mM EDTA, 0.215mM octyl sulfonic acid (Fluka, Milwaukee, WI), and 4% methanol (v/v). It was filtered and degassed under vacuum; pH was 3.45. Data were collected using a PC, running Gilson Medical Electronics Unipoint system controller software, which also controlled the pump parameters.

Data analysis

The mean of the three baseline samples were used as the baseline measure and all values were converted to percentage of baseline. Data were analyzed by repeated measures analyses of the variance (ANOVA) with the condition (BL, RSCM, FEMALE [COP/NOCOP], and POST) as the repeated factor and Copulation (Copulators vs. Non-Copulators) as the independent factor. Additionally, correlation and regression analyses were performed to determine if DA levels during pre-copulatory interactions (RSCM)

predict future DA release and consummatory behavior frequency as well as cloacal gland size. Effects were considered significant for $p < 0.05$ and when appropriate, pairwise post-hoc comparisons were performed using Fisher's least significant difference (LSD) test. All analyses were carried out with Windows version of the software SPSS, version 17.0.

Results

Although all subjects copulated in the pretests following the surgery, not all subjects copulated while undergoing microdialysis (ten quail copulated [Copulators] while four did not [Non-copulators]). This outcome made it possible to compare the effects of copulation (between-subjects variable) on the concentration of DA in the mPOA during different testing conditions (within-subjects variable). Statistical analyses using a mixed design ANOVA of percent change from baseline revealed no main effect of copulation ($p > 0.05$; probably due to the smaller number of Non-copulators) but there was a significant main effect of the testing condition ($F_{3,36} = 3.665$, $p = 0.021$; figure 1A,B). Post-hoc analyses revealed that this change was significantly higher in FEMALE samples compared with POST. Additionally, among the ten males who copulated, all birds provided both COP and NOCOP samples (refer to Methods for a description). The within-subject analysis of percent change from baseline within these birds revealed a significant main effect of testing condition ($F_{4,36} = 5.152$, $p = 0.002$; figure 1C). Post-hoc analyses revealed that both the RSCM and COP samples contained a higher percent change of DA as compared to baseline and this change was also significantly higher in RSCM, COP, and NOCOP samples when compared to POST. Interestingly, there was no significant difference between COP and NOCOP samples.

The ranges of frequencies of RSCM in each of the three RSCM samples were: RSCM1: 0–320, RSCM2: 0–95, RSCM3: 0–77; and the ranges of frequencies of CCM's in each of the three FEMALE samples were: F1: 0–4, F2: 0–2, F3: 0–3. In an effort to gain a better understanding of how the DA levels during pre-copulatory interactions may predict future DA levels and appetitive and consummatory behavior frequency as well as presumed testosterone levels (as measured by cloacal gland area), correlation and regression analyses were performed. The cloacal gland area for one outlier was removed from analysis. Notably, only mean DA in RSCM samples showed any correlations with other variables (see table 1). Multiple regression analyses were then used to establish the unique contribution of mean DA percent change from baseline in RSCM samples to predict mean DA percent change in FEMALE samples, mean CCM frequency during FEMALE samples, as well as the cloacal gland area of each bird. A series of multiple regressions were performed to determine which variables best predicted the variables mentioned above. As presumed, the best predictor of all was indeed DA percent change from baseline in RSCM samples. As such, results revealed a significant regression model for mean DA during FEMALE ($F_{1,13} = 10.803$, $p = 0.006$; figure 2A) as well as for mean CCM frequency ($F_{1,13} = 10.752$, $p = 0.007$; figure 2B). Each model independently accounted for 47% of their variances (43% adjusted). Furthermore, results also revealed a significant regression model for cloacal gland area ($F_{1,12} = 6.718$, $p = 0.025$; figure 2C) which accounted for 38% of the variance (32% adjusted). In all cases, none of the other variables (mean RSCM frequency, mean CCM frequency, mean DA percent change from baseline in FEMALE samples, and cloacal gland area) significantly contributed to any of the regression models, $p > 0.1$ for all.

Finally, four animals were found to have cannulae placement outside the mPOA and were thus removed from analysis. The data from these birds showed a significant main effect of testing condition ($F_{3,9} = 5.258$, $p = 0.023$), however post-hoc analysis revealed that this effect was due to a significant decrease in DA during POST samples as compared to BL. This significant difference could be a result of a small sample size. However, because there was

no change in DA in any other testing condition from baseline, it suggests regional specificity of the DA response seen in animals with correct cannulae placement.

Discussion

The present experiment provides results consistent with our previous report (Kleitz-Nelson, Dominguez et al., 2010). By measuring DA levels during pre-copulatory interactions with a female and by correlating them with an estimate of testosterone levels, this experiment further provides evidence of the link of mPOA DA levels with motivational aspects of male sexual behavior as well as hormonal influences on this DA release and subsequent behavioral output. First, we observed in this study an increase in DA levels in the mPOA of male quail in response to the presence of a female that they could see by not physically interact with. These DA levels then returned to baseline after she was removed (figure 1A). This significant rise in DA occurred only in quail that subsequently went on to copulate with the female (figure 1B). In our previous study we only studied DA release in male quail that had the opportunity to copulate with females (Kleitz-Nelson, Dominguez et al., 2010). Furthermore, in this study, within birds that copulated, no change was detected among sampling periods in which they did or did not mate (figure 1C). Hence, in the presence of a female, the elevated DA concentration persisted regardless of the behavioral response of the male. Importantly, the data from the animals with cannulae placement outside the mPOA showed no changes in levels of DA release compared to baseline, supporting the regional specificity of the DA response seen in animals with correct placement.

One of the novel and interesting findings in the present experiment therefore is that copulating versus non-copulating males could be discriminated based on levels of DA that were assessed before mating could even be initiated. These pre-copulatory levels of DA were collected in the presence of a female where the male could see, hear, and smell her, but they could not interact physically. If the male exhibited a significant rise in DA in the mPOA in response to viewing the female, he then proceeded to copulate. If the rise in mPOA DA did not occur, then he failed to copulate. Correlation and regression analyses further supported this observation, revealing that levels of DA during the pre-copulatory interaction can predict DA levels when the male is in the physical presence of the female as well as predict the average CCM frequency (figure 2A,B). Thus, higher levels of DA in the mPOA during the pre-copulatory interaction predicted a greater likelihood that the male would mate successfully.

Furthermore, our results also revealed a significant relationship between cloacal gland area and DA levels during the pre-copulatory period of interaction, such that the larger size of the cloacal gland predicted higher DA levels during the RSCM samples (figure 2C). As previously described, the cloacal gland is an androgen-sensitive structure, and its size has been shown to be an accurate indicator of circulating testosterone levels (Delville et al., 1984; Delville et al., 1985; Sachs, 1967). Thus, our findings suggest that increases in testosterone predict higher DA levels in the mPOA during pre-copulatory interactions. This implies that circulating testosterone may contribute to the ability of distal cues from the female to activate hypothalamic DA neurons. Further, these findings provide support for the hypothesis that DA action is downstream of testosterone in the causal change of events that result in the activation of male-typical sexual behavior (reviewed in Balthazart, Baillien, & Ball, 2002; Balthazart & Ball, 1998a; Hull et al., 1999; Hull et al., 2006). Interestingly, the cloacal gland size did not correlate with any other DA or behavioral measurement, thereby suggesting that the release of DA during pre-copulatory interactions is the event most closely linked to the presumed testosterone action. Additionally, our present results corroborate previous microdialysis experiments in rats (Hull et al., 1995), which showed that the presence of a female enhanced DA release in the mPOA in a testosterone-dependent

fashion. It was suggested in that study that the presence of testosterone increased the probability that copulation will occur by enhancing the release of DA during the pre-copulatory interaction (Hull et al., 1995). This present report also provides support for theories about the interaction of testosterone and DA action (reviewed in Balthazart et al., 2002; Balthazart, Baillien, Cornil, & Ball, 2004; Balthazart & Ball, 1998a, 1998b), however future studies should be conducted using castrated quail with or without testosterone replacement in order to more specifically support these claims.

Specifically, in the quail preoptic area, aromatase-immunoreactive neurons are almost exclusively located within the boundaries of the POM (Balthazart & Ball, 1998b; Panzica, Viglietti-Panzica, & Balthazart, 1996). Importantly, testosterone must be aromatized within this nucleus to exert its behavioral effects (Balthazart et al., 2004). Further, rapid changes in aromatase activity have been observed after exposure to DA and dopaminergic compounds in quail hypothalamic homogenates and explants (Absil, Baillien, Ball, Panzica, & Balthazart, 2001; Balthazart et al., 2002). In addition, aromatase is expressed at high levels in the quail brain, and it is well established that testosterone increases aromatase activity in the preoptic area of all species of birds and mammals examined so far (Balthazart, Baillien, Charlier, Cornil, & Ball, 2003). Thus, it is reasonable to assume that in the present experiment the quail with larger cloacal glands (and therefore presumed higher testosterone levels) had greater aromatase activity than the birds with a smaller cloacal gland area. As such, we discovered with regression analysis that the cloacal gland size significantly predicts the DA levels during the pre-copulatory interaction while the male can see, hear, and smell the female but can't physically interact with her.

Interestingly, aromatase has been referred to as a multifunctional enzyme (Balthazart & Ball, 1998b) because estrogen can also be metabolized into catechol estrogens (MacLusky, Philip, Hurlburt, & Naftolin, 1984), and evidence suggests that this reaction is catalyzed by aromatase acting as a bi-functional enzyme (Balthazart, Stoop, Foidart, Granneman, & Lambert, 1994; Osawa, Higashiyama, Shimizu, & Yarborough, 1993). These catechol estrogens have been reported to bind to neural membranes and D2 receptors (Weisz, 1991) and are powerful competitive inhibitors of catechol(-)-methyltransferase (COMT). COMT is an enzyme which degrades catecholamines (including DA), and thus by blocking the metabolism of catecholamines, catechol estrogens lead to the accumulation of catecholamines in the synaptic cleft and therefore to an enhancement of their action (MacLusky, Naftolin, Krey, & Franks, 1981; Timmers & Lambert, 1989; Weisz, 1991). Thus, based on the results of the present experiment, we hypothesize that the higher testosterone concentrations (as assessed by the larger cloacal gland size) resulted in greater aromatase activity which can, in turn, metabolize estrogen into catechol estrogens, which are competitive inhibitors of COMT, resulting in an accumulation of DA and enhanced behavioral output. As such, the birds with larger cloacal glands had higher DA levels during the pre-copulatory interaction, and this increased DA also predicted higher DA levels during the copulatory interactions as well as higher frequency of the consummatory response (CCM frequency).

An alternative hypothesis involves the gaseous messenger molecule nitric oxide (NO). Both basal and female-stimulated DA release in the mPOA of rats is regulated by NO (Lorrain & Hull, 1993; Lorrain, Matuszewich, Howard, Du, & Hull, 1996). Furthermore, neuronal NO synthase (nNOS) is hormonally regulated, such that estradiol up-regulates nNOS in the mPOA, with the resulting production of NO maintaining DA release and preventing DA reuptake, thereby facilitating copulation (reviewed in Hull & Dominguez, 2006). In order to further explore the effects of DA on hormonally primed behavioral responses, mPOA DA levels and behavioral frequencies should be compared in birds with no testosterone (castrated), with testosterone replacement (castrated plus exogenous testosterone) and with

an aromatase inhibitor (e.g. fadrozole) to investigate the hypothesis of aromatase effects on DA activity. Furthermore, the injection of compounds which alter the effects of NO activity (e.g. NOS inhibitors) could be administered using the quail model developed in this experiment while measuring the effects on DA release in the mPOA and the subsequent behavioral output. These future experiments remain essential to further elucidate the interaction of hormone-neurotransmitter interactions in the mPOA to regulate male sexual behavior.

Numerous similarities exist between mammalian and avian species related to the organization of male sexual behavior and its neural control, suggesting that many of the features of the neural substrates regulating male sexual behavior have been evolutionarily conserved. For example, RSCM contractions can be considered functionally equivalent to 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 distal female cues (Balthazart and Ball, 1998a). Furthermore, the general organization of the DA system (e.g., anatomical localization of the cell bodies, projections, and distribution of the receptor subtypes) is similar in birds and mammals (Smeets and Reiner, 1994). In particular, the A14 TH cell group innervates the mPOA in a manner similar to what has been observed in mammals (Reiner, Karle, Anderson, and Medina, 1994). The neural substrate responsive to steroid hormone action is also quite similar in quail and rats (Ball and Balthazart 2004, 2010) and the anatomical location of the mPOA is also highly conserved in that it is located just anterior to the hypothalamus and receives indirect sensory input from almost all sensory modalities and sends connections back to those sources in both taxa (Simerly and Swanson, 1986; Panzica, Viglietti-Panzica, & Balthazart, 1996). Lastly, bilateral lesions of the preoptic area in males eliminate sexual activity in virtually every vertebrate species examined (for a review, see Hull et al., 1999). Taken together, the present findings add to the growing body of evidence which strongly suggests that this is a highly conserved excitatory sexual system.

This experiment is the first reported case investigating the temporal pattern of DA release in the mPOA in relation to the pre-copulatory exposure to a female and to the expression of both appetitive and consummatory aspects of male sexual behavior in Japanese quail. Furthermore, we indicate that DA itself does not elicit behavior directly, but allows stimuli to have easier access to hormonally primed output pathways. Overall, these results support the hypothesis that DA action in the mPOA is specifically linked to sexual motivation as compared to exclusively copulatory behavior *per se*.

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Figure 1.

Extracellular DA in the mPOA changes during pre-copulatory and copulatory exposure to a female. *A,B*, Mean change in mPOA DA during baseline (BL), RSCM test (RSCM), in the physical presence of a female (FEMALE), and after the female was removed (POST); Copulators $n=10$, Non-Copulators $n=4$. *C*, Mean change in mPOA DA during BL, RSCM, while copulating (COP), while in the presence of a female but not copulating (NOCOP), and POST within the same birds; $n=10$. Samples were collected at 6 min intervals. Values are % baseline and expressed as mean $\pm$ SEM.

a, $p<0.05$ as compared to BL; b, $p<0.05$ as compared to POST.

Figure 2.

Scatterplot of mean mPOA DA during RSCM with mean mPOA DA during FEMALE (A), mean CCM frequency during FEMALE (B), and cloacal gland area (C); $n=14$ for A,B; $n=13$ for C. DA samples were collected at 6 min intervals and are % baseline. Cloacal gland areas were measured for each bird on test day and are reported as mm^2 .

Simple correlation values (Pearson’s Correlation, r) for each condition, significance (p), and sample size (n) for each correlation.

Table 1

Simple Correlations						
	Mean RCSM DA	Mean FEMALE DA	Cloacal Gland Area	Mean RCSM Frequency	Mean CCM Frequency	
Mean	r	1	0.688**	0.368	0.687**	
RCSM	p		0.006	0.195	0.007	
DA	n	14	14	14	14	
Mean	r	0.688**	1	0.123	0.297	
FEMALE	p	0.006		0.676	0.303	
DA	n	14	14	14	14	
Cloacal	r	0.616*	0.153	0.458	0.503	
Gland	p	0.025	0.617	0.115	0.080	
Area	n	13	13	13	13	
Mean	r	0.368	0.123	1	0.453	
RCSM	p	0.195	0.676		0.104	
Frequency	n	14	14	14	14	
Mean	r	0.687**	0.297	0.453	1	
CCM	p	0.007	0.303	0.104		
Frequency	n	14	14	14	14	

Mean DA levels are % baseline and cloacal gland area is mm².

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).