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Is sexual motivational state linked to dopamine release in the medial preoptic area?

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Abstract

The medial preoptic area (mPOA) is a key site for the dopaminergic enhancement of male sexual behavior. Dopamine release increases in the rat mPOA with mating, supporting the critical stimulatory role played by preoptic dopamine on male sexual behavior. However, it has been questioned whether dopamine is specifically related to the occurrence of male sexual behavior and not simply involved in general arousal. To address this question, we ask whether dopamine release in the mPOA is linked to the production of male sexual behavior in Japanese quail, a species that exhibits a much shorter temporal pattern of copulation than rats and does not have an intromittent organ, resulting in a very different topography of their sexual response. Extracellular samples from the mPOA of adult sexually experienced male quail were collected every six minutes before, during, and after exposure to a female using *in vivo* microdialysis and analyzed using HPLC-EC. Extracellular dopamine significantly increased in the presence of a female and returned to baseline after removal of the female. However, subjects who failed to copulate did not display this increased release. These findings indicate that it is not solely the presence of a female that drives dopamine release in males, but how a male responds to her. Further, in subjects that copulated, dopamine release did not change in samples collected during periods of no copulation. Together, these findings support the hypothesis that dopamine action in the mPOA is specifically linked to sexual motivation and not only copulatory behavior or physical arousal.

Keywords

microdialysis; male sexual behavior; birds; neuroethology; neuroendocrinology

Introduction

The medial preoptic area (mPOA) 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 (Hull et al., 1999). Based primarily on pharmacological findings, the catecholamine neurotransmitter dopamine (DA) appears to facilitate male sexual behavior in rats and other mammals partly through its action in the mPOA

(for a review, see Hull et al., 2006). For example, in rats, DA agonists microinjected into the mPOA facilitate sexual behavior (Hull et al., 1986; Pehek et al., 1988; Pehek et al., 1989; Scaletta and Hull, 1990; Markowski et al., 1994) whereas microinjections of DA antagonists impair copulation, genital reflexes, and sexual motivation (Pehek et al., 1988; Warner et al., 1991). Whether DA is related specifically to the occurrence of male sexual behavior has, however, recently been questioned (Paredes and Ågmo, 2004). These authors argue that DA is important for motor behavior or general arousal but not specifically related to the control of male sexual behavior (Paredes and Ågmo, 2004).

One critical argument in favor of the involvement of DA in the control of sexual behavior is provided by the assessment of DA release during sexual interactions. In rats, it has been shown that the presence of an estrus female enhances extracellular DA in the mPOA (Hull et al., 1995) as measured by *in vivo* microdialysis followed by HPLC with electrochemical detection (HPLC-EC). Males that exhibited a substantial precopulatory increase in DA in the mPOA copulated with females, but in the absence of this rise in DA they did not (Hull et al., 1995). These data thus support the hypothesis that a rise in DA in the mPOA is specifically related to the occurrence of male sexual behavior.

Investigations of DA release in relation to male sexual behavior in the mPOA have been limited to rodents. For over 30 years, quail have been valuable in illustrating the cellular basis of androgen metabolites activating male-typical sexual behavior and have also proved to be a useful model of dopaminergic regulation of male sexual behavior (for reviews, see Balthazart and Ball, 1998; Absil et al., 2001; Balthazart et al., 2002). Quail exhibit robust male sexual behaviors but display a faster temporal sequence as compared to rats. Importantly, they lack an intromittent organ, so the topography of sexual behavior is quite different from mammals. Because quail do not exhibit erections, the detection of a change in the release of DA in the mPOA can not 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 studies using quail are important to better understand the role of DA release in the mPOA for the control of male sexual behavior. The current report is the first reported case examining whether DA levels, as measured by *in vivo* microdialysis in the mPOA in quail, is linked to the expression of sexual behavior specifically.

Materials and Methods

Subjects

A total of 21 adult male Japanese quail (*Coturnix japonica*) and 15 female stimulus quail were obtained from a local breeder (CBT Farms, Chestertown, MD) and were experimentally and sexually naive prior to experimental procedures. All birds received 5 pre-test trials after the cannula implantation surgery (see below) for copulatory behavior to insure 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 and Adler, 1972). 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. 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), and food and water available *ad libitum*.

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, made of 23 gauge thin-wall stainless steel tubing, 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. An obturator, cut the same length as the guide cannula, was inserted into the guide cannula until microdialysis experiments began. 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 constructed according to previous procedures (Yamamoto and Pehek, 1990). The dialysis membrane (Spectra/Por *in vivo* microdialysis hollow fibers; Spectrum, Gardena, CA) had an outer diameter of 170 μm, an inner diameter of 150 μm, an active dialyzing length of 1mm, and an 18,000 molecular weight cutoff. A Teflon-covered tether encased the inflow tubing. 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.

Nine males were used in the pilot study conducted to identify an appropriate flow rate in which to perform the experiment. Samples were collected every 3 min with a flow rate of 1.0μl/min yielding 3μl of dialysate per sample, immediately frozen (−80°C), and later assayed using HPLC-EC by an experimenter who was blind to the experimental conditions. Three of the nine animals in this pilot study had inadequate dialysate volume for analysis so their samples were excluded. Analysis of percent change from baseline revealed no significant effect of sample taken in 3 min intervals ($F_{2,10}$=1.79, p=0.216). We determined that the flow rate of 1μl/min was too quick to allow for proper collection of dialysate, and therefore we decreased the flow rate to 0.5μl/min and the samples were collected every 6 min 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 microdialysis chamber without a female for one hour to allow the birds to habituate to the chamber. On the day of testing, the probe was implanted, the subject was placed in the microdialysis chamber, and the probe was then attached to the perfusion line. Six hours later, three baseline (BL) samples were collected. The female was then placed into the chamber, where they could copulate. During this period, six 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 36 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 (NO COP). After the last sample was collected, the female was removed and three final samples were collected (POST period). The following samples were assayed using HPLC-EC: three BL; six FEMALE, samples that included COP and NO COP; and three POST.

At the conclusion of the experiment, cannula placements were verified histologically. Animals were anesthetized with isoflurane gas anesthetic, and, using the same probe that was used for microdialysis, a dye solution was reverse-dialyzed into the mPOA. Animals were immediately

euthanized by rapid decapitation, and their brains were removed, frozen, and sectioned (40μm) using a cryostat. Sections including the mPOA were mounted on slides and examined for cannula placement. No lesions of the mPOA were discovered. 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 Acurate 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 (300μm inner diameter, 5cm long, packed with 3μm 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 Acurate microflow processor split the flow, so that flow through the analytical column was ~7μl/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, FEMALE [*COP/NO COP*], and POST) as the repeated factor and Copulation (Copulators vs. Non-Copulators) as the independent factor. Effects were considered significant for $p<0.05$. All analyses were carried out with Windows version of the software SPSS, version 16.0.

Results

An example chromatogram from a representative bird is illustrated in figure 1. The within subjects analysis of percent change from baseline showed a significant effect of the presence of a female ($F_{2,16}=4.224$, $p=0.034$; figure 2A,B). Post-hoc analyses revealed that this change was significantly higher in FEMALE samples compared with baseline. Further, although all subjects copulated in the pretests following the surgery, not all subjects copulated in the microdialysis setting (six quail copulated [Copulators] while four did not [Non-copulators]) thus making it possible to compare the effect of copulation (between variable) on the concentration of DA in the preoptic area. This analysis revealed a main effect of copulation ($F_{1,8}=6.153$, $p=0.038$) and an interaction of female presence and copulation ($F_{2,16}=3.802$, $p=0.045$) such that there was only a significant rise in DA in quail who copulated. The ranges of frequencies of CCM's in each of the six FEMALE samples are: F1: 0-3, F2: 0-1, F3: 0, F4: 0-1, F5: 0-3, F6: 0. Although none of the birds copulated in samples F3 or F6, it is interesting to note in figure 2A that the DA levels from these samples remain high in the “Copulators”. Additionally, among the six males who copulated, four birds provided both COP and NO COP samples (refer to Methods for a description). The analysis of percent changes from baseline within these birds revealed no change ($t=0.064$, $p=0.953$) during periods of copulation as compared to periods when no copulation occurred (figure 2C).

Finally, two animals were found to have cannulae placement outside the mPOA and were thus removed from analysis. Interestingly, the data from these two birds showed no change in DA release from baseline, suggesting the regional specificity of the DA response.

Discussion

This study represents the first attempt at performing *in vivo* microdialysis in the mPOA investigating extracellular DA release during male sexual behavior in any species other than in rodents. Our first challenge was to identify an appropriate flow rate in which to perform these experiments. Using a flow rate of 0.5μl/min collected at six minute intervals, we discovered an increase in DA levels in the mPOA of male quail in the presence of a female, which then decreased back to baseline after the female was removed (figure 2A). This significant rise in DA only occurred in quail who copulated (figure 2B). Furthermore, within birds that copulated, no change was detected between sampling periods during which they did or did not mate (figure 2C). Hence, in the presence of a female, the elevated DA concentration persists regardless of the behavioral response of the male. This suggests that the consummatory behavior *per se* does not modulate the release of DA in the mPOA; rather it is the presence of a female only if the male is motivated and able to copulate. Specifically, all birds were exposed to the female, but only the males who eventually engaged in copulation showed the significant rise in DA. Thus, it is not sufficient for the male to see a female, but rather it is whether or not he will eventually respond to her that correlates with this DA response in the mPOA. These data are consistent with the conclusion that DA release in the mPOA is specifically linked to sexual motivation. As in rodents, the mPOA in quail is bidirectionally connected to many brain areas, receiving inputs from a variety of sensory and regulatory areas and sending outputs to "neurovegetative" centers and to brain regions directly connected to motor pathways (Panzica et al., 1996; Simerly and Swanson, 1986; Simerly and Swanson, 1988). These connections support its role as an integrative center for coordinating sexual motivation with its appropriate behavioral output.

As is the case in rats, these data indicate that the rise in mPOA DA occurs in the presence of a female only if the male successfully copulates (Hull et al., 1995). Also similar to what has been observed in rats, removal of the female leads to a rapid decrease in DA release. In the study by Hull et al. (1995) copulating versus non-copulating males could be discriminated based on their precopulatory DA levels. Precopulatory levels were collected in the presence of a female where the male could see, hear, and smell her, but could not interact physically with her. If the male exhibited a rise in DA in the mPOA in response to the female he would then be able to go on and copulate. If he did show this precopulatory rise, he did not engage in copulation. In our current study, we did not collect a similar precopulatory measure. However, we observed 6 min sampling bins among copulating males when the male and female were together but not engaging in copulation. We uncovered no differences in the release of DA during periods in which the male quail is copulating and in the presence of the female as compared to when a female is still present but the male is not copulating.

In addition to the hypothesized actions on sexual motivation, some actions of DA in the mPOA of rats appear to be related directly to the facilitation of penile erections (for a review, see Hull et al., 2006). Dominguez and Hull (2005) hypothesized that as a result of a sexually exciting stimulus and/or sexual activity in rats, the mechanism of a low-threshold of extracellular DA in the mPOA is mediated by D2 receptors that disinhibit the tonic brake on genital reflexes. A moderate threshold mechanism activates D1 receptors and facilitates penile erections, while a high threshold mechanism, activated by stimulation of D2 receptors, facilitates seminal emissions and inhibits erections. It has been further hypothesized that these mechanisms may be activated sequentially by increasing levels of DA release or longer duration of DA action in the mPOA (Dominguez and Hull, 2005). Because quail lack an intromittent organ but still

exhibit a robust pattern of sexual motivation, quail are a useful model for studying different components of sexual behavior. In this species, gamete transfer occurs via the male mounting the female and contacting his cloaca to hers, but does not require male-typical neuromuscular control as is the case with mammals (Seiwert and Adkins-Regan, 1998). In the present experiment, DA levels increases in males who copulated, but contrary to rodents, quail do not need an erection to successfully perform the behavioral sequence. Thus, the DA rise occurs in the absence of the need of erection, further supporting a role of DA in the control of male sexual behavior rather than only erection and ejaculation.

Similar to what we have just reviewed for rats, specific activations or inhibitions of male sexual behavior in quail have been observed following systemic injections of D1 or D2-like agonists and antagonists (Balthazart et al., 1997; Castagna et al., 1997). Thus, in quail and in rats, DA can both inhibit and facilitate male sexual behavior. However, given the differences in the topography of male-typical sexual behavior in rats versus quail, while the release of DA in the mPOA occurs in the presence of a female in both species, the functional consequences of DA changes may vary between the species. For example, in rats, DA in the mPOA is thought to play a role in the control of erections and ejaculation as well as a role in sexual motivation. We have observed in male quail who will engage in copulation a pattern of mPOA DA release in the presence of a female similar to that observed in rats. In particular in this current study, we compared DA levels in male quail that were in the presence of female and either engaged or did not engage in copulatory behavior. A novel finding of our study is that in birds that either had or eventually would copulate, DA was high in the presence of a female even during sampling periods when they were not copulating. In other words, these “Copulators” always exhibit high levels of mPOA DA in the presence of a female even when they were not actually engaging in copulatory behavior. “Non-copulators,” on the other hand, never copulated and never exhibited a rise in mPOA DA in the presence of a female similar to what was reported in rats (Hull et al., 1995). These findings support a role of DA release in the control of sexual motivation in quail. Given the lack of a penis in quail, their expression of copulatory behavior is likely to be affected by tactile stimuli from the genital area in a manner that is quite different from what is observed in mammals (Balthazart and Ball, 1998).

In summary, the results from the current experiment suggest that consummatory behavior *per se* does not modulate the release of DA in the mPOA. Rather, when the male is motivated and able to copulate, it is the presence of a female that appears to correlate with an increase in DA levels. Specifically, we observed a rise in DA only in subjects who copulated, but this was not strictly correlated with the performance of the behavior, suggesting a link to motivation. In a critical review, Paredes and Ågmo (2004) have questioned whether DA is specifically linked to the control of male sexual behavior. They argue that the effects of DA manipulations on male sexual functioning can be explained via the modulation of general arousal or motoric function rather than a specific role on sexual behavior. However, the present experiment shows that the rise in DA occurs in quail, a species that has no need for an erection, linking the release of DA in the mPOA to sexual behavior and not solely physical arousal. Overall, these data are consistent with the notion that DA in the mPOA is specifically linked to sexual motivation.

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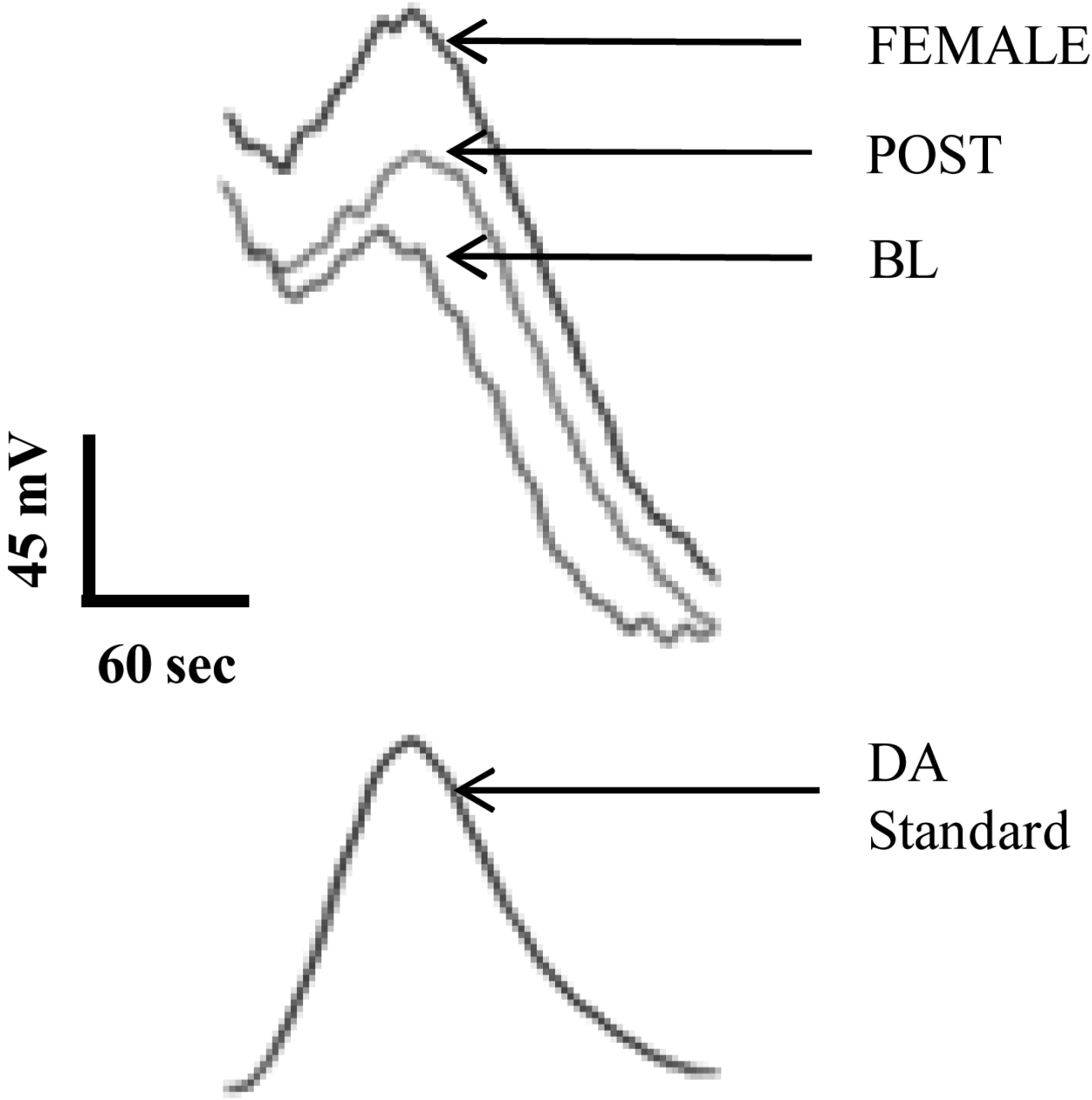

Figure 1.
Comparison of chromatograms collected from a representative animal during baseline (BL), in the presence of a female (FEMALE), and after the female was removed (POST), with standard (DA Standard).

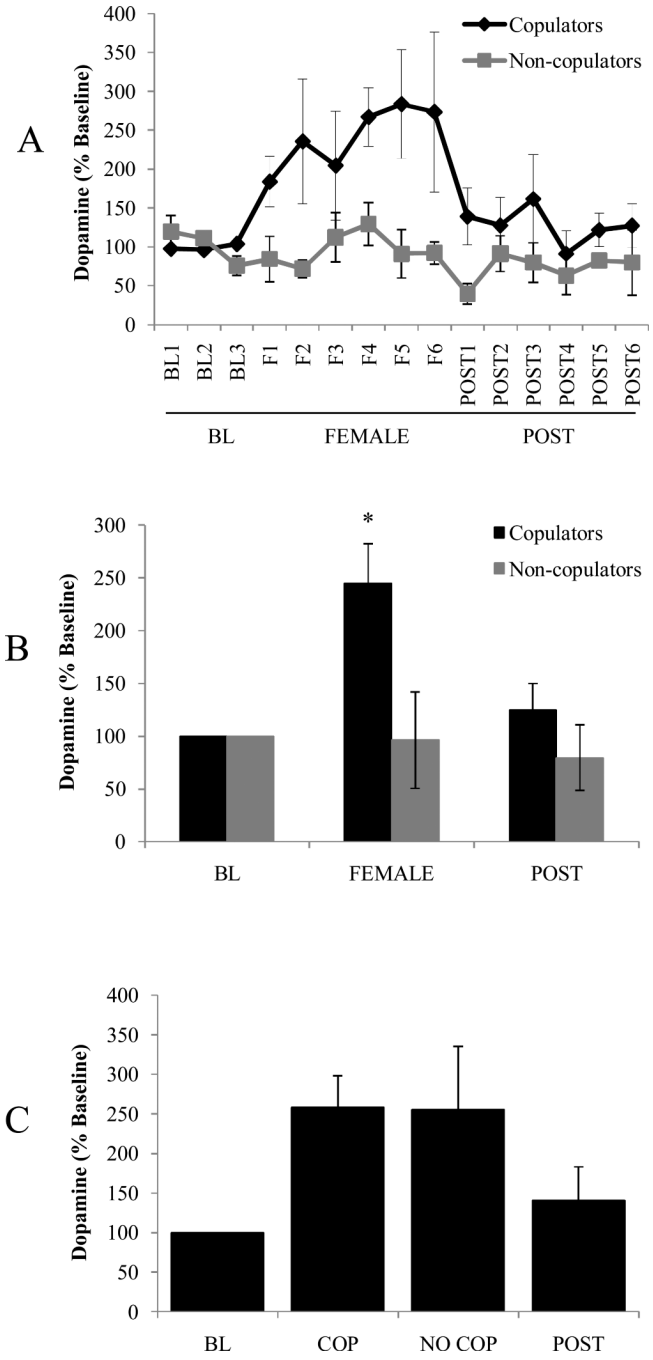

Figure 2.

Extracellular DA in the mPOA changes in the presence of a female (FEMALE). *A*,*B*, Mean change in mPOA DA during baseline (BL), FEMALE, and after the female was removed (POST); Copulators n=6, Non-Copulators n=4. *C*, Mean change in mPOA DA during COP and NO COP, within the same birds; n=4.

Samples were collected at 6 min intervals. Values are % baseline and expressed as mean +/− SEM. *p<0.05.