Psychoneuroendocrinology (2013) 38, 789—799

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Dynamic changes in brain aromatase activity following sexual interactions in males: Where, when and why?

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Received 15 July 2012; received in revised form 1 September 2012; accepted 3 September 2012

KEYWORDS
Aromatase;
Estrogen synthesis;
Non-genomic action of estrogens;
Copulatory behavior;
Sexual motivation

Summary It is increasingly recognized that estrogens produce rapid and transient effects at many neural sites ultimately impacting physiological and behavioral endpoints. The ability of estrogens to acutely regulate cellular processes implies that their concentration should also be rapidly fine-tuned. Accordingly, rapid changes in the catalytic activity of aromatase, the limiting enzyme for estrogen synthesis, have been identified that could serve as a regulatory mechanism of local estrogen concentrations. However, the precise anatomical localization, time-course, triggering stimuli and functional significance of these enzymatic changes *in vivo* are not well understood. To address these issues as to where, when and why aromatase activity (AA) rapidly changes after sexual interactions, AA was assayed in six populations of aromatase-expressing cells microdissected from the brain of male quail that experienced varying durations of visual exposure to or copulation with a female. Sexual interactions resulted in a rapid AA inhibition. This inhibition occurred in specific brain regions (including the medial preoptic nucleus), in a context-dependent fashion and time-scale suggestive of post-translational modifications of the enzyme. Interestingly, the enzymatic fluctuations occurring in the preoptic area followed rather than preceded copulation and were tied specifically to the female's presence. This pattern of enzymatic changes suggests that rapid estrogen effects are important during the motivational phase of the behavior to trigger physiological events essential to activate mate search and copulation.

1. Introduction

Estrogens profoundly affect a multitude of physiological and behavioral responses such as social behaviors, nociception and neuroprotection (Evrard and Balthazart, 2004; Trainor et al., 2006; Garcia-Segura, 2008; Ball and Balthazart, 2009; Hull and Rodriguez-Manzo, 2009). Estrogen effects are classically attributed to the transcriptional activity of their liganded nuclear receptors (Tsai and O'Malley, 1994), usually

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http://dx.doi.org/10.1016/j.psyneuen.2012.09.001

arise slowly (hours—days) and coincide with the slow fluctuations in circulating concentrations of gonadal steroids associated with specific physiological states (*e.g.* estrus cycle, breeding cycle; Ball and Balthazart, 2009). Estrogens also rapidly regulate neuronal activity through membrane-initiated events (Maggi et al., 2004; Vasudevan and Pfaff, 2007) that translate into fast (within minutes) and transient modulations of physiological and behavioral responses (Cornil and Charlier, 2010; Roepke et al., 2011). Estrogen's ability to trigger rapid and reversible responses implies that mechanisms exist to regulate their concentrations on a comparable time-scale.

Estrogens are synthesized from androgens by aromatase, an enzyme present in various tissues (gonads, muscles, adipose tissue, etc.) but also in discrete brain regions (Foidart et al., 1995; Ball and Balthazart, 2009). Previous research on brain aromatase largely focused on the regulation of aromatase concentration in relation to different reproductive states. It was recently demonstrated that aromatase activity (AA) can also be rapidly and reversibly modulated by calcium-dependent phosphorylations resulting from neuronal depolarization or by glutamate release (Balthazart et al., 2003, 2006; Remage-Healey et al., 2008, 2011; Charlier et al., 2011). Moreover, rapid AA fluctuations occur *in vivo* in response to social interactions (Cornil et al., 2005; Remage-Healey et al., 2008, 2009) or acute stress (Dickens et al., 2011). This acute control of AA preferentially occurs at the synapse, and could thus serve as a rapid and spatially restricted control mechanism of local estrogen concentrations (Remage-Healey et al., 2011; Cornil et al., 2012). However, the precise anatomical localization, time-course, triggering stimuli and functional significance of these rapid enzymatic changes *in vivo* are not well understood.

To address these issues as to where, when and why AA rapidly changes after sexual interactions, AA was quantified in aromatase-rich regions that were microdissected from the brains of male Japanese quail exposed to varying durations of visual exposure to or copulation with a female. Since this species expresses much higher AA than rodents and is the system most consistently demonstrating the fastest fluctuations in AA (Cornil et al., 2005), it constitutes an exquisite model to study these questions. Changes in enzyme kinetics were analyzed in parallel with the behavior exhibited by males in different social conditions. Results identified rapid, region- and context-specific changes in AA, occurring on a time-scale suggestive of post-translational modifications of the enzyme. The careful analysis of where and when these enzymatic changes occur with respect to the timing of sexual behavior suggests that rapid changes in brain estrogens mediate fast changes in sexual motivation but not in copulatory performance, which would be affected only by slower genomic effects of steroids.

2. Materials and methods

2.1. Subjects

198 male Japanese quail (*Coturnix japonica*) were used as subjects. Animals were obtained from a local breeder in Belgium or derived from our breeding colony at the University of Liège. All animals were adult (>8 weeks), individually housed, maintained on a long day photoperiod (16 h light and 8 h dark), provided with food and water *ad libitum* and kept gonadally intact. Experiments complied with the Belgian laws on the "Protection of experimental animals" and were approved by the Ethics Committee for the Use of Animals at the University of Liège (Protocol #1235).

2.2. Experimental procedures

Four separate experiments were performed to determine the timing and neuroanatomical specificity of female-induced changes in AA. Prior to each experiment, subjects were pre-tested for copulatory behavior until they had gained sufficient experience (see below) and then assigned to different experimental groups matched for their cloacal gland size and behavioral performance during the pre-tests. During the experimental tests, subjects were exposed for a given duration (see below) to a sexually experienced female that they had never been paired with. In all experiments, control (CTL) birds were killed immediately after being removed from their home cage without exposure to a female. Immediately after these manipulations, birds were killed by rapid decapitation. Trunk blood was collected and stored at 4 °C, while the brain was rapidly (<2 min) collected, frozen on dry ice and stored at −80 °C. On the next day, blood samples were centrifuged (9 min at 9000 × *g*), the plasma was collected and stored at −80 °C.

In Experiment 1, 65 males were allowed to copulate with a female during 2 (n = 14), 5 (n = 15), 10 (n = 15) or 15 (n = 6) min before brain collection or left in their home cage as a control (n = 15). Experiment 2 tested whether the rapid changes in AA observed in Experiment 1 directly result from the intense sexual activity occurring during the first 2 min of interaction. Males (n = 57) were allowed to copulate with a female for 2 min, and left in the empty experimental arena for 0 (n = 11), 3 (n = 11), 8 (n = 11) or 13 (n = 12) min before brain collection or left in their home cage as a control (n = 12). Experiment 3 investigated whether the visual interaction in the absence of physical contacts with a female produces changes in AA similar to those observed after copulation. Males (n = 49) were allowed to view a female for 2 (n = 10), 5 (n = 9), 10 (n = 10) or 15 (n = 10) min before brain collection or left in their home cage as a control (n = 10). Finally, Experiment 4 assessed whether rapid changes in AA are reversible in a time-scale compatible with changes in the enzyme's concentration or enzyme catalytic activity. Males (n = 28) were allowed to copulate for 5 min with a female. Brains were collected either immediately after the sexual interaction (n = 10) or after 115 additional min in their home cage (n = 8) or in birds left in their home cage as a control (n = 8).

2.3. Behavioral tests

Appetitive sexual behavior was assessed by measuring the frequency of rhythmic cloacal sphincter movements (RCSM). The procedure was described previously (Taziaux et al., 2004). Briefly, tests were performed in an aquarium (40 cm × 20 cm × 25 cm) placed above a mirror at a 45° angle, which provides an unobstructed view of the male's cloacal area. The aquarium was divided into two chambers by