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Estradiol transfer from male big brown bats (*Eptesicus fuscus*) to the reproductive and brain tissues of cohabiting females, and its action as a pheromone

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ABSTRACT

The powerful estrogen, 17β-estradiol, has been found to pass from male excretions to the reproductive organs, brain, and other tissues of cohabiting females in laboratory mice. The current studies were designed to examine whether this phenomenon also occurs in big brown bats (*Eptesicus fuscus*), a mammal appropriate for testing cross-species generality because of its phylogenetic distance from mice. When tritiated estradiol (^{3}H-E_2) was administered directly on the nasal area of adult female bats, radioactivity was reliably observed in the uterus and ovaries, and also in the brain and other tissues. When ^{3}H-E_2 was applied to the skin, radioactivity was observed in reproductive and other peripheral tissues. We injected male bats with minute quantities of ^{3}H-E_2 and housed each of them directly with groups of adult females for 48 h. We then measured radioactivity in male and female bat tissues. In each of several replications of one male housed with three females, radioactivity was reliably observed in the uterus of all females, and in many other tissues in almost every female. Measurement in the organs of males directly exposed to ^{3}H-E_2 showed high levels of radioactivity in the testes and especially the epididymides. These data indicate that estradiol is transferred from males to females, likely via absorptions from males' excretions and potentially also via intravaginal exposure during mating. Given the potency of estradiol in regulating female reproductive physiology and behavior, our data strongly suggest the potential for pheromonal action whereby male mammals induce sexual receptivity and ovulation in females.

1. Introduction

Recently it was demonstrated that 17β-estradiol (E_2) can transfer between cohabiting mice (*Mus musculus*). When adult males were injected with small quantities of ^{3}H-E_2 and each housed in a cage with a female conspecific, radioactivity was subsequently observed throughout the female's body, especially in tissues rich in estrogen receptors (ER) such as the uterus (Guzzo et al., 2012, 2013). Unconjugated (bioactive) E_2 is reliably present in the urine of male mice (deCatanzaro et al., 2006, 2009), which provides a vector for transmission to proximate conspecifics (deCatanzaro et al., 2009; Guzzo et al., 2010, 2012, 2013). The hormone E_2 is highly lipophilic with a low molecular mass (272.4 Da) and high chemical stability, which permits percutaneous and nasal absorption into blood circulation, where it largely remains in an unconjugated, bioavailable form (Bawarshi-Nassar et al., 1989; Goldzieher and Baker, 1960; Guzzo et al., 2010, 2012; Hueber et al., 1994; Scheuplein et al., 1969). Nasal and percutaneous absorption of ^{3}H-E_2 is much more efficient than that of ^{3}H-progesterone or ^{3}H-testosterone (Guzzo et al., 2012), and although ^{3}H-progesterone can also transfer between cagemates, it does so much less effectively than ^{3}H-E_2 (Guzzo et al., 2013). E_2 is exceptionally potent in the regulation of mammalian female reproductive physiology and behavior, and very low doses of exogenous E_2 can mimic pheromonal actions including novel-male-induced disruption of early pregnancy (deCatanzaro et al., 2001, 2006) and male-induced promotion of female sexual maturation (Bronson, 1975; Thorpe and deCatanzaro, 2012).

This study was designed to test the generality of E_2 transmission between cohabiting conspecifics in a species that is phylogenetically distant from mice. Bat fossils have been identified from as early as 52.5 million years ago (Simmons et al., 2008; Veselka et al., 2010). Modern genomic methods suggest that the Order Chiroptera is part of the Superorder Laurasiatheria, which separated from the Superorder that includes rodents (Euarchontoglires) much earlier, during the mid-Cretaceous (Murphy et al., 2004;

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Springer et al., 2004; Zhou et al., 2012). The big brown bat, *Eptesicus fuscus*, is one of the most common and widely distributed bat species in North America (Kurta and Baker, 1990). Breeding in *E. fuscus* is seasonal, with one litter of 1–2 pups per year (Kurta and Baker, 1990). Mating in temperate regions typically occurs in autumn, but can also occur during brief periods of awakening during winter hibernation or in early spring (Oxberry, 1979). Females store sperm, then ovulate and become pregnant in the spring (Christian, 1956; Oxberry, 1979; Racey, 1979; Wimsatt, 1944), with the time of parturition varying but typically occurring in June (Christian, 1956; Schowalter and Gunson, 1979). In captivity, individuals of both sexes readily mate with multiple partners (Mendonça et al., 1996). Indeed, many bat species are polygynous or promiscuous (McCracken and Wilkinson, 2000), and while the mating patterns of *E. fuscus* have not been studied in the wild, evidence of multiple paternity within litters indicates promiscuity (Vonhof et al., 2006). Given their highly social nature and seasonally-produced secretions, it has been suggested that pheromonal activity is likely in some bat species (Heideman, 2000); however, to date pheromonal investigations in bats have focused only on individual recognition and orientation toward the home colony (Bloss et al., 2002; Safi and Kirth, 2003).

We studied E_2 transmission among individual big brown bats from a captive research colony during the autumn when mating is frequent. As sex steroids can enter the body via nasal or percutaneous absorption in mice (*e.g.,* Guzzo et al., 2012), we hypothesized that this could also occur in bats. Accordingly, we first looked at the distribution of radioactivity after a single direct intranasal or cutaneous administration of tritiated estradiol (^{3}H-E_2) to females. We also hypothesized that physical contact during cohabitation would be sufficient to cause E_2 injected into male bats to arrive in the reproductive and brain tissues of untreated females. To test for this, we injected male *E. fuscus* with ^{3}H-E_2 and measured the distribution of radioactivity in female tissues after 48 h of cohabitation with these males. We focused on the presence of ^{3}H-E_2 in the ovaries and uterus because these tissues have high concentrations of alpha and beta ER (ERα and ERβ; Couse et al., 1997; Kuiper et al., 1997), and because of their known roles in mediating the Bruce and Vandenbergh effects (*e.g.,* Thorpe and deCatanzaro, 2012). We also looked for radioactivity in the hypothalamus because of its potential roles in pheromonal effects (Baum and Bakker, 2013) and concentrations of ER in the ventromedial nucleus and preoptic area (Sar and Parikh, 1986; Simerly et al., 1990). We included the liver and kidneys for their roles in estrogen metabolism and excretion, and other brain and peripheral tissues for comparison.

2. Materials and methods

2.1. Animals and housing

Wild *E. fuscus* were captured from buildings in southern Ontario and housed in a husbandry facility that permitted animals to fly (Faure et al., 2009). Colony temperature and lighting varied with ambient conditions. Bats selected from the colony were brought into the lab and housed in small (28 × 22 × 18 cm) stainless steel wire mesh holding cages. Bats in holding cages had *ad libitum* access to mealworms (*Tenebrio molitor*) and water, except where otherwise stated. All procedures were approved by the Animal Research Ethics Board of McMaster University and conformed to guidelines of the Canadian Council on Animal Care.

2.2. Chemicals and materials

SOLVABLE solubilization cocktail, Ultima Gold scintillation cocktail, 8 ml midi-vial scintillation vials, and [2,4,6,7-[^{3}H](N)]-E_2 (stock solution dissolved in ethanol, 1.0 μCi/μl, 72.1 Ci/mmol) were obtained from PerkinElmer, Waltham, MA, USA.

2.3. Experiment 1: Direct intranasal exposure of female bats to ^{3}H-E_2

On day 1 of the experiment, adult female bats were randomly selected from the research colony, assigned to either the hormone treatment or contamination control conditions (n = 5 per condition), and kept overnight in separate holding cages. On day 2, each female in the hormone treatment condition was intranasally administered 10 μCi ^{3}H-E_2 (corresponding to 37.7 ng E_2 per animal) via a micropipette tip inserted directly into one nostril. This dose represents a fraction of the endogenous concentration of the hormone, based on extrapolation of data from mice (Guzzo et al., 2013). Control females were intranasally administered 10 μl 95% ethanol vehicle. Immediately following intranasal administration, each female was individually housed in a standard polypropylene mouse cage (28 × 16 × 11 cm) with a wire-grid lid, without access to food or water.

One h after intranasal administration of ^{3}H-E_2 or ethanol, each bat was deeply anesthetized by isoflurane inhalation and euthanized by perfusion with 20 ml phosphate-buffered saline. Tissue samples were collected and placed in pre-weighed 8 ml scintillation vials. Reproductive tissues included the whole uterus and both ovaries. Neural tissues included samples of olfactory bulbs, the cerebellum, bilateral sections of the frontal cortex, and a section of the hypothalamus on the ventral brain surface anterior to the pituitary stalk and posterior to the optic chiasm. Other tissues sampled included the heart, lung, external intercostal muscle, abdominal adipose tissue, liver, and a cross-section of the kidney encompassing both the cortex and medulla. Following collection, sample vials were re-weighed and wet tissue mass was recorded.

Tissue samples were solubilized by adding 1 ml of SOLVABLE to each vial. After 10 min of agitation, vials were placed in a 50 °C water bath for 2 h. Vials were then re-agitated for 10 min and then left in the water bath for an additional 2–3 h until the samples had completely dissolved. The samples were then removed from the bath and permitted to cool for 10 min, after which 5 ml of Ultima Gold scintillation cocktail was added to each vial. Vials were re-agitated for 10 min to promote mixing of the solubilized tissue sample and scintillation cocktail. Each vial was then stored in the darkness chamber of a TriCarb 2910 TR Liquid Scintillation Analyzer with a high sensitivity option (PerkinElmer, Waltham, MA) for 5 min to eliminate residual heat and luminescence. The radioactivity in each vial was measured for 5 min, with the final adjusted estimate quantified in disintegrations per minute (DPM) calculated by Quanta-Smart software. Radioactivity measures were standardized to the wet tissue mass and reported as DPM/mg tissue.

2.4. Experiment 2: Direct cutaneous exposure of female bats to ^{3}H-E_2

As in Experiment 1, on day 1 adult female bats were randomly selected from the colony, assigned to the hormone treatment or contamination control conditions (n = 5 per condition), and kept overnight in the lab in holding cages. On day 2, female bats were cutaneously administered either 10 μCi ^{3}H-E_2 (corresponding to 37.7 ng E_2 per bat) or 10 μl 95% ethanol, applied directly to the midline surface of the abdomen via a micropipette tip (*cf.* Guzzo et al., 2012). All other procedures, including isolation of the bats, anesthesia, perfusion, tissue collection, sample processing, and scintillation counting, were identical to Experiment 1.

2.5. Experiment 3: Direct exposure of females to ^{3}H-E_2-injected males

Experiment 3A was conducted in November 2012. Nine females from the colony were brought into the laboratory and exposed to a