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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A B S T R A C T

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.

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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;

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 ($28 \times 22 \times 18$ 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 ($28 \times 16 \times 11$ 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 QuantaSmart 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

single ^{3}H-E$_2$-treated male. On day 1, one adult male and a cohort of three adult females were randomly selected from the colony. The three females were housed together and the male was housed independently, both in clean holding cages. On day 2, the male was administered 50 µCi ^{3}H-E$_2$ in 150 µl phosphate-buffered saline (corresponding to 188.5 ng of E$_2$) via intraperitoneal (i.p.) injection (*cf.* Guzzo et al., 2013). Based on extrapolation from work with mice (Guzzo et al., 2013), this dose represents a fraction of the endogenous concentration of the hormone. After 1 h of isolation to prevent accidental E$_2$ transfer from the injection site, the three females were placed in the male's cage. After 48 h of cohabitation, the females were removed, anesthetized via an i.p. injection of sodium pentobarbital, and euthanized by perfusion with 20 ml phosphate-buffered saline. Tissues were processed as described above.

Subsequently, two additional cohorts of three adult females each were consecutively exposed to the same male, with at least 72 h between replications to allow for clearance of the previous ^{3}H-E$_2$ injections from the male's system (*cf.* Guzzo et al., 2013) and to restore his sexual interest assuming a post-copulatory refractory period (*cf.* McGill and Blight, 1963). Between replicates, the male was re-injected i.p. with 50 µCi ^{3}H-E$_2$ in 150 µl phosphate-buffered saline and isolated for 1 h prior to being housed with each subsequent new cohort of females. After 48 h of cohabitation, females were anesthetized, euthanized, and perfused. After collecting tissues from the third cohort of females, the male was sacrificed and his tissues were similarly analyzed, with the testes and epididymides being taken in lieu of the ovaries and uterus.

Experiment 3B was conducted to test the generality across males of the capacity to transfer ^{3}H-E$_2$ to females. In November 2013, two new adult males were each given an i.p. injection of 50 µCi ^{3}H-E$_2$, and 1 h later each was housed with three adult females in a clean holding cage as described in Experiment 3A. After 48 h of cohabitation, females were anesthetized with isoflurane and their blood was collected via cardiac puncture. Each female was sedated with isoflurane and euthanized by perfusion with 20 ml phosphate-buffered saline. In mid-December, one of the injected males was re-treated with 50 µCi ^{3}H-E$_2$. At 1 h after the injection, the male was housed with three young females born in the colony earlier that year. At the start of treatment, one female was 177 days old and the other two were 187 days old. After 48 h of cohabitation, each female and the male were anesthetized, sampled for blood, and euthanized as described above.

Tissue collection and sample processing were identical to Experiment 1. Blood samples were centrifuged at 1500g for 10 min, after which 10 µl of serum was added to vials containing 5 ml Ultima Gold. Measurement of radioactivity, reported as DPM/mg tissue or DPM/µl serum, was performed as described above. Samples of food and water from the holding cage of the treatment group were analyzed for radioactivity to determine whether ^{3}H-E$_2$ had contaminated the food and water. Given our experimental design, it was impossible to concurrently measure levels of radioactivity in treatment and control bats paired with the same male; however, dry swipes of the dissection table, dissection tools, and anesthesia induction chamber were taken between dissections of females to ensure that radioactivity had not contaminated our dissection station, equipment, and general experimental area.

2.6. Statistical considerations

Radioactivity counts substantially exceeding background levels in the natural environment are not possible unless inadvertent contamination were to occur. Radioactivity measures from control animals were essentially at or near zero after automatic correction for background levels; hence, unequal variances occur between ^{3}H-E$_2$-treated and control conditions, violating a critical assumption of parametric statistics. Where $n \geqslant 5$ for each of two conditions, DPM values in completely non-overlapping ranges will always reach statistical significance ($P < 0.05$) by a Wilcoxon Rank-Sum non-parametric test. For inferences of our research, any DPM reading substantially above background radiation indicates meaningful ^{3}H-E$_2$ transfer, obviating the need for statistics. Accordingly, the data from Experiment 3 were considered in the context of single-subject research (*e.g.*, Kazdin, 1982), with replication consistently demonstrated across different male stimulus animals and different female subjects.

3. Results

3.1. Experiment 1: Direct intranasal exposure of female bats to ^{3}H-E$_2$

Direct intranasal administration of ^{3}H-E$_2$ to females resulted in radioactivity 1 h later in all tissues from all 5 exposed subjects (Fig. 1, *upper panel*). In contrast, all 5 control subjects had DPM/mg readings at or near zero. Data from the two treatment conditions were entirely distinct, in completely non-overlapping ranges. Among ^{3}H-E$_2$-treated females, the highest values were observed in the ovaries, uterus, and liver, and values from these tissues were in a completely non-overlapping range from that of all other samples from the olfactory bulb, cerebellum, frontal cortex, hypothalamus, heart, lung, and muscle.

3.2. Experiment 2: Direct cutaneous exposure of female bats to ^{3}H-E$_2$

Direct cutaneous administration of ^{3}H-E$_2$ to females yielded radioactivity in all tissues (Fig. 1, *lower panel*). Data from the 5 treatment bats were in a completely distinct range from that of the 5 control subjects, which were all at or near zero DPM/mg. Unlike the nasal exposure results of Experiment 1, there was a substantial range of values among ^{3}H-E$_2$-exposed females in certain tissues (muscle 2.2–121.5; adipose 7.4–1010.2; uterus 5.5–228.4; ovary 6.6–454.6). Relatively little radioactivity was observed in brain tissues, although the values there did not overlap with those of controls. Among ^{3}H-E$_2$-treated females, all DPM/mg values obtained from the ovary, uterus, and abdominal adipose were in a completely non-overlapping range from the values obtained in the olfactory bulb, cerebellum, frontal cortex, and hypothalamus.

3.3. Experiment 3: Direct exposure of females to ^{3}H-E$_2$-injected males

In each of 3 cohorts where 3 adult females were exposed to a ^{3}H-E$_2$-injected adult male (Male 1) for 48 h in Experiment 3A, radioactivity levels were substantially above background in all 9 females (Fig. 2). The male was given at least 3 days rest between the end of one exposure and reinjection with ^{3}H-E$_2$ before cohabitation with a new cohort. Considering all females from all cohorts, mean DPM/mg values were greatest in the lung, muscle, liver, ovary, and uterus, in that order. Radioactivity counts for tissue samples from Male 1 taken directly after the last exposure were: olfactory bulb, 303.2; cerebellum, 253.1; frontal cortex, 335.3; hypothalamus, 345.1; heart, 149.4; lung, 133.8; muscle, 214.1; adipose, 57.7; testis, 910.8; epididymis, 30737.4; and liver, 270.0.

Values for each individual are presented for each replicate cohort of 3 adult females housed with a ^{3}H-E$_2$-treated male for 48 h in Experiment 3B (Fig. 3). Radioactivity was observed in all 9 females, but its distribution was idiosyncratic. Radioactivity above background levels was observed in all individuals exposed to Male 2 (*Panel A*). Male 3 (*Panel B*) was repeatedly observed to be in direct physical contact with one of the 3 females (Female
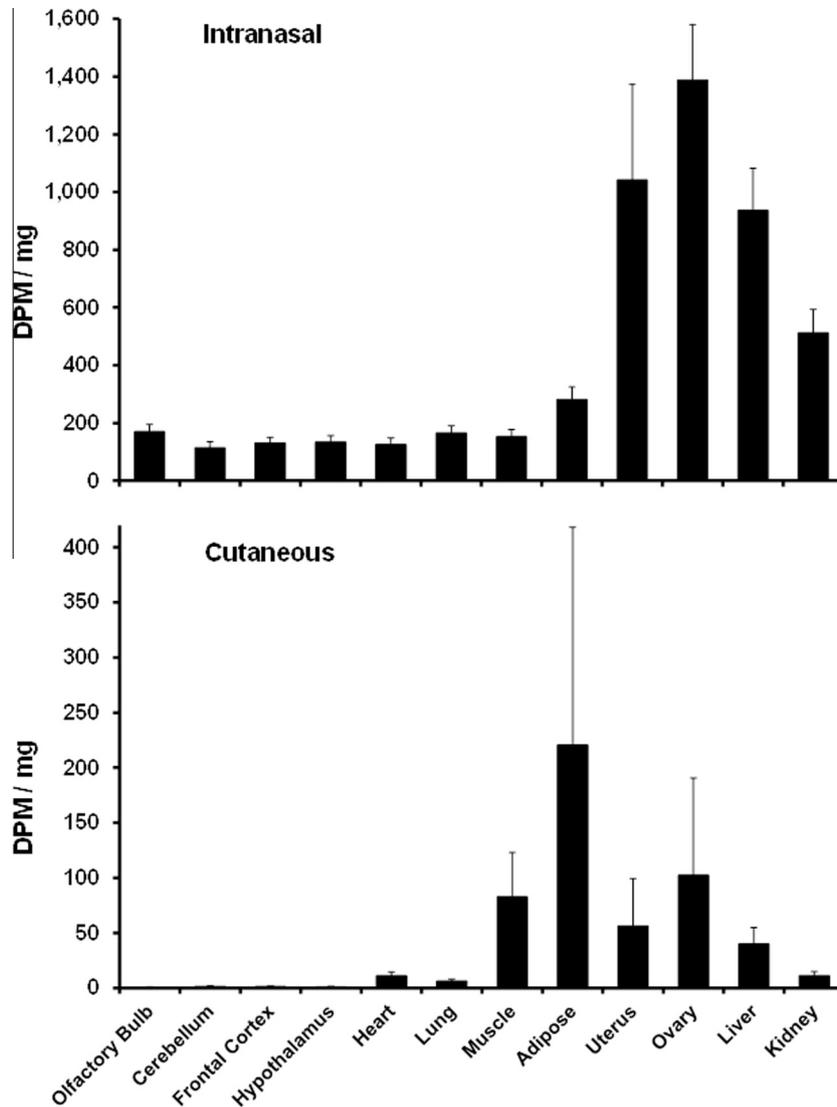
Fig. 1. Radioactivity (mean DPM + SEM) measured in solubilized tissues of adult female bats 1 h after direct (*upper panel*) intranasal administration (*n* = 5) and (*lower panel*) cutaneous administration (*n* = 5) of 10 μCi (37.7 ng) ^{3}H-E$_2$ in Experiments 1 and 2. Control bats given ethanol intranasally (*n* = 5) showed values of 0 DPM/mg in all tissues, except for 3 individual tissue samples (muscle, 0.12; uterus, 0.27; liver 0.07 DPM/mg), each in a different bat. Intranasal control and ^{3}H-E$_2$-treated conditions differed significantly for all tissues (*P* < 0.05). Control bats given ethanol cutaneously (*n* = 5) showed values of 0 DPM/mg in all tissues, except for 3 individual tissue samples (muscle, 0.05, and adipose, 0.25, in one bat; uterus, 0.09, in another). Cutaneous control and ^{3}H-E$_2$-treated conditions differed significantly for all tissues (*P* < 0.05).

6) but not the others, corresponding to substantially higher levels of radioactivity in that female and relatively little in the other two. Tissues from all 3 younger, laboratory-born females in the second cohort exposed to Male 3 also showed radioactivity (*Panel C*). Serum measures of radioactivity were taken for each female and the values (DPM/μl) were respectively 1.9, 0.0, and 3.4 for Females 1, 2, and 3; 1.1, 0.0, and 19.9 for Females 4, 5, and 6; and 6.0, 3.6, and 3.5 for Females 7, 8, and 9. The values (DPM/mg) for tissues from Male 3, which was twice directly injected with ^{3}H-E$_2$, were: cerebellum, 243.1; frontal cortex, 236.9; hypothalamus, 247.4; heart, 131.4; lung, 121.4; muscle, 198.8; adipose, 920.3; testis, 2024.2; epididymis, 3525.2; liver, 323.9; kidney, 226.4. Serum from this male measured at 501.0 DPM/μl.

Dry swipes of our surgical tools, the work tray, the dissection table, and the anesthetic induction chamber taken after dissections in Experiment 3B showed zero or negligible DPM values indicative of background radiation, demonstrating no external contamination. Samples taken from the water dish and mealworms from the food dish also showed levels at background, indicating that

contamination of the water or food was not responsible for ^{3}H-E$_2$ transfer between conspecifics.

4. Discussion

Steroids are typically assumed to operate solely within the body of the individual whose glands produce the hormones. Here we show evidence that E$_2$, arguably the most powerful steroid, can originate in one individual and arrive in the tissues of another. Injection of minute quantities of ^{3}H-E$_2$ into male bats during the mating season reliably produced radioactivity in the reproductive and brain tissues of cohabiting females. This effect was replicated in all 18 females and was produced by all three ^{3}H-E$_2$-treated stimulus males in every replication with a new cohort of cohabiting females. Radioactivity was found in the uteri and lungs of all male-exposed females, and in the ovaries and livers of all but three exposed females. In brain tissues, levels of radioactivity were quite variable among male-exposed females. The profile across tissues
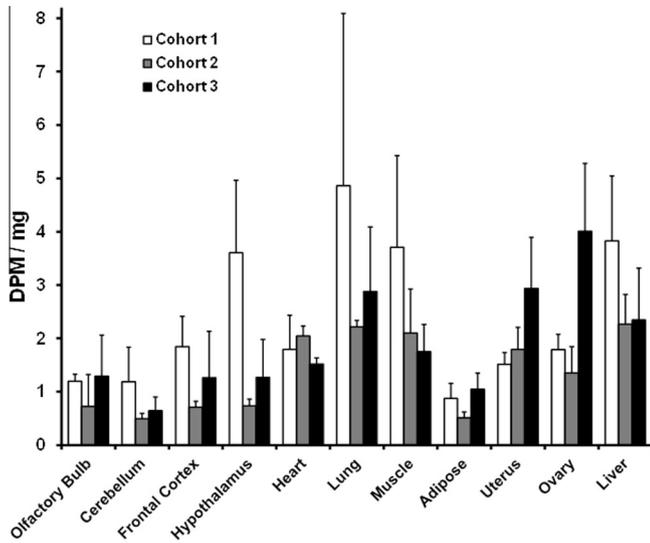
Fig. 2. Radioactivity (mean DPM + SEM) measured in solubilized tissues of 3 cohorts of adult female bats after 48 h of cohabitation with a ^{3}H-E$_2$-treated adult male in Experiment 3A, conducted in 2012. Each cohort consisted of 3 females concurrently exposed to the same male (Male 1), which was re-injected (i.p.) with 50 µCi (188.5 ng) ^{3}H-E$_2$ 1 h prior to cohabiting with each new cohort (total *n* = 9).

Fig. 3. Radioactivity (DPM) measured in solubilized tissues of adult female bats (*n* = 9) after 48 h of cohabitation with a ^{3}H-E$_2$-treated adult male in Experiment 3B, conducted in 2013. *Panel A* shows 3 females concurrently exposed to Male 2. *Panel B* shows 3 different females exposed to Male 3. Each male was injected (i.p.) with 50 µCi (188.5 ng) ^{3}H-E$_2$ 1 h prior to cohabiting with the females. *Panel C* shows three young laboratory-bred female bats, born during the previous spring, after 48 h of cohabitation with Male 3 after re-injection with 50 µCi (188.5 ng) ^{3}H-E$_2$ 1 h prior to cohabitation with the females. *The olfactory bulb and cerebellum were not examined from Female 9.

was similar to that observed with female mice exposed to ^{3}H-E$_2$-treated males (Guzzo et al., 2012, 2013). The similarity of E$_2$ transmission between conspecifics in mice and bats, despite their evolutionary separation by scores of millions of years, indicates that this phenomenon is highly likely to occur in many mammalian species.

The doses of ^{3}H-E$_2$ employed in these experiments were designed to represent just a fraction of the estimated endogenous concentrations of E$_2$ based upon extrapolation from comparisons of urinary E$_2$ from endogenous and exogenous sources in mice (Guzzo et al., 2010, 2012, 2013). There are few studies of endogenous E$_2$ in *E. fuscus*, but one study (Mendonça et al., 1996) shows plasma concentrations (from females during October in Alabama) that are substantially higher than in published plasma samples for female laboratory mice (Bronson and Desjardins, 1974; Nelson et al., 1992). One study in mice (Thorpe and deCatanzaro, 2012) found that a dose of 60 µg E$_2$ i.m. every two days was necessary to restore urinary E$_2$ to normal levels in castrated males. That dose was much greater than the 37.7 ng of ^{3}H-E$_2$ used in Experiments 1 and 2, or the 188.5 ng of ^{3}H-E$_2$ used in Experiment 3, even considering that the bat weighs approximately 50–80% of the weight of a mouse.

Experiment 1 shows that ^{3}H-E$_2$ readily passes into female circulation after nasal exposure, as it does in mice (Guzzo et al., 2010, 2012). Notably, the vomeronasal organ, while found in a number of bats, is absent in the big brown bat (Cooper and Bhatnagar, 1976). It has long been thought that the vomeronasal organ is critical for pheromonal activity in mice (Baum and Bakker, 2013; Leinders-Zufall et al., 2004; Oboti et al., 2014); however, the exposed nasal vasculature is on its own sufficient to promote absorption of small molecules, especially if they are lipophilic (Arora et al., 2002; Türker et al., 2004). Some lipophilic substances can also pass from the nasal cavity directly into cerebrospinal fluid (Sakane et al., 1991). Radioactivity was observed in the lungs of all females in Experiment 3, with average counts higher than in most other tissues. It is possible that intranasal exposure could lead to ^{3}H-E$_2$ absorption via the lungs, although radioactivity in the lungs could also reflect the presence of ERβ there (Kuiper et al., 1997).

In Experiment 2, percutaneous absorption of ^{3}H-E$_2$ was observed in all exposed female bats, although absorption was more variable across individuals than after intranasal exposure in

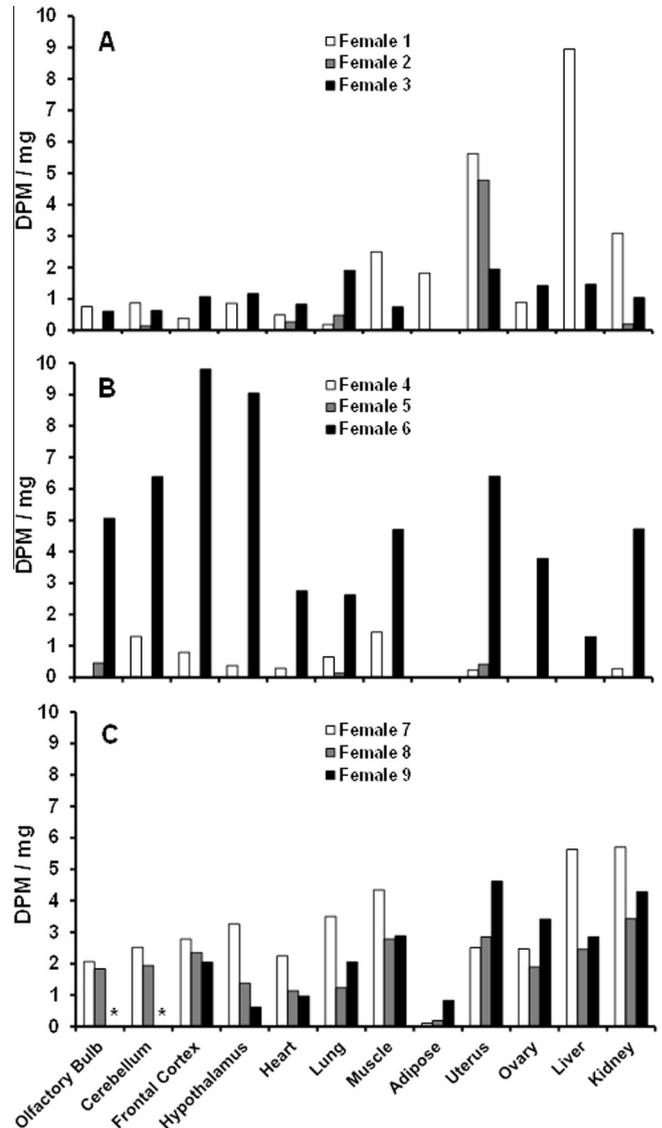Experiment 1, and more variable than previously found after cutaneous exposure in mice (Guzzo et al., 2010, 2012). Percutaneous absorption of a variety of molecules into mammalian circulation is well known, and is affected by solubility, polarity, and molecular size (Schaefer et al., 1982; Wester and Maibach, 1983).

It is also possible that E$_2$ of male origin arrived in females intravaginally. We did not systematically observe our bats for mating, as the presence of human observers and higher lighting levels could perturb natural behavior in this nocturnal species. Informally, we saw many instances of physical contact between males and females (*e.g.*, they roosted together in the same corner of the holding cage). Importantly, we measured radioactivity in the testes and epididymides of two males in Experiment 3, and levels in the epididymides were extraordinary compared to all other tissues. We previously observed substantial radioactivity in the epididymides of ^{3}H-E$_2$-treated male mice (Guzzo et al., 2012), consistent with

the presence of ERα and ERβ in that tissue (Couse et al., 1997). The possibility that E_2 could accompany sperm during mating is worthy of further scientific attention, as direct intrauterine delivery would bypass the general circulation and act directly on ER in the female reproductive tract.

Many chiropterans, including big brown bats, show some asynchrony of reproductive functions that in many other mammals are closely associated (Gustafson, 1979; Martin and Bernard, 2000). In males, peak levels of testosterone and spermatogenesis occur during summer and early fall, and can precede mating by some months (Gustafson, 1979). Females mate months before ovulation, and when aroused during the winter they may spontaneously ovulate, with increasingly greater frequency near the end of hibernation (Oxberry, 1979). Such ovulations permit fertilization by sperm stored from copulations during the autumn or from brief arousals during hibernation (Oxberry, 1979; Racey, 1979; Wimsatt, 1944). In rodents, ovariectomy causes females to become asexual, while injections of E_2 followed by an acute surge in progesterone will induce sexual receptivity in those females (Barfield and Lisk, 1974; Pfaff, 1980). However, in captive big brown bats, ovariectomy does not reduce female sexual activity (Mendonça et al., 1996). The presence of estrogens and progesterone a few months after ovariectomy in female *E. fuscus* is suggestive of non-ovarian hormone sources, such as the adrenal cortex or abdominal adipose (Mendonça et al., 1996).

On the basis of our findings in bats, we suggest that male-sourced steroids could also contribute to the induction of estrus in females. Considering our previous data showing E_2 transfer in mice (Guzzo et al., 2012, 2013), we suggest that this may occur in many other mammals, consistent with evidence that exposure to males can promote regular estrous cycling in mice (Whitten, 1956), goats (Rivas-Muñoz et al., 2007), and hamsters (Dodge et al., 2002). Specifically, male-to-female transfer of E_2 could explain how male exposure can induce sexual receptivity, luteinizing hormone (LH) release, and ovulation. The actions of E_2 in the hypothalamus are critical for the induction of female sexual receptivity (Pfaff, 1980). Moreover, E_2 triggers LH surges from the pituitary gland in female mammals, which in turn induces ovulation (*e.g.,* Ferin et al., 1969; Meikle et al., 2001; Robker and Richards, 1998). As in other mammals, ovulation in bats can be induced by exogenous estrogens and LH (Oxberry, 1979). We therefore suggest that E_2 transmission from males to females could be a major trigger of behavioral estrus and ovulation.

Exogenous E_2 has several other powerful influences on female reproduction. The presence of males can hasten pubertal development in juvenile females of many mammals. The Vandenbergh effect is well studied in mice (Vandenbergh, 1967), but has also been found in many other mammalian species (*e.g.,* Brooks and Cole, 1970; Harder and Jackson, 2003; Izard and Vandenbergh, 1982; Beehner and Lu, 2013). Evidence has implicated E_2 in this effect since the work of Bronson (1975). Castrated male mice cannot induce the Vandenbergh effect, but restoring urinary levels of E_2 by intramuscular injections reinstates the capacity of males to hasten uterine growth in proximate females (Thorpe and deCatanzaro, 2012). Endogenous E_2 is critical for maturation of the female reproductive tract (*e.g.,* Ogasawara et al., 1983; Quarmby and Korach, 1984) as E_2 increases DNA synthesis and cell proliferation in the uterus, thereby stimulating uterine growth (Cooke et al., 1997; Mukku et al., 1982; Ogasawara et al., 1983; Quarmby and Korach, 1984). The Vandenbergh effect has not yet been studied in bats. We examined three laboratory-bred female bats born in June of the same year, and found ^3H-E_2 transfer from the male to each female. The age of full reproductive maturity of female big brown bats is not fully known. One study (Christian, 1956) found that ovaries taken in October from females born during the previous spring or summer were indistinguishable from those of adults, however another study (Schowalter and Gunson, 1979) found that such young females are less likely to be parous compared to older females.

Exogenous E_2 can also have strong effects on ovoimplantation in inseminated female mammals. E_2 helps to prepare the mammalian uterus for blastocyst implantation; however, supraoptimal levels of E_2 can perturb the timing of arrival of ova at the uterus (Ortiz et al., 1979), reduce blastocyst survival (Valbuena et al., 2001), and render the uterine epithelium refractory to implantation (Ma et al., 2003). These latter facts are pertinent to the role of male-sourced E_2 and the capacity of novel males to disrupt peri-implantation pregnancy in the Bruce effect (*e.g.,* deCatanzaro et al., 2001, 2006; Guzzo et al., 2010, 2012; Rajabi et al., 2014). Castrated male mice cannot induce the Bruce effect, but restoring urinary levels of E_2 by intramuscular injections reinstates their capacity to disrupt blastocyst implantation (Thorpe and deCatanzaro, 2012). The Bruce effect is found in many mammals (*e.g.,* Clulow and Langford, 1971; Roberts et al., 2012; Rohrbach, 1982), and while it is unknown whether bats have a Bruce effect, there is evidence of intermale competition during fertilization (Vonhof et al., 2006).

The radioactivity profile observed in female bats after exposure to ^3H-E_2-treated males is largely consistent with the distribution of ER, as was true in mice (Guzzo et al., 2012). Female mammals have an abundance of ER in the uterus and ovaries, but they also occur at some level in the kidneys, lungs, hypothalamus, olfactory bulbs, and cerebral cortex, among other tissues (Couse et al., 1997; Kuiper et al., 1997). The E_2 molecule is extremely potent and can pass in unconjugated form into excretions such as urine, saliva, and perspiration (*e.g.,* deCatanzaro et al., 2009; Muir et al., 2008). It can also be absorbed via nasal, cutaneous, and other routes by cohabiting conspecifics due to its highly lipophilic nature and low molecular mass. E_2 plays critical roles in female reproductive physiology and behavior, and many male mammals have evolved the ability to influence females' reproductive state to their advantage through excretions that contain E_2.

The capacity of male bats to transmit such a powerful steroid to female conspecifics sets the stage for pheromonal activity. The extraordinary concentration of E_2 in the males' epididymides and their ability to transmit E_2 to the reproductive tract and brain of cohabiting females both suggest that male bats have evolved to use E_2 as a pheromone. Among other possibilities, absorbed E_2 could be involved in the induction of sexual maturation, estrus, sexual receptivity, and ovulation.

Acknowledgments

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