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Neonatal DHT but not E2 speeds induction of sexual receptivity in the musk shrew

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Abstract

Neural aromatization of testosterone (T) to estrogen during development is thought to be important for sexual differentiation of many altricial mammals. We evaluated the effects of neonatal injections of the non-aromatizable androgen dihydrotestosterone propionate (DHTP) and estradiol (E2) on the copulatory behavior of the female musk shrew, an altricial insectivore. Following adult ovariectomy and replacement T, animals were paired with a stimulus female for two 60-minute copulatory behavior tests. The latency to induce sexual receptivity (in the form of tail-wagging by the female), mount latency and total number of mounts were recorded in experimental females and in a group of untreated control males. While neither hormone treatment significantly affected mounting behavior, DHTP-treated animals induced receptivity faster and with latencies not significantly different from intact males, suggesting that early non-aromatizable androgens can have masculinizing actions by either increasing sexual motivation or making the treated animal more attractive to the stimulus female. Reliance on androgenic rather than estrogenic metabolites for the differentiation of courtship behaviors conforms to the pattern seen more typically in primates than rodents.

Keywords

masculinization; dihydrotestosterone; altricial; copulatory behavior; aromatization; sexual differentiation; insectivore

Introduction

Developmentally-present steroids are recognized as essential components of mammalian sexual differentiation [1]. Testosterone (T) has three metabolic pathways. It can bind and act via androgen receptors (AR), be aromatized to estradiol (E2), which acts via estrogen receptors (ER), or be irreversibly reduced to the non-aromatizable dihydrotestosterone (DHT), which also acts via AR. While both AR- and ER- dependent pathways likely contribute to sexual differentiation, their relative importance has been debated and likely varies by species. In rats, aromatization is clearly a necessary step for some aspects of masculinization. ER, but not AR blockers eliminate differentiation of the rat sexually

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dimorphic nucleus of the preoptic area [2], while androgen-insensitive (*Tfm*) rats show few impairments in male-typical mounting behavior, suggesting ER action alone can masculinize this behavior [3]. However, AR mutations in mice disrupt both male copulatory behaviors and male-typical attraction to female odors [4–5]. DHT exposure during the week before and after birth masculinizes copulatory behaviors in wild-type but not AR-knockout female mice [5], suggesting that androgen need not be aromatized to masculinize mouse copulatory behavior and that developmental AR activation is essential to this process.

Estrogenic pathways, particularly those involving ER α also contribute to behavioral masculinization in mice [6]. Preventing neonatal aromatization of T to E2 disrupts male partner preference for females and male-typical copulatory behaviors in rats [7]. review [8] of sexual differentiation literature of 3 altricial and 4 precocial species concluded that in most altricial mammals, developmentally-present T requires conversion to E2 to fully differentiate copulatory behaviors. While there is some evidence for a role for early aromatization in sexual differentiation of some precocial species (guinea pigs and pigs), there is no evidence that estrogenic metabolites were essential for male sexual differentiation in non-human primates, as aromatizable and non-aromatizable seem to both be effective masculinizers [8–10].

The musk shrew (*Suncus murinus*) is an altricial insectivore with an unusual hormonal profile. Sexual behavior in the female musk shrew is activated much like male sexual behavior in other species [11]. Female musk shrews have no behavioral estrus cycle, do not show the lordosis reflex and are tonically sexually receptive; mating is activated by ovarian and adrenal T [12–13]. In a typical copulatory behavior test for this species, the male is placed in a test box for a brief (15 minute) acclimation period, then a stimulus female is introduced. The male courts the female by approaching, following, chasing and investigating the anogenital area. Females initially respond to this attention with aggression in the form of male-directed vocalizations, lunges and attacks that are occasionally fierce enough to bloody his nose. Eventually, the females' behavior shifts from aggression to receptivity, as indicated by "tail-wagging" behavior (presentation of the rump and a characteristic swishing movement of the tail) and reduced aggression toward the male [11,13]. Shortly after tail-wagging begins, the male typically achieves his first successful mount. Tail-wag latency is usually 10–15 minutes but is longer with sexually inexperienced females [14].

In the musk shrew, neural aromatization of gonadal T to E2 in the medial preoptic area is essential to the activation of copulatory behavior in both sexes [11]. Neonatal testosterone propionate (TP) treatment of female musk shrews both masculinizes (as measured by a decrease in mount latency and an increase in mount rate) and defeminizes (as measured by an increase in male-directed aggression during tail-wagging and a resultant reduced intromission efficiency by the male) copulatory behavior [15], but whether T had this organizational effect via AR or after conversion to E2 was unknown. We tested whether aromatization was necessary for masculinization of musk shrew copulatory behavior by treating newborn females with either the non-aromatizable androgen dihydrotestosterone propionate (DHTP) or estradiol benzoate (EB) and then testing their copulatory behavior in adulthood after gonadectomy and long-term activational T-treatment.

Methods

Animals

Musk shrews used for this study were bred in a colony at Mary Baldwin College. Adult shrews were housed in single plastic cages (43 × 21 × 19 cm) with pine shaving bedding and food (10:1 mixture of Purina cat chow and Milk Specialties mink chow) and water provided *ad lib*. All cages were checked daily for food and water supply and cleaned on a bi-weekly

basis. The colony is maintained on a 14L:10D light cycle (lights on at 0700) at a temperature range of 20–23 degrees C. Female pups were housed in with their dams and male siblings until weaning at 21–30 days.

Treatment

On postnatal days 1–5, female pups received either DHTP (0.1 mg in 0.02 ml sesame oil, s.c., N=7), EB (0.01 mg in 0.02 ml sesame oil, s.c., N=7), or vehicle (N=6) injections. The DHTP dosage was chosen based on the testosterone propionate dosage found to be effective at masculinizing musk shrew copulatory behavior [15]; the EB dose was based on other developmental studies [16–18] that used EB at 1/20th to 1/5th the dosage of DHTP. To ensure equivalent activation hormonal levels, at 2–4 months of age, all female animals were ovariectomized under sevoflurane anesthesia and implanted with Silastic capsules containing T (5mm, od 2.08 mm × id 0.98 mm). This size implant was previously shown to be effective in the activation of male sexual behavior by providing a simulation of normal male circulating T levels [13,19]. A group of untreated, intact, sexually-inexperienced, similarly aged males was used as a positive control. All procedures were approved by the Mary Baldwin College Institutional Animal Care and Use Committee and are consistent with the *NIH Guide for the Care and Use of Laboratory Animals*.

Behavior testing

Behavior testing occurred 2–4 weeks post-surgery. Tests for male copulatory behavior were conducted during the animals' dark period in a clear plastic test box (40 × 19.5 × 12.5 cm). The experimental female was placed in the test box to acclimate for 5 minutes, and a stimulus female (a dam who had previously mated and given birth to at least one litter) was introduced. Animals were paired with different females in the two trials. Animals were given two 60-minute copulatory behavior tests by an experimenter blind to hormone treatment; blindness to sex was not feasible given the much larger body size and obvious penile reflexes of male shrews.

Behaviors recorded included the latency to induce sexual receptivity of stimulus females, as indicated by female "tail-wagging" behavior [13], latencies of first mount, and number of complete mounts. For control males, the latency to first intromission and ejaculation and the number of mounts with successfully placed intromissions was also recorded and the test was terminated if the male ejaculated before 60 minutes elapsed. The data were analyzed using a 4×2 (treatment by trial) mixed-design ANOVA, with the two trials considered as a repeated measure, followed by post-hoc testing using Fisher's protected t-test. Statistical significance was defined as $p < 0.05$.

Results

Physical effects of early DHT and E2 on genitalia

All female animals, regardless of hormone treatment, had normal open vaginas within the cloacal slit. Examination at sacrifice showed slightly enlarged phalluses in the DHT-treated females, but no erections were observable during copulatory behavior testing.

Copulatory behavior

All neonatally-treated DHT females successfully induced tail-wagging in the stimulus animal on both tests, as did all but one of the control males (83.33%). One male failed to induce tail-wagging on his first test. In the E2 group, 3 of 7 (43.86%) induced tail-wagging on the first test and 4 of 7 (57.14%) on at least one test. In the oil-treated controls, 1 of 6 (16.67%) induced tail-wagging on the first test and 2 of 6 (33.33%) on at least one test. A two-tailed Fisher's exact test found that DHT-treated animals were more likely than oil-

treated females to induce tail-wagging than controls on both the first test ($p = .004$) and over both tests ($p = 0.02$). E2-treated animals were intermediate between DHT and control females and not significantly different from either, while DHT-treated females and intact males were equally likely to induce tail-wagging ($p = 0.46$).

Six out of 7 (85.71%) DHT-treated shrews mounted the stimulus animals on at least one test, compared 4 of 7 (57.14%) E2-treated shrews, 2 of 6 (33.33%) female oil controls, and 7 of 7 (100%) control males. Fisher's exact probability test found control males significantly more likely to mount than oil-treated females ($p = 0.02$), while both E2- and DHT-treated females were intermediate between control females and males and not significantly different from either.

Tail-wag latency

Results are summarized in Figure 1. ANOVA showed a main effect of treatment [$F(3,22) = 7.91, p = 0.029$] but no effect of trial [$F(1,22) = 2.719, p = 0.113$] and no interaction [$F(1,22) = 0.459, p = 0.714$]. Post-hoc tests found male shrews initiated tail-wagging over 3 times faster than oil treated females [$t(22) = 3.13, p < 0.05$]. DHT [$t(22) = 2.19, p < 0.05$] but not E2 [$t(22) = 0.82, p > 0.05$] reduced average tail-wag latency compared to oil-treated females. There was no significant difference between DHT-treated females and male controls [$t(22) = 1.06, p > 0.05$]. E2-treated females were intermediate between oil controls and DHT-treated females [$t(22) = 1.42, p > 0.05$] and not significantly different from either, though they induced tail-wagging slower than intact males. [$t(22) = 2.42, p < 0.05$].

Mount latency

Results are summarized in Figure 2. ANOVA showed main effects of treatment [$F(3,22) = 5.71, p = 0.005$] and trial [$F(1,22) = 5.84, p = 0.025$] with shorter mount latencies on trial 2. The interaction of trial with treatment did not reach statistical significance. [$F(1,22) = 2.06, p = 0.134$]. Post hoc tests found that control males had shorter mount latencies than either oil [$t(22) = 3.14, p < 0.05$] or E2-treated females [$t(22) = 2.45, p < 0.05$], but were not significantly different from DHT-treated females [$t(22) = 1.68, p < 0.05$]. DHT did not masculinize mount latency compared to either control [$t(22) = 1.57, p > 0.05$] or E2-treated females [$t(22) = 0.80, p > 0.05$], but produced a mount latency intermediate between the males and control females. E2-treated females were not significantly different from oil-treated females [$t(22) = 0.81, p > 0.05$].

Mounting behavior

Results are summarized in Figure 3. ANOVA showed a main effect of treatment [$F(3,22) = 8.803, p = 0.001$], but no effect of trial [$F(1,22) = 1.97, p = 0.175$] and no interaction [$F(1,22) = 0.581, p > 0.663$]. Post-hoc tests showed higher numbers of mounts in males than in oil- [$t(22) = 3.38, p < 0.05$], E2- [$t(22) = 2.91, p < 0.05$], or DHT-treated females [$t(22) = 2.56, p < 0.05$]. No other group differences reached statistical significance.

Discussion

Early DHT treatment of female musk shrews increased the likelihood and shortened the initiation of tail-wagging behavior in a stimulus female to nearly the levels of intact male controls, although it did not produce an increase in mounting behavior as in previous studies with early T [15]. Neonatal E2 had no significant effect on any behavioral measure. This suggests that AR-dependent processes either organize the musk shrew brain to produce more of the male-typical courtship behaviors (chasing, following) or makes the shrew more attractive to the stimulus female by altering some sensory signal, either through the nervous

system or the periphery. This behavioral masculinization occurs in the absence of noticeable masculinization of the genitals.

One possibility is that the DHTP-treated animals are more sexually motivated and therefore more persistent in pursuing the stimulus female during the aggressive, pre-tail-wagging phase of courtship. Early DHT treatment could also have changed the experimental animals' scent during adulthood in a way that triggered the females' shift to receptivity more quickly. Pre-exposure to male bedding odors has been shown to reduce tail-wag latency in the musk shrew [14,20]. A third, unexplored possibility is that the DHT-treated animals stimulate receptivity in the female via ultrasonic calling. Such calls by males are a typical courtship behavior in a variety of rodents and, like have been shown to be both organized and activated by T or one of its metabolites [21–23]. AR-mediated processes have been specifically implicated in display of this behavior in rats [23, review]. Future studies will attempt to distinguish between these separate, though non-exclusive, explanations.

In contrast to the neonatal T-treatments of a previous study [15], neither DHT nor E2 masculinized mounting behavior over that seen in oil-treated controls. However, the previous study used a different testing protocol where experimental animals were tested with females who had already been pre-induced to tail-wag by an intact male. It is likely that this enhancement of receptivity facilitated mounting behavior in those animals relative to ours. It is also possible that a combination of both metabolites is needed to fully masculinize mounting behavior in the musk shrew. In monkeys, ferrets [8] and mice [5] a combination of estrogenic and androgenic action during development can masculinize copulatory behavior better than either metabolite alone. ERs and ARs are also expressed in a nucleus of aromatase-like immunoreactivity (AROM-ir) in the medial preoptic area in the musk shrew preoptic area, so this area is likely responsive to both androgenic and estrogenic T-metabolites [24–25].

Alternatively, it is possible mounting behavior is not especially differentiated in musk shrews and that normal female shrews, like many rodent species [26–27], maintain the capacity for substantial mounting behavior when exposed to high T levels in adulthood and provided with a receptive partner. Such a capacity may be typical of species that are exposed to some T from the placenta or from male siblings during prenatal development [26,28]. Given the role of tonic T in activating female sexual behavior in the musk shrew [29] and the suggestion that T levels may actually increase during pregnancy [11], it is possible that female musk shrews receive substantial T exposure as fetuses. This view is consistent with the observation of mounting behavior in some control animals in this and previous studies [15] and also with our observations (unpublished) that female musk shrews, like rats [28], occasionally mount unresponsive males. The capacity for some mounting behavior by normal females does not preclude postnatal T from further increasing that behavior [27] or developmentally-present steroids from being important for masculinizing other important aspects of copulation, such as soliciting of and attractiveness to females. Furthermore, it should be remembered that male shrews, unlike our experimental females, receive sensory feedback in the form of penile stimulation from erections and intromissions. This is presumably a source of positive reinforcement that encourages increased mounting behavior, at least until ejaculation occurs.

The ability of neonatal DHT but not E2 to masculinize courtship behaviors is unusual among altricial mammals. In many altricial rodents, estrogenic T-metabolites play a larger role than androgenic ones in behavioral masculinization [8]. For example, male rats castrated and given neonatal replacement DHT show reduced partner preference for estrous females as adults compared to those receiving T neonatally [30]. Female gerbils treated neonatally with DHT do not display T-induced mounting behavior as adults, but those

treated with either neonatal T or E2 do [31]. While DHT can act via the AR to masculinize copulatory behavior in mice [5], that study used both pre- (5 day) and postnatal (8 day) treatments, while our animals were treated only over the first 5 postnatal days. Furthermore, studies of ER knock-out mice suggest that ER-mediated pathways are crucial to the masculinization of the male-typical preference for female odors [32].

While E2 did not organize male-typical mating behaviors, aromatization is clearly an important component in activating such behaviors in the musk shrews [11]. Compared to females, males have higher circulating T-levels [19,29], higher brain aromatase activity [33] and a larger AROM-ir nucleus in the medial preoptic area [34]. Neonatal T-treatment in females increases the size of the AROM-ir nucleus in females while neonatal castration of males reduces it [34]. Taken together, it is reasonable to hypothesize that early T is responsible for the male-like differentiation of both the hypothalamic structures and adult copulatory behavior.

Overall, primates show greater evidence of masculinization by developmentally-present non-aromatizable androgens than do rodents [9–10]. In the female Rhesus monkey, prenatal DHT masculinizes juvenile mounting and play behaviors and increases displays of male-like mounting behavior in adulthood [35–36]. Importantly, early T and DHT treatment of female Rhesus monkeys increase the capacity of adult T to stimulate not only mounting, but also the courtship displays preceding copulation, including lip-purse gestures and female-directed approaches [37]. Such courtship displays may be especially critical to reproductive success in species like Rhesus monkeys and musk shrews, where females are not immobilized by the lordosis reflex when mounted [10]. Our data suggest that musk shrews, despite their altricial nature, show a sexual differentiation pattern similar to that of primates: one in which non-aromatizable androgens are more effective neonatal masculinizers of courtship behavior than E2.

Shrews (*Soricidae*) are the largest family within the insectivore order and the second-largest family of mammals after the murid rodents. Traditionally, they have been thought of as “primitive” placental mammals, perhaps resembling the earliest ancestral mammals that underwent adaptive radiation at the extinction of the dinosaurs. Recent molecular and historical morphological data provide conflicting evidence in determining the exact phylogeny of insectivores and their relationship to modern primates and rodents [38]. Ancestries based on morphological analysis [38] have traditionally viewed insectivores as more closely related to primates than either order is to rodents; some molecular analyses, particularly those based on mitochondrial DNA, hold a similar view [39–40]. Other genetic phylogenies [41] place primates and rodents closer to each other and more distant from the insectivores. Whatever the case, the capacity of the developing brain to be efficiently masculinized by AR activation instead of, or in addition to, ERs may be a trait that has been retained by shrews and primates, but evolved into a more aromatization-dependent pathway in many rodent species.

Masculinization and defeminization, though both triggered by developmentally-present sex steroids, are considered separate and independent processes [42, review]. Aromatization is essential for defeminization of most altricial rodents examined, but not for primates and certain other precocial species [8], leading some to hypothesize that aromatization-dependent defeminization processes are a widespread trait in altricial mammals. Pilot data (Ewton and Freeman, unpublished) suggests that neonatal DHT treatment of female musk shrews leads to longer tail-wag latencies and increased male-directed aggression when they are paired with a stud male, mimicking the defeminizing effects of neonatal T [15]. Future studies will examine the effects of DHT/E2 combinations on both masculinization and defeminization in the musk shrew.

Conclusion

In musk shrews, non-aromatizable DHT increases the likelihood and shortens the latency to initiate sexual receptivity in a female partner when given to female neonates, producing a pre-mounting courtship behavior similar to the intact male. This lack of dependence on aromatization suggests that sexual differentiation of courtship behaviors in shrews more closely resembles the pattern seen in primates than that of the more commonly studied laboratory rodents.

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References

1. Phoenix C, Goy R, Gerall A, Young W. Organizing action of perinatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology* 1959;65:369–382. [PubMed: 14432658]
2. Dohler K, Coquelin A, Davis F, Hines M, Shryne J, Sickmoller P, Jarzab B, Gorski RA. Pre- and postnatal influence of an estrogen antagonist and an androgen antagonist on differentiation of the sexually dimorphic nucleus of the preoptic area in male and female rats. *Neuroendocrinology* 1986;42:443–448. [PubMed: 2939360]
3. Olsen, KL. Genetic influences on behavioral sexual differentiation. In: Gerall, A.; Moltz, H.; Ward, IL., editors. *Sexual Differentiation: a Life-Span Approach*, Handbook of Behavioral Neuroendocrinology. New York: Plenum Press; 1992. p. 1-40.
4. Bodo C, Rissman EF. Androgen receptor is essential for sexual differentiation of responses to olfactory cues in mice. *Eur J Neurosci* 2007;25:2182–2190. [PubMed: 17419752]
5. Sato T, Matsumoto T, Kuwano H, Watanabe T, Uematsu Y, Sekine K, et al. Brain masculinization requires androgen receptor function. *Proc Natl Acad Sci U S A* 2004;101(6):1673–1678. [PubMed: 14747651]
6. Wersinger SR, Sallen K, Villalba C, Lubahn DB, Rissman EF, De Vries GJ. Masculine sexual behavior is disrupted in male and female mice lacking a functional estrogen receptor alpha gene. *Horm Behav* 1997;32:176–183. [PubMed: 9454668]
7. Brand T, Kroonen J, Mos J, Slob AK. Adult partner preference and sexual behavior of male rats affected by perinatal endocrine manipulations. *Horm Behav* 1991;25:323–341. [PubMed: 1937426]
8. Wallen K, Baum M. Masculinization and defeminization in altricial and precocial mammals: Comparative aspects of steroid hormone action. *Horm Behav* 2002;4:385–423.
9. Thorton J, Zehr JL, Loose MD. Effects of prenatal androgens on Rhesus monkeys: a model system to explore the organization hypothesis in primates. *Horm Behav* 2009;55:633–644. [PubMed: 19446080]
10. Wallen K. Hormonal influences on sexually differentiated behavior in nonhuman primates. *Front Neuroendocrinol* 2005;26:7–26. [PubMed: 15862182]
11. Freeman LM, Rissman EF. Neural aromatization and the control of sexual behavior. *Trends Endocrinol Metab* 1996;7:334–338. [PubMed: 18406768]
12. Fortman M, Dellovade TL, Rissman EF. Adrenal contribution to the induction of sexual behavior in the female musk shrew. *Horm Behav* 1992;26:76–86. [PubMed: 1563731]
13. Rissman EF, Crews D. Hormonal correlates of sexual behavior in the female musk shrew (*Suncus murinus*): the role of estradiol. *Physiol. Behav* 1988;44:1–7.
14. Rissman EF. Social variables influence female sexual behavior in the musk shrew (*Suncus murinus*). *J. Comp. Psychol* 1987;101:3–6.

15. Freeman LM, Arora T, Rissman EF. Neonatal androgen affects copulatory behavior in the musk shrew. *Horm Behav* 1998;34:231–238. [PubMed: 9878272]
16. Breedlove SM, Jacobson CD, Gorski RA, Arnold AP. Masculinization of the female rat spinal cord following a single neonatal injection of testosterone propionate but not estradiol benzoate. *Brain Res* 1982;237:173–181. [PubMed: 7074356]
17. Han TM, De Vries GJ. Organizational effects of testosterone, estradiol and dihydrotestosterone on vasopressin mRNA expression in the bed nucleus of the stria terminalis. *J Neurobiol* 2003;54:502–510. [PubMed: 12532400]
18. Isgor C, Sengelaub DR. Prenatal gonadal steroids affect adult spatial behavior, CA1 and CA3 pyramidal cell morphology in rats. *Horm Behav* 1998;34:193–194.
19. Rissman EF. Gonadal influences on sexual behavior in the male musk shrew (*Suncus murinus*). *Horm. Behav* 1987;21:132–136.
20. Rissman EF, Li X. Olfactory bulbectomy blocks mating-induced ovulation in musk shrews (*Suncus murinus*). *Biol. Reprod* 2000;62:1052–1058.
21. Matochik JA, Barfield RJ. Hormonal control of precopulatory sebaceous scent marking and ultrasonic mating vocalizations in male rats. *Horm Behav* 1991;25:445–460. [PubMed: 1813373]
22. Holman SD, Hutchinson JB. Differential effects of neonatal castration on the development of sexually dimorphic brain areas in the Mongolian gerbil. *Brain Res Dev Brain Res* 1991;61(1):147–150.
23. Zuloaga DG, Putz DA, Jordan CL, Breedlove SM. The role of androgen receptors in the masculinization of brain and behavior: what we've learned from the testicular feminization mutation. *Horm Behav* 2008;53:613–626. [PubMed: 18374335]
24. Veney SL, Rissman EF. Co-localization of estrogen receptor and aromatase enzyme immunoreactivities in adult musk shrew brain. *Horm Behav* 1998;33:151–162. [PubMed: 9698499]
25. Veney SL, Rissman EF. Immunocolocalization of androgen receptors and aromatase enzyme in the adult musk shrew brain. *Neuroendocrinol* 2000;72:29–36.
26. Baum MJ. Sexual differentiation of pheromone processing: links to male-typical mating behavior and partner preference. *Horm Behav* 2009;55:579–588. [PubMed: 19446074]
27. Martel KL, Baum MJ. Adult hormone treatment but not surgical disruption of vomeronasal function augments male-typical sexual behavior in female mice. *J Neurosci* 2009;29:7658–7666. [PubMed: 19535577]
28. Afonso VM, Pfaus JG. Hormonal and experiential control of female-male mounting in the female rat. *Horm Behav* 2006;49:30–37. [PubMed: 15955535]
29. Brand T, Slob A. Neonatal organization of adult partner preference behavior in male rats. *Physiol Behav* 1991;49:107–111. [PubMed: 2017462]
30. Rissman EF, Clendenen A, Krohmer R. Role of androgens in the regulation of sexual behavior in the female musk shrew. *Neuroendocrinol* 1990;51:468–473.
31. Ulibarri C, Yahr P. Effects of androgens and estrogens on sexual differentiation of sex behavior, scent marking, and the sexually dimorphic area of the gerbil hypothalamus. *Horm Behav* 1996;30:107–130. [PubMed: 8797020]
32. Kudwa AE, Michopoulos V, Gatewood JD, Rissman EF. Roles of estrogen receptor alpha and beta in differentiation of mouse sexual behavior. *Neuroscience* 2006;138(3):921–928. [PubMed: 16338079]
33. Rissman E, Harada N, Roselli C. Effect of vorozole, an aromatase enzyme inhibitor, on sexual behavior, aromatase activity and neural immunoreactivity. *Neuroendocrinol* 1996;8:199–210.
34. Veney SL, Rissman EF, Freeman LM. Perinatal organization of a sexually dimorphic aromatase enzyme-containing immunoreactive nucleus. *Neuroreport* 2000;11:3409–3412. [PubMed: 11059911]
35. Goy, R. Development of play and mounting behavior in female rhesus virilized prenatally with esters of testosterone and dihydrotestosterone. In: Chivers, DJ.; Herbert, J., editors. *Recent Advances in Primatology Behavior*. London: Academic Press; 1981. p. 449–462.
36. Goy, R. Patterns of juvenile behavior following early hormonal interventions. In: Magnusson, D.; Greitz, T.; Nilsson, LG.; Winblad, B.; Hokfelt, T.; Terenius, L., editors. *The Lifespan*

Development of Individuals: Behavioral, Neurobiological and Psychosocial Perspectives. Cambridge/New York: Cambridge Univ. Press; 1996. p. 296-314.

37. Pomerantz SM, Goy RW, Goy MM. Expression of male-typical behavior in adult female pseudohermaphroditic Rhesus: comparisons with normal males and neonatally gonadectomized males and females. *Horm Behav* 1986;20(4):483–500. [PubMed: 3793029]
38. Symonds MRE. Phylogenies and life histories of the “Insectivora:” controversies and consequences. *Biol Rev Camb Philos Soc* 2005;80:93–128. [PubMed: 15727040]
39. Butler, P. Phylogeny of the insectivores. In: Benton, M., editor. *The phylogeny and classification of the tetrapods: Mammals*. Vol. 2. Oxford: Clarendon Press; 1988. p. 117-141.
40. Arnason U, Adegoke JA, Bodin K, Born EW, Esa YB, Gullberg A, Nilsson M, Short RV, Xu X, Janke A. Mammalian mitogenomic relationships and the root of the eutherian tree. *Proc Natl Acad Sci U S A* 2002;99:8151–8156. [PubMed: 12034869]
41. Stanhope MJ, Waddell VG, Madsen O, de Jong W, Hedges SB, Cleven GC, Kao D, Springer MS. Molecular evidence for multiple origins of Insectivora and for a new order of endemic African insectivore mammals. *Proc Acad Sci U S A* 1998;95:9967–9972.
42. McCarthy MM, Wright CL, Schwarz JM. New tricks by an old dogma: mechanisms of the organizational/activational hypothesis of steroid mediated sexual differentiation of brain and behavior. *Horm Behav* 2009;55:655–665. [PubMed: 19682425]

Figure 1.

Neonatal dihydrotestosterone (DHT-) treated female shrews initiated sexual receptivity (tail-wagging) faster than female sesame oil-treated controls ($p < 0.05$, Fisher's protected t-test.) but were not significantly different from intact males. Estradiol (E2-) treated females were intermediate between control and DHT-treated females, but were slower than males in initiating tail-wagging ($p < 0.05$). Bars indicate means $\pm$ standard error of the mean.

Figure 2.

Mount latencies overall were shorter on the second trial than on the first ($p < 0.05$, ANOVA). Control males mounted faster than either neonatally sesame oil-treated control or estradiol (E2-) treated females ($p < 0.05$, Fisher's protected t-test.) while dihydrotestosterone (DHT-) treated females were intermediate. Bars indicate means $\pm$ standard error of the mean.

Figure 3.

Intact control males achieved more mounts than females treated with either neonatal dihydrotestosterone (DHT-), estradiol (E2-) or sesame oil ($p < 0.05$, Fisher's protected t-test.). No other group differences were significant. Bars indicate means $\pm$ standard error of the mean.