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Is human brain masculinization estrogen receptor-mediated? Reply to Luoto and Rantala

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ABSTRACT

Human genetic males are unlike rodent males in that neither the ability to convert testosterone to estrogen nor a functional estrogen receptor (ER) appears necessary for male-typical behavior, but a functional androgen receptor (AR) is required. Brain masculinization is probably mainly AR-mediated in human genetic males. ER binding may nevertheless have important masculinizing or defeminizing effects in human genetic females. Probably the strongest available evidence on this issue is derived from females exposed to synthetic estrogens in utero due to their mother's treatment with DES. As we review, the totality of evidence from this population indicates little or no effect of estrogens on sexuality in genetic females. In addition, if brain masculinization were ER-mediated in humans, it seems unlikely that sex hormone-binding globulin would bind estrogens so effectively as to prevent them from masculinizing the brain. In sum, current evidence suggests that estrogen plays a limited role in masculinizing the human brain and behavior.

Louto and Rantala (2017; henceforth, L&R) highlight an underappreciated point in behavioral endocrinology—that human brain masculinization is primarily androgen receptor (AR)-mediated, rather than estrogen receptor (ER)-mediated, as it is in rodents (reviewed in Zuloaga et al., 2008). It is now well-established in rodents that testosterone produced by the testes crosses the blood-brain barrier where it is converted to estrogen via the enzyme aromatase. In a somewhat surprising quirk of biology, it is estrogen binding to ER that is primarily responsible for sending neural development down a pathway to engender a masculine brain (Morris et al., 2004). Brains are not masculinized by females' high estrogen levels because a protein called alpha-fetoprotein (AFP) binds estrogen in the blood and prevents it from crossing the blood-brain barrier (Bakker et al., 2006; McEwen et al., 1975; Puts et al., 2006). Although it is often assumed that this "aromatization hypothesis" applies equally to humans, we reviewed evidence that sexual differentiation in the human brain is instead mediated mainly through androgen binding to AR (Motta-Mena and Puts, 2017).

L&R disagree and offer what they view as evidence of ER mediation. Their primary evidence consists of two studies (Ehrhardt et al., 1985; Meyer-Bahlburg et al., 1995) suggesting that women who were exposed prenatally to the synthetic estrogen diethylstilbestrol (DES) were masculinized behaviorally, in that they were more likely than controls to exhibit non-heterosexual orientation. However, these results were not replicated in subsequent larger studies (Newbold, 1993), including one by the same authors (Lish et al., 1991). The largest investigation of psychosexuality in women exposed prenatally to DES included 3946 women exposed prenatally to DES and 1740 women not exposed (Titus-Ernstoff et al., 2003, cf. 30 women in each group in Ehrhardt et al.). In contrast to earlier findings, the DES-exposed women were slightly *less* likely than unexposed women to have had sex with a female partner. As Hines (2011) points out, this study has limitations despite its impressive sample size. For example, sexual orientation was assessed via a single question regarding sexual behavior, and there were other group differences that might raise questions about the comparability of DES participants to controls. There is evidence that DES produces reproductive-tract abnormalities in women, as well as increasing the odds of psychiatric disease (Vessey et al., 1983). It would not be particularly surprising if DES had some effect on sexuality. Yet

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studies relating DES to sexual orientation and sexual behavior have not led to clear findings, either in detailed small-sample studies or large epidemiological surveys.

McCarthy (2008) notes that the apparent lack of effect of DES on psychosexuality in women is consistent with experimental data from nonhuman primates, in which testosterone and dihydrotestosterone (DHT) have similar effects on sexually differentiated behaviors among female rhesus monkeys (reviewed in Wallen, 2005). Because DHT is non-aromatizable, Wallen (2005) concluded that aromatization and ER binding are not critical to brain masculinization and defeminization in rhesus monkeys. Likewise, we pointed out that men lacking aromatase report no difference in sexual behavior or orientation. This conclusion was supported by a review of eight studies (Cooke et al., 2017), as L&R acknowledge. The fact that men can have masculine behavior despite the inability to aromatize testosterone to estrogen strikes us as powerful evidence that ER mediation is not critical for masculinizing the human brain. L&R counter that this evidence "is based on genetic males and cannot be extrapolated to females." While some effects of estrogens may differ in a genetically female brain vs. a genetically male brain, this is generally not the case. In rodents, hormones influence sexually dimorphic brain and behavioral traits largely independently of chromosomal sex (De Vries et al., 2002).

L&R use this same argument that evidence in genetic males cannot be extrapolated to genetic females to dismiss evidence from human genetic males (46, XY) with complete androgen insensitivity syndrome (CAIS). In CAIS, testosterone production by the testes is male-typical, but androgen receptors are nonfunctional, and brain and behavior are female-typical. Again, this seems to represent strong evidence that AR rather than ER is critical to produce masculine brain and behavior in humans. Ngun et al. (2011) comment that this represents an "important difference" with rats, in which XY rats with AR mutations behave sexually like wild-type males. They conclude that, while a role for estrogen cannot be completely eliminated, "the implication is that androgens play an important role in masculinizing the human brain." To this, we would add that the scanty available evidence indicates that men lacking a functional ER are male-typical in gender identity and sexual orientation (Smith et al., 1994).

One final point concerns the role of AFP, which prevents estrogen from crossing the blood-brain barrier to masculinize and defeminize the brains of female rodents, as noted above. We pointed out in our review that AFP has low affinity for estrogen in humans (Swartz and Soloff, 1974), so women's high estrogen levels would seemingly masculinize and defeminize their brains if these were ER-mediated. L&R charge that we "disregard that the sex hormone-binding globulin (SHBG) has a similar function to rodent AFP in humans, binding to endogenous estrogens with high affinity." We note here that human SHBG binds testosterone and DHT approximately 5 and 30 times, respectively, as strongly as it binds 17-beta-estradiol (Hong et al., 2015). Moreover, rat AFP binds 17-beta-estradiol over 2.5 times as strongly as human SHBG does. L&R cite this study but omit these important details. According to the other paper cited by L&R on this point (Varshney and Nalvarte, 2017), human SHBG may indeed have a similar function to rodent AFP "*with the exception that it has higher affinity for androgens than [estrogens], and may thus protect the female brain from masculinization by androgens*" (emphasis added). To us, these caveats represent critical omissions.

In sum, human genetic males are unlike rodent males in that neither the ability to convert testosterone to estrogen nor apparently a functional ER is necessary for male-typical gender identity and sexuality, but a functional AR is required. It seems clear that brain masculinization is mainly AR-mediated in human genetic males. This leaves open the possibility that ER binding has important masculinizing or defeminizing effects in human genetic females. Probably the strongest available evidence on this issue is derived from females exposed to synthetic estrogens in utero due to their mother's treatment with DES. As we have reviewed, the totality of evidence from this population indicates little or no effect of estrogens on sexuality in genetic females. In addition, if brain masculinization were ER-mediated in humans, it seems unlikely that SHBG would bind estrogens so effectively as to prevent them from masculinizing the brain. Thus, while we acknowledge that available human data are correlational, we find little reason to question our initial suggestion that estrogen plays a limited role in masculinizing the human brain and behavior.

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