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COMMENTARY

Alternative Views on the Role of Sex Steroid Hormones on the Emergence of Phenotypic Diversity in Female Sexual Orientation

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In their Target Article, Luoto, Krams, and Rantala (2018) used the four questions of Tinbergen to gain a better understanding of the evolution and developmental origin of female sexual orientation and the variety of homosexual phenotypes reported in women. In their endeavor, Luoto et al. propose a compelling model to explain the origin of female homosexuality, or what they carefully call female non-heterosexuality to take into account the greater flexibility that women exhibit regarding the sex of their sexual partner. Most studies on the origin of sexual orientation consider it as a binary variable with exclusive heterosexuals and homosexuals placed at the two extremes of a continuum. This might be true for male sexual orientation which is characterized by a bimodal distribution with the highest frequencies at the two extremes. By contrast, female sexual orientation shows a more spread out distribution of frequencies of intermediate phenotypes and a higher prevalence of bisexuality than in men. Luoto et al. propose a provocative model to explain the emergence of different phenotypes along this continuum in women. The butch phenotype is characterized by masculine morphological and psychological traits and is considered as the manifestation of exclusive homosexuality, whereas the femme phenotype (feminine lesbians) and bisexual women constitute intermediary phenotypes between butches and exclusive heterosexual women. Their model proposes that the different phenotypes represented along this masculinity–femininity continuum arise from different exposures to sex steroid hormones during fetal development: exposure to low levels of estrogens would lead to the most feminine phenotype (i.e., strict heterosexuality), whereas exposure to higher estrogen levels associated with progestins and/or androgens would lead to more masculine profiles, and finally exposure to high levels of androgens would lead to the most masculine phenotype. This model might seem appealing at first sight, but it raises numerous issues and does not seem to be viable when placed in a more refined context. Indeed, we believe that it ignores important aspects of the literature regarding: (1) the different factors at the origin of sex differences, (2) the role of the different sex hormones in these processes, and (3) the well-known species differences regarding the implicated hormones. We thus believe that alternative views should be considered to explain the emergence of these different homosexual phenotypes.

First, evidence coming from animal studies strongly points to two main factors in the sexual differentiation of the brain and behavior: the genetic difference arising from the different chromosomal complements (XX vs XY chromosomes) in each and every cell (Arnold et al., 2016) and the differential exposure to sex steroid hormones during development and in adulthood (McCarthy, 2012). The classical theory of sexual differentiation of the brain and behavior posits that males and females are exposed to different sex steroid hormones during development resulting in the establishment of robust sex differences that are activated by rising levels of circulating sex steroids at puberty. In a manner similar to the differentiation of the internal and external genitalia, this theory holds that male-typical neural and, by extension, psychosexual characteristics are organized primarily by exposure to testicular androgens, including testosterone (T). Depending on the species, this can occur via the direct action of androgens in the brain or following aromatization of androgens into estrogens. By contrast, female-typical neural and psychosexual characteristics develop in the absence of sex hormones, i.e., by default (McCarthy, 2012). The male-typical sexual differentiation of the human brain is thought to proceed during two different perinatal periods when testosterone levels are high in developing boys. A first peak in testosterone occurs during mid-pregnancy, between weeks 12

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and 18, and a second peak takes place in the first 3 months after birth (Nagamani, McDonough, Ellegood, & Mahesh, 1979; Reyes, Boroditsky, Winter, & Faiman, 1974). Thus, in humans, mostly based on clinical evidence from the study of disorders of sex development, heterosexual orientation and other sex-typical behaviors are thought to be programmed during these two periods of high testosterone, whereas the development of gender identity, i.e., the feeling of being a man or a woman, is thought to develop primarily during the first peak of testosterone (reviewed in Swaab, 2007).

The key role of central T aromatization in brain masculinization is supported by a wealth of data from the rodent literature, including, but not limited to, the preoptic area and the regulation of partner preference (Baum, 2006; Ngun, Ghahramani, Sanchez, Bocklandt, & Vilain, 2011). However, regardless of the identity of the regions and responses influenced by central aromatization during early development, this conclusion is limited to rodents as research on sheep and primates has not demonstrated much support for the role of estrogens in brain masculinization and on sexual partner preference in particular (Baum, 2006; Wallen, 2005). In sheep particularly, a sexually dimorphic subregion has been found in the preoptic area (called oSDN-POA for ovine sexually dimorphic nucleus of the preoptic area) that is reduced (i.e., more feminine) in males that prefer to mount other males (Roselli, Larkin, Resko, Stellflug, & Stormshak, 2004). Inhibiting aromatase during gestation failed to affect the volume of this nucleus as well as sexual partner preference (Roselli & Stormshak, 2009), thereby not supporting a role for estrogens in the development of this nucleus and perhaps sexual orientation. Furthermore, in humans, there is at present no evidence that estradiol plays such a role: men with gene mutations in either the estradiol receptor (Smith et al., 1994) or aromatase gene (Jones, Boun, Proietto, & Simpson, 2006) are heterosexual and have a male-typical gender identity. Nevertheless, Luoto et al. (2018) propose that high estrogen exposure during development would lead to a male-typical INAH-3 and, consequently, to the feminine ("femme") homosexual phenotype. This idea is based solely on a review of the rat literature regarding the role of estrogens in the development of the POA, and in particular the sexually dimorphic nucleus (SDN) of the POA (e.g., Dohler et al., 1984). Yet, definitive evidence supporting such a role for early estrogens in the development of the INAH3 is lacking. At present, one study has reported a smaller, female-typical, INAH3 in homosexual compared to heterosexual men (LeVay, 1991), but this result was only partially replicated (Byne et al., 2001). These data are subject to high individual variations since they are obtained in postmortem tissues and it remains difficult to parse out whether the volume of this structure had causally determined the behavior or whether the behavior had affected the size of the structure (Balthazart & Court, 2017). It is of course very tempting to directly link the volume of the human INAH-3, which is thought to be homologous to the rat or ovine SDN, to sexual partner preference, particularly since studies in rats and ferrets have shown that lesioning the SDN (or the equivalent in ferrets, the male nucleus) switched the sexual preference of males from females to males (Paredes & Baum, 1995; Paredes, Tzschentke, & Nakach, 1998). However, it should be noted that these lesions included but were not restricted to the SDN. Finally, at present, no data exist on the INAH-3 in non-heterosexual women. Therefore, we believe that extrapolating observations made in male rodents or ferrets to women is too premature.

However, it is surprising that a role for estrogens in the feminization of the brain and behavior was not considered. Indeed, although the classical theory of sexual differentiation of brain and behavior considers that the female brain develops in the absence of developmental hormonal exposure, recent evidence actually points to an active role for estrogens in brain feminization as well as the existence of different critical, hormone-sensitive, periods for the masculinization versus feminization of the rodent brain (Bakker, Honda, Harada, & Balthazart, 2002; Bakker, Pierman, & Gonzalez-Martinez, 2010; Brock, Baum, & Bakker, 2011; Brock, Douhard, Baum, & Bakker, 2010; Mohr, DonCarlos, & Sisk, 2017; Mohr, Garcia, DonCarlos, & Sisk, 2016). Thus, a male-typical brain is primarily organized prenatally and early postnatally under the influence of testosterone/estradiol, whereas the organization of a female-typical brain results from the combined absence of perinatal androgens and estrogens together with the postnatal influence of estradiol which might perhaps even extend into puberty (Bakker & Brock, 2010). In their defense, Luoto et al. (2018) do mention a potential role of estrogens in brain feminization, i.e., a minimal amount of estrogens is required for the normal development of female sexuality, but this is unfortunately limited to the legend of Fig. 1 showing their pendulum swing model, whereas it clearly warrants further discussion. In our opinion, this omission does not only impede the understanding of the model as a whole but also eludes an important alternative hypothesis to explain the emergence of various female phenotypes of non-heterosexuality. Instead of looking at the glass being half empty, it could be half full. In other words, the different female phenotypes could reflect different degrees in feminization by estrogens rather than different degrees of masculinization. Perhaps the butch phenotype does not result from any over-exposure to androgens prenatally, but from a lack of (or an incomplete) feminization by estrogens after birth or some combination of both. Whether estrogens exert a feminizing role in the development of the human female brain is, however, unknown, although some evidence from girls with Turner syndrome (characterized by the absence of one X-chromosome and non-functional ovaries) is in favor of this hypothesis (e.g., Ross, Roeltgen, Feuillan, Kushner, & Cutler, 1998). Whereas further studies are obviously still required before any conclusion can be drawn about the contribution of estrogens to brain feminization and perhaps sexual

orientation in women, we believe that this possibility deserves serious consideration.

Luoto et al. (2018) also propose a potential role for progestins in the development of non-heterosexual orientation (see Fig. 1 of their Target Article). This is mainly based on one recent study that reported more non-heterosexual contacts in men and women whose mother took a synthetic progestin lutocyclin compared to non-exposed subjects (Reinisch, Mortensen, & Sanders, 2017). However, lutocyclin can have both estrogenic and androgenic effects and by consequence cannot be considered as a specific activator of the progesterone receptor during pregnancy. Moreover, no effect on measures of heterosexual behavior and attraction to females was found. There could be many possible explanations for this phenomenon, but perhaps most importantly, this result was obtained with a very small sample size (17 women and 17 men). It might thus be tempting to propose a role for progesterone, but there is just no strong evidence from the animal literature that progesterone is important during development in establishing sexual behavior, including partner preference, at least in males (reviewed in Wagner, 2006). Indeed, progesterone-receptor (PR) knockout male mice do not show impaired sexual behavior. Similarly, blocking PR signaling during development does not seem to affect male sexual behavior. By contrast, in light of its important role in female reproductive and sexual functioning, a potential role for progesterone in inducing a heterosexual orientation in females could be proposed. The study by Desroziers, Brock, and Bakker (2017) recently showed that blocking PR during prepubertal development actually decreased female sexual behavior in female mice, suggesting a potential role for PR (in addition to estradiol) in brain feminization. However, olfactory mate preferences did not seem to be affected in either sex, but perhaps a more in-depth analysis of the role of progesterone and its receptor in the feminization of the brain using state-of-the-art transgenic mouse models (such as a temporary blocking of PR over development) would lead to new important insights. At the time, it remains, however, too speculative to propose such a role for progesterone in the development of sexual orientation in humans.

Finally, we would like to mention the interplay that seems to exist between neuroimmune and neuroinflammatory processes and the sexual differentiation of the brain (Lenz & Nelson, 2018; Thion, Gihoux, & Garel, 2018). Specifically, the masculinizing actions of estrogens in the preoptic area of rodents involve prostaglandin E2 (i.e., a pro-inflammatory hormone known for its role in fever induction), as well as microglia, the brain-resident macrophage (i.e., immune cells activated in response to injury and inflammation), and mast cells (i.e., another type of immune cells activated in allergic reactions) (Amateau & McCarthy, 2004; Lenz, Nugent, Haliyur, & McCarthy, 2013; Lenz et al., 2018). Although it is currently not known whether similar processes play a role in the sexual differentiation of the human brain, it is interesting to note that several disorders of the central nervous system (e.g., autism spectrum disorders, schizophrenia, attention deficit disorder) that are sexually differentiated have been associated with increased neonatal inflammation supporting the existence of similar crosstalk between the neuro-immune system and brain sexual differentiation in humans (Lenz & Nelson, 2018). In addition, men express higher levels of genes associated with microglia and inflammatory processes in the brain compared to women (Werling, Parikshak, & Geschwind, 2016). Consequently, any factor that would stimulate the neuroimmune system of developing females might interfere with the normal organization of their brain leading to some degree of masculinization (Lenz et al., 2018). In this context, it could be imagined that factors, as benign or harmless as a mild inflammation or an allergic reaction, encountered during key periods of brain development could also contribute to the phenotypic variability of sexual preference in women, but also between sexes.

In conclusion, the question of the biological bases of sexual orientation has been the center of many heated debates and is far from being resolved. Among the many issues associated with its study is the fact that homosexuals (and heterosexuals for that matter) do not seem to constitute a homogeneous population. This is true for both men and women (Balthazart & Court, 2017; Luoto et al., 2018). Understanding how this phenotypic diversity emerges among non-heterosexuals could be important as it might help to clarify how homosexuality (and also heterosexuality) emerges in the first place. The idea that phenotypic variety in sexual partner preference in women may arise from differential hormonal exposure thus constitutes a first step in this direction. However, we argue that the current model is too strongly based on specific findings reported in rodents, which are difficult to extrapolate to humans at this stage. Furthermore, the current model would have benefitted from a more comprehensive analysis of the data from the animal literature. In particular, we would like to propose that the phenotypic diversity observed in lesbians might also result from an incomplete feminization and thus invite the authors to look at this question from a different angle. Finally, we should not forget that genetics and epigenetics are also involved in the sexual differentiation of the brain (Forger, 2018; Ngun & Vilain, 2014). Hormones might thus not explain everything.

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