Testosterone Levels as an Indicator of Sexual Orientation in Women **Monique Gilmore**

Sexual orientation is biologically conferred during the first trimester of pregnancy (O'Hanlan et al., 2018). Sex hormones have been shown to affect the development of male- and female-specific phenotypes (Wang et al., 2019). Specifically, it appears that the human fetal brain develops into either the male or female direction through either direct action of testosterone or the absence of such an action, respectively (Bao and Swaab, 2011).

In line with this, girls with congenital adrenal hyperplasia (CAH), exposure to high testosterone levels in the womb, tend to show male-typical characteristics, even after CAH is treated (Bao and Swaab, 2011). Similarly, an all-sample meta-analysis showed that outside of increased testosterone levels, there is little discernable difference in sex hormones between lesbian, bisexual, and heterosexual women (Harris et al., 2020).

In animal models, treating both pregnant guinea pigs and rhesus macaques with testosterone masculinized the reproductive behavior of their female offspring (Hines, 2020). Figure 1 visualizes this effect of high prenatal levels of testosterone in mice (Balthazart, 2011).

I propose a project to help further elucidate the factors that influence homosexual and bisexual behavior/identity in women. Sexual orientation in women is understudied, and there is especially little research regarding bisexuality. I hypothesize that higher levels of testosterone present prenatally & early postnatally will correlate with homosexual behavior/identity in both animal models and humans, while a level of testosterone equal to the level of estrogen present prenatally & early postnatally will correlate with bisexual behavior/identity in animal models and humans. This hypothesis will be explored through the following specific aims:

1. **Female offspring of female mice with 1) testosterone levels higher than their level of estrogen will exhibit a greater number of attempts to copulate with mice of the same gender, 2) testosterone levels that are the same as their level of estrogen will exhibit an equal number of attempts to copulate with mice of the same and opposite gender, and 3) no increased levels of testosterone will exhibit a greater number of attempts to copulate with mice of the opposite gender.** Pregnant female mice (n = 12) will be separated equally into the three groups listed above. The level of testosterone will be changed on day 11 of gestation. Each group will be housed together, for syncing of the estrous cycle. As offspring are born, the mother and offspring will be housed together separate from the other mothers, until four weeks. Then, the offspring will be tagged with which of the three groups they are in, separated from the mother, and housed together with the offspring of the other groups. It will then be observed the number of times a given female mouse attempts to copulate with another mouse, as well as both the group and gender of both mice. Any offspring will be removed every two weeks. Statistical analysis will be conducted using ANOVA.
2. **Female children of women exhibiting 1) typical testosterone levels prenatally will associate with female-typical behavior, heterosexual identity, and male partners; 2) high levels of testosterone prenatally but a fetus not diagnosed with CAH will associate with male-typical behavior, homosexual identity, and female partners; 3) fetuses diagnosed with CAH will associate with male-typical behavior, homosexual identity, and female partners; and 4) levels of testosterone equal to the levels of estrogen will associate with both female- and male-typical behavior, bisexual identity, and both male and female partners.** In this longitudinal study,100 women pregnant with a female child will be selected around week 18-22 of pregnancy and placed into one of the four groups listed above. As the children develop postnatally, data will be collected on 1) how much the child exhibits male-typical behavior, and 20-25 years postnatally, 2) what sexual orientation the child identifies with given the options of homosexual, heterosexual, bisexual, none, and other, as well as 3) how many and the

gender of the partner(s), sexual and non-sexual, the individual has had. Statistical analysis will be conducted using ANOVA.

3. **Newborn female mice not injected with the drug Testofen will exhibit a greater number of attempts to copulate with male mice, while newborn female mice injected weekly with Testofen will exhibit a greater number of attempts to copulate with female mice, with a greater percentage being other female mice from this same group.** Female mice born with normal levels of testosterone (n = 30) will be separated into the two groups listed above. Both groups will be kept with their mothers for four weeks, during which injections will begin. The mice will then be separated from their mothers and housed together, for syncing of the estrous cycle. After another four weeks, the injections will end, and both groups will then be housed with male mice. It will then be observed how often each group attempts to copulate with female mice compared to male mice, as well as whether the mice copulated with are part of the experimental group, the control group, or the male mice. This observation will continue for eight weeks. Any offspring will be removed every two weeks. Statistical analysis will be conducted using independent t-tests.

References

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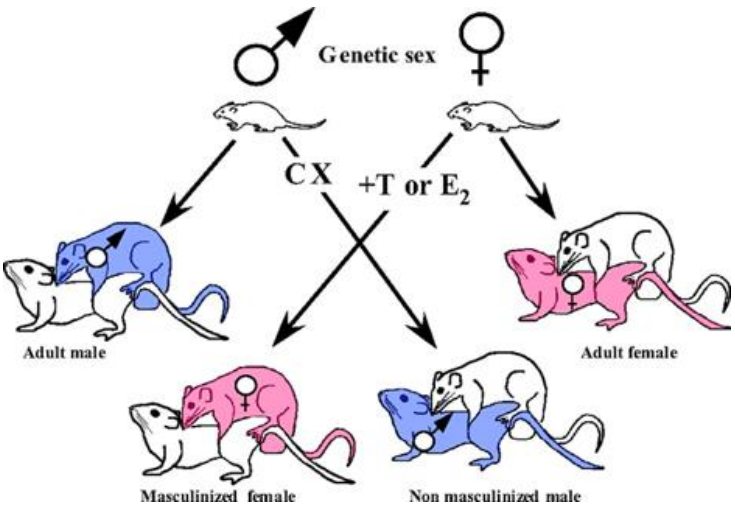
Figure 1: Effect of Early Exposure to Testosterone in Mice (Balthazart, 2011).



Genetic males and females are shaded in blue and pink, respectively. Other subjects represent test stimuli. Increased testosterone (T) appears to lead to male-typical behavior (attraction to females) in genetic females. This indicates that levels of testosterone are able to affect sexual behavior in female mammals, providing the basis for the hypothesis that levels of testosterone may affect sexual orientation and behavior in female humans.