No association between adult sex steroids and hand preference in humans

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Abstract
Background: There is ongoing debate about the effects of hormones on the lateralization of the developing brain. In humans, there are conflicting theories of how testosterone during development should affect lateralization. Empirical studies linking prenatal and postnatal testosterone levels to hand preference (a proxy for lateralization) are similarly mixed. Links between hand preference and health may also suggest a mediating role of steroid hormones such as testosterone and estradiol. Studies to date of adult steroid hormones and handedness have been hindered by samples that contain small numbers of non-right-handers.

Results: In the largest study of the phenomenon to date, I find that the testosterone ($n = 7290$) and estradiol ($n = 3700$) levels of left- and mixed-handed adults are no different to those of right-handers. All Bayesian 95% highest density intervals contained 0.

Discussion and conclusions: The results have implications for studies that show elevated risk of hormonal-related mental and physical disorders in left-handers.

1 | BACKGROUND

There is ongoing debate around the effects of prenatal and postnatal hormones, particularly testosterone (T) on the lateralization of the developing brain (Grimshaw et al., 1995, Lust et al., 2011; Hollier et al., 2014; Beking et al., 2018; Mercure et al., 2009; Pfannkuche et al., 2009). Because atypical lateralization is often present in left-handers (Papadatou-Pastou, 2011), some have proposed that prenatal T exposure could influence handedness, though theories disagree on the predicted direction of the effect (see Papadatou-Pastou et al., 2017 and Richards et al., 2021 for reviews).

The few studies conducted on the links between prenatal testosterone, handedness and laterality show mixed results (Grimshaw et al., 1995; Lust et al., 2011; Richards et al., 2021; Tan & Tan, 2001). As prenatal T is difficult to measure, some have used the ratio of the lengths of the 2nd and 4th digits of the hand (2D4D) as a proxy of prenatal testosterone exposure. However, a recent, large meta-analysis found small, inconsistent effects between 2D4D and handedness (Richards et al., 2021). Another program of research has used levels of salivary or serum T in children and adults as a proxy. These studies are similarly inconsistent. Some studies find that left-handers have lower testosterone than right- or mixed-handers (Moffat & Hampson, 1996a, $n = 80$; Moffat & Hampson, 2000, $n = 180$; Hampson & Sankar, 2012, $n = 180$), while others find no effect (Vuoksimaa et al., 2010, $n = 771$; Beaton et al., 2011, $n = 40$; Papadatou-Pastou et al., 2017,
terone levels (Faurie et al., 2011, n = 64) and some are inconclusive (Tan, 1990, total n = 120). One study found that left-handedness was associated with increased testosterone levels (Faurie et al., 2011, n = 64). While there have been many studies, the questions of whether and how testosterone is associated with handedness are unanswered.

One complicating factor of previous studies is that there is not good evidence that postnatal testosterone is a robust indicator of prenatal testosterone. Prenatal testosterone differences may persist into adulthood, but it is unclear whether this relationship is strong enough to justify using adult T as a proxy for prenatal T. About half of the variance in T levels is attributable to genetic effects (Caramaschi et al., 2012; Hoekstra et al., 2006), so between-individual variation may be somewhat stable. On the other hand, Beking et al. (2018) did not find significant correlations between prenatal and pubertal T in boys or girls.

Some studies have also proposed that estrogens such as estradiol may be related to handedness. There is some evidence that prenatal exposure to synthetic estrogens is associated with left-handedness (Scheirs & Vingerhoets, 1995; Smith & Hines, 2000; Titus-Ernstoff et al., 2003). However, Richards et al. (2021) found no clear links to between prenatal estradiol and handedness. Studies of the link between adult estradiol levels and handedness are slim. Some studies show that adult E is negatively linked to right hand preference (Negrev et al., 2001; Tan, 1992), but other studies find no relationship (Hodgetts et al., 2015; Vuoksimaa et al., 2010; Wisniewski et al., 2005).

Studying links between handedness and adult hormone levels may be interesting regardless of whether adult hormones are effective proxies for prenatal ones. Non-right handedness has long been linked to various physical and mental health issues (Bryden et al., 2005; but see Bryden et al., 1994; Geschwind & Behan, 1982, 1984), and postnatal steroid hormones may play a mediating role. For example, studies have shown that left-handed women are more likely to develop breast cancer than right-handed women (Fritsch et al., 2007). Higher estradiol levels in left-handers might provide a mechanism to explain this association, as high estradiol has been linked to the development of breast cancer (Bernstein, 2002). In addition, a meta-analysis revealed higher incidence of non-right-handedness in schizophrenic individuals (Sommer et al., 2001), who may also have lower testosterone and estrogen levels (Akhondzadeh et al., 2006; Markham, 2012; Moore et al., 2013). Testosterone suppresses many components of the immune system (Trigunaite et al., 2015). If non-right handers have higher testosterone, this may help explain their higher incidence of some physical illnesses.

Furthermore, recent studies have found that left- and mixed-handers report being more aggressive and engaging in more fights than right-handers (van der Feen et al., 2020; Zickert et al., 2018). The 'Fighting Hypothesis' of left-handedness proposes that left-handedness has been maintained in humans by natural selection due to left-handers having an advantage in ritual fighting and war (Raymond et al., 1996; Richardson & Gilman, 2019). Left-handers' increased fighting success is thought to be due to their rarity giving them a negative frequency dependent advantage. However, few studies have examined this directly, leaving open the possibility of other explanations. Testosterone has long been linked with aggression (though correlations are often smaller than assumed, see Geniole et al., 2020), raising the question of whether adult levels of testosterone are responsible for increased aggression among left- and mixed-handers. Overall, understanding how adult hormone levels are related to handedness may shed light on diverse phenomena, and was the purpose of this study.

Small sample sizes may have resulted in the failure of many previous studies to find a relationship between steroid hormones and handedness. This limits their statistical power unless effects are large. One exception is Vuoksimaa et al. (2010) (n = 771), though as their sample consisted of twin pairs (whose data are not independent of each other) their effective sample size may be far lower than the participant number suggests. This is further compounded by the fact that unless researchers actively recruit non-right-handers, they might expect as little as 10% of their sample to be non-right-handed (Papadatou-Pastou et al., 2020). This imbalance of group sizes reduces statistical power further. For example, while Hoppler et al. (2018) included data from 256 men, only 36 of these men were not right-handed (22 left-handers, 14 mixed-handers), so their power to detect group differences may be low. The small sample size of previous studies is due in part to the use of time-consuming and expensive (though rigorous) brain imaging (Papadatou-Pastou et al., 2017) as well as detailed and/or behavioral measures of handedness and laterality, such as the peg moving task (Beaton et al., 2011). The literature reviewed above suggests that research testing associations between hand preference and serum testosterone should be high powered to be maximally informative.

The present study aims to complement previous work with a high-powered test of any links between hand preference and adult testosterone and estradiol levels in a representative western sample with a relatively large number of non-right handers.
2 | METHODS

2.1 | Sample

This study used data from participants examined as part of 2 waves of the U.S. National Health And Nutrition Examination Survey (NHANES) in 2011–2012 and 2013–2014. Participants were included if handedness and testosterone data were available and they were aged 18 and over. Women were only included if they reported having a menstrual period in the last 12 months. This excludes post-menopausal women and women who have had hysterectomies.

Overall, the sample included 7290 participants, 2451 females (mean age = 42.54, SD = 17.48 years). The sample contained 6441 right-handers (88.4%), 588 left-handers (8.1%), and 261 mixed-handers (3.6%). Estradiol was only available for participants who were examined in 2013 (n = 3700, 1217 women), 3293 right-handers (89%), 289 left-handers (8%), and 118 mixed-handers (3%).

2.2 | Measures

Handedness was measured with a single item question that had three options: “Are you 1) Right-handed, 2) Left-handed, or 3) Do you use both hands equally?”. This is a measure of hand preference direction and does not include data on strength of preference, except in so far as someone who uses both equally has a preference strength of roughly 0. It also does not tell us about relative hand skill, which is also the subject of much research in laterality (e.g., Crow et al., 1998).

Serum testosterone (T) and estradiol (E) were measured using isotope dilution liquid chromatography tandem mass spectrometry in nanograms per deciliter (ng/dl) and picograms per milliliter (pg/ml), respectively. Full details of reliability, validation, and quality control are given in the Centre for Disease Control and Prevention’s laboratory manual (CDC, 2014).

2.3 | Analysis

R code that downloads the data from the CDC website, then cleans and analyzes it, is available on the Open Science Framework (t.ly/pg6V). The distributions of both testosterone and estradiol were strongly non-normal. The data were transformed to bring both skew and kurtosis as close to 0 as possible. The distributions of male T and E were both best corrected by a square root transform. The female distributions were best made normal by a natural log transform. As such, each sex was analyzed separately, and model outputs are not easily comparable. All further references to T and E refer to their transformed values. T and E were also both standardized for all analyses, so effects of predictors are in standard deviation units.

I fitted several Bayesian linear models, with T or E as outcome variables and handedness as a predictor, using the rstanarm package in R (Goodrich et al., 2020). Handedness was dummy-coded as two binary variables comparing (a) the effect of being left-handed versus right-handed and (b) the effect of being mixed-handed versus right-handed. One exception is when analyzing female estradiol levels, where data on mixed-handers was insufficient (n = 16). In this case, left- and mixed-handers were combined into a single non-right-handers group (n = 103).

Participant age, the year the examination took place (2011 or 2013), and when the hormone measurement was taken (three levels: morning, afternoon and evening) were all included as control variables. Time of day was included to account for diurnal variation in hormone levels (see Bremner et al., 1983). However, as T levels may fall dramatically even over the course of the morning (Brambilla et al., 2009), I also re-ran all testosterone models excluding participants who were tested in the morning. Year of examination was not included for the E analysis because data were available for only 1 year. Results of these covariates are not reported here as their effects on steroid hormones are well established and discussed in other studies and are not the focus of the present study. For the interested reader, they are given in Appendix S1, or can be obtained by running the provided R code.

All predictors were modeled with priors defined by Cauchy distributions with a width of 2.5 (the default used by rstanarm). A Cauchy distribution was used as its ‘heavy tails’ make it relatively robust to outliers, which are common in hormonal data (Pollet & van der Meij, 2017). A width of 2.5 represents no strong expectations about the direction and size of the effect, often referred to as ‘weakly informative priors’ (Lemoine, 2019). The large amount of data is highly likely to overwhelm all but the strongest priors (Kruschke, 2013). Indeed, reducing the prior distribution width by two orders of magnitude to 0.025 (reflecting massively increased expectation that the effect is near 0) did not change results noticeably. I report the median of the posterior distribution (similar in practice to a regression beta coefficient) and 95% highest density intervals (HDI), which represent the interval that we can be 95% certain contains the population coefficient, given the data.

3 | RESULTS

Figure 1 shows violin plots of transformed hormone values for different levels of handedness. Separate plots are given for men and women and for both T and E. In men, neither left-handers (posterior median = 0.07, 95%
HDI = -0.03 to 0.17), nor mixed-handers (median = 0.07, 95% HDI = -0.07 to 0.20) showed T levels significantly different from right-handers as all credibility intervals contained 0. Similarly, in women, neither left-handers (median = 0.01, 95% HDI = -0.13 to 0.17) nor mixed-handers (median = -0.01, 95% HDI = -0.26 to 0.32) showed T levels significantly different from right-handers. Excluding those whose testosterone was measured in the morning (2350 men and 1170 women excluded), did not change the results qualitatively (95% HDIs still contained 0).

In men, neither left-handers (posterior median = 0.002, 95% HDI = -0.13 to 0.15) nor mixed-handers (median = 0.09, 95% HDI = -0.11 to 0.28) showed E levels significantly different from right-handers. Similarly, in women, non-right-handers did not show E levels significantly different from right-handers (median = -0.01, 95% HDI = -0.22 to 0.18).

**FIGURE 1** Residual, transformed hormone levels for individuals with different handedness, where age of participant, time of testing session and year of sampling have been controlled. The left and right panels correspond to data for men and women, respectively. The top and bottom panels correspond to testosterone and estradiol, respectively. Note that hormone levels have been transformed differently for males and females and as such cannot be directly compared without back-transformation.
4 | DISCUSSION

Previous research on the link between hormones and handedness has produced mixed results. Here, I find evidence that neither adult serum testosterone nor estradiol are related to hand preference direction. In all cases, for both men and women, Bayesian 95% highest density intervals contained 0, and posterior medians indicated that any effects are likely very small if they exist at all. Some 95% HDIs were still moderately wide, (such as when testing the difference between mixed- and right-handers) which underscores the need for very large samples to obtain reliable effect estimates. Nonetheless, I failed to find associations despite a representative sample larger than all previous studies on the topic.

The findings here that adult testosterone and estradiol are unrelated to handedness indicate that hormones may not mediate links between handedness and health (e.g., Bryden et al., 2005; Fritschi et al., 2007), at least not in a linear way. In addition, the present findings suggest that the mechanism by which handedness is linked to aggression is unlikely to be through a common cause of static adult testosterone levels (dynamic measures of T may be more informative, see Geniole et al., 2020). Further research is needed to understand how and why non-right-handedness is related to illness and aggression.

The present study has limitations. First, as estradiol changes over the menstrual cycle (and to a lesser extent, so does testosterone), not controlling for cycle phase will have introduced random noise to the data. There may also be systematic noise if participants in certain cycle phases are less likely to consent to a testing session, though this is untested. The study used a simplistic measure of handedness. Participants self-reported only the direction of their hand preference. While this is not the only study to have used such a measure (e.g., Hoppler et al., 2018; Vuoksima et al., 2010) it does not capture the full diversity of handedness phenotypes present in the population, as well as individual differences in strength of handedness. It is not clear to what extent participants interpreted “use both hands equally” to refer to using their left hand for some tasks and their right hand for others, or whether it was asking if they use both hands equally within each given task. Participants may have interpreted this question only to refer to their writing hand. Older, left-handed participants may have been forced to write with their right hand, which would reduce observed group differences in hormones if any exist. Self-report measures may not be highly consistent with behavioral measures (Bryden et al., 2000; Papadatou-Pastou et al., 2013). The large sample employed here likely makes up for power lost due to random measurement error, but it is possible that systematic error might affect results. It would be difficult to get behavioral measures from as many participants as the present study but would nonetheless be ideal. Larger neuroimaging studies would also be valuable as they are more direct evidence of cerebral lateralization.

Here I demonstrate that neither adult testosterone nor estradiol are related to handedness in a large representative sample. This suggests that the link between handedness and health may not be mediated by serum steroid hormones in any simple way. Future studies of handedness and hormones should use direct measures of prenatal hormones, as well as more detailed measures of handedness/laterality.

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DATA AVAILABILITY
The data used in this study are publicly available on the United States Center for Disease Control (CDC) website: https://wwwn.cdc.gov/nchs/nhanes/Default.aspx. Alternatively running the R-script provided from the author will download the data to your computer (t.ly/pg6V).

ETHICS STATEMENT
The NHANES observes high ethical standards in data collection, including obtaining informed consent, anonymization of data and ensuring the privacy of participants is protected. Details can be found on the CDC website, particularly here: https://www.cdc.gov/nchs/nhanes/participant/participant-confidentiality.htm.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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