Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Sex Steroids and the Organization of the Human Brain

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Review of Lombardo et al.

Studying the biological mechanisms underlying sexual differentiation in the human brain provides important insights into the etiology and trajectory of neurodevelopmental disorders in males and females (Baron-Cohen et al., 2011). Sex steroid hormones, the end products of the hypothalamus-pituitary-gonadal axis, exert powerful effects on the organization and sexual differentiation of brain structures. From animal studies, it has become clear that during early development, exposure of the brain to testosterone and estradiol leads to irreversible changes in the nervous system (McCarthy et al., 2012). Moreover, fetal exposure to sex steroids has a major impact on the sexual differentiation of the brain (McCarthy et al., 2012). For example, high levels of fetal testosterone (FT) result in brain masculinization in experimental animals, such as enlargements of the volume and soma size of the suprachiasmatic nucleus, bed nucleus of the stria terminalis, and ventromedial hypothalamus (Zuloaga et al., 2008).

In humans, studies of the effects of FT often rely on indirect measures such as the ratio between the index finger (2D) and ring finger (4D) or on opposite-sex twin studies. Specifically, a smaller 2D:4D ratio correlates with higher FT exposure, and through the intrauterine presence of a male fetus, opposite-sex twin girls are exposed to higher FT levels than same-sex twin girls. Using the latter indirect measure of FT, earlier reports showed that total brain volume and cerebellum volume, typically found to be larger in males, were positively correlated with higher FT exposure (Peper et al., 2009).

A recent paper by Lombardo et al. (2012) provided direct evidence for an association between FT levels and sexual differentiation of brain gray matter in humans. In this pioneering study of 28 developing boys, FT levels were determined from amniotic fluid. Amniocentesis was performed between 13 and 20 weeks of gestation, which is a critical period of brain masculinization. When these boys were 8–11 years old, a structural MRI was made. Using voxel-based morphometry, gray matter regions within the whole brain of these 28 boys were identified showing significant correlations with FT levels. The amygdala and hypothalamus were included as a priori regions of interest. Then, in a second normative sample of 217 (101 boys) children (NIH Pediatric MRI Data Repository), sexual dimorphisms in gray matter were determined. Finally, a conjunction analysis was performed to isolate brain regions whose direction of the FT correlation was congruent with the direction of sexual dimorphism.

Lombardo et al. (2012) hypothesized that the size of brain areas that were normally larger in males than in females would correlate positively with FT, whereas the size of brain areas that are normally larger in females would correlate negatively with FT. Results showed that higher levels of FT were associated with larger right temporal/parietal junction and posterior superior temporal sulcus. As predicted, these brain areas were larger in males than in females in the normative sample. Conversely, FT level was negatively correlated with gray matter volumes within the planum temporale/parietal operculum and within the posterior lateral orbitofrontal cortex. Again, in line with the hypothesis, these brain areas were larger in females than males in the normative sample.

Having unique access to direct levels of FT, Lombardo and colleagues (2012) provide the first human evidence that FT contributes to the organization of gray matter structures in a sexually dimorphic way. The sexually dimorphic brain areas that Lombardo and colleagues (2012) found to be associated with FT could provide insight into sex differences found in cognitive and affective functioning, including language processing, mentalizing, social attention, and empathy. In summary, sex differences in these mental functions might result at least partially from FT effects on the underlying brain structures.

FT levels did not correlate with volume in all sexual dimorphic gray matter areas in Lombardo et al. (2012), however. For example, the hypothalamus and subregions of the amygdala were normally larger in males, but these volumes were not correlated with FT levels. This is not wholly surprising, because numerous factors likely contribute to sex differences in

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brain morphology, such as estrogens, proteins encoded on sex chromosomes, and environmental factors (McCarthy et al., 2012).

In contrast, FT levels did correlate with gray matter volume in a subregion of the amygdala (i.e., the ventromedial area of the amygdala) that is not sexually dimorphic. Interestingly, it has recently been proposed that some phenotypic endpoints that do not show sex differences nonetheless can be affected by different factors in males and females (McCarthy et al., 2012). Therefore, FT could affect the size of the ventromedial amygdala in males, but its size in females is affected by other factors. To test this hypothesis however, the inclusion of female (hormonal) data is required.

Influences of sex steroid hormones on fetal brain development such as those identified by Lombardo et al. (2012) are thought to set the stage for additional effects of such hormones that occur during puberty and adolescence. Animal literature increasingly suggests that puberty represents a second critical period during which sex-steroid-related brain reorganization takes place. For example, in male rodents, testosterone treatment before and during adolescence, but not after adolescence, caused reorganization in parts of the amygdala and hypothalamus—brain areas involved in social behavior (Schulz et al., 2009). These data indicate that the adolescent brain remains sensitive to the organizational effects of steroid hormones. In humans, sex differences in subcortical and cortical gray matter become more prominent during puberty, but the contribution of sex hormones to this process seems to be sex- and region-specific (Peper et al., 2011). Animal studies have also demonstrated that sex steroids affect myelination by acting on glial cells (Garcia-Segura and Melcangi, 2006). In humans, white matter sexual dimorphisms also become more prominent during puberty and adolescence: in boys, white matter microstructure increases more steeply than in girls (Bava et al., 2011), possibly under the influence of pubertal hormones. Recent human evidence indicates that the pubertal reorganization of white matter pathways is associated with increased levels of pubertal sex steroid hormones (Herting et al., 2012). Lombardo and colleagues (2012) point out that some of their participants (8–11 years) might have already entered puberty. If so, pubertal testosterone might have interacted with gray matter sex differences established during the prenatal period. The authors argue that by correcting their analyses for age, possible current testosterone effects on gray matter should have been controlled (as age and testosterone are highly correlated during puberty). However, after controlling for age, pubertal testosterone has been associated with individual differences in structural brain development (Peper et al., 2011). For example, a larger amygdala and hippocampus volume are related to increased levels of testosterone in both sexes regardless of age (Neufang et al., 2009).

The importance of studying the effects of pubertal sex steroids on human brain structure is further underlined by the fact that there is a sexual differentiation in vulnerability to mental disorders before and around puberty (Zahn-Waxler et al., 2008). Sex differences in child and adolescent mental disorders can be roughly divided into two groups: (1) disorders with a marked male preponderance arising before puberty, such as conduct disorder, autism, and attention deficit-hyperactivity disorder; and (2) disorders with a marked female preponderance (2:1) arising during puberty, such as mood and anxiety-related disorders (Zahn-Waxler et al., 2008). Eating disorders show an even higher incidence in females and often arise in the course of puberty. Moreover, an earlier onset of pubertal development is associated with increases in eating and mood symptoms. It might be argued that during puberty, previously organized brain circuits are activated by changing gonadal hormone environments, possibly setting the stage for sex differences in vulnerability to these mental disorders. Although sex steroids are not the single cause of these complex disorders, puberty might have a profound impact on the developmental trajectories of these neuropsychiatric illnesses (Zahn-Waxler et al., 2008).

In conclusion, studying the influence of sex steroids on human brain structure not only gives important insights into the etiology of healthy brain maturation, but can also serve as a model for the development of neuropsychiatric illnesses with a skewed sex ratio. As Lombardo and colleagues (2012) emphasize, FT is an important developmental mechanism contributing to sexual differentiation of brain anatomy. Later surges of sex steroid hormones during puberty might play a vital role in further refining gray and white matter observed during this period.

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