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A testosterone-related structural brain phenotype predicts aggressive behavior from childhood to adulthood

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ABSTRACT

Structural covariance, the examination of anatomic correlations between brain regions, has emerged recently as a valid and useful measure of developmental brain changes. Yet the exact biological processes leading to changes in covariance, and the relation between such covariance and behavior, remain largely unexplored. The steroid hormone testosterone represents a compelling mechanism through which this structural covariance may be developmentally regulated in humans. Although steroid hormone receptors can be found throughout the central nervous system, the amygdala represents a key target for testosterone-specific effects, given its high density of androgen receptors. In addition, testosterone has been found to impact cortical thickness (CTh) across the whole brain, suggesting that it may also regulate the structural relationship, or covariance, between the amygdala and CTh. Here, we examined testosterone-related covariance between amygdala volumes and whole-brain CTh, as well as its relationship to aggression levels, in a longitudinal sample of children, adolescents, and young adults 6–22 years old. We found: (1) testosterone-specific modulation of the covariance between the amygdala and medial prefrontal cortex (mPFC); (2) a significant relationship between amygdala-mPFC covariance and levels of aggression; and (3) mediation effects of amygdala-mPFC covariance on the relationship between testosterone and aggression. These effects were independent of sex, age, pubertal stage, estradiol levels and anxious-depressed symptoms. These findings are consistent with prior evidence that testosterone targets the neural circuits regulating affect and impulse regulation, and show, for the first time in humans, how androgen-dependent organizational effects may regulate a very specific, aggression-related structural brain phenotype from childhood to young adulthood.

1. Introduction

Structural covariance, the examination of anatomic correlations between brain regions, has emerged recently as a useful addition to existing modalities investigating brain function and structure (Alexander-Bloch et al., 2013). Maturational coupling has been demonstrated in both structural covariance and functional connectivity networks (Raznahan et al., 2011), reflecting progressive network integration between 5 and 18 years old (Zielinski et al., 2010). Such findings have significantly contributed toward support for the use of structural covariance as a valid measure of developmental brain changes. Yet the exact biological processes leading to changes in covariance, and the relation between such covariance and behavior, remain largely unexplored.

The steroid hormone testosterone represents a compelling mechanism through which this anatomical covariance may be developmentally regulated in humans (Nguyen et al., 2013a). Indeed, brain masculinization of limbic brain regions primarily

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relies on a perinatal testosterone surge in rodents (Zuloaga et al., 2008). In addition, testosterone can act on both androgen and estrogen receptors through its conversion to other steroid hormones (Zuloaga et al., 2008), potentially carrying out widespread effects in the CNS.

Many limbic structures have been shown to be exquisitely sensitive to the effects of testosterone, but among them, the amygdala shows the most significant reorganization through androgen receptor-dependent processes during the pubertal transition (De Lorme et al., 2012; Johnson et al., 2013). More recent evidence shows that the cortex also displays a high density of androgen receptors (Goldstein et al., 2002), and there have been several reports of testosterone-related differences in cortical maturation in children, adolescents and young adults (Herting et al., 2014; Nguyen et al., 2013a). Taken together, these findings suggest that testosterone may regulate the organization and function of both the amygdala and the cortex.

In particular, both exogenously administered and endogenous testosterone levels have been associated with a decrease in functional connectivity between the amygdala and medial prefrontal cortex (mPFC), effectively uncoupling the amygdala from the regulatory influences of the mPFC (Bos et al., 2012; Goetz et al., 2014; Stanton et al., 2009; van Wingen et al., 2009; Volman et al., 2011). Testosterone-related changes in amygdala-mPFC functional connectivity were, in turn, shown to correlate with levels of aggression, risk-taking and reactivity to threat, establishing a direct link to behavioral changes (Peters et al., 2015; Spielberg et al., 2015). Interestingly, our group also found a negative structural covariance between the amygdala and mPFC (Albaugh et al., 2013). This anatomical relationship may well represent the structural foundation underlying the functional connectivity between the amygdala and mPFC.

Herein, we aim to examine whether testosterone levels during childhood, adolescence and young adulthood is associated with structural covariance between amygdala volume and cortical thickness (CTh) in a longitudinal sample of 216 developmentally healthy subjects 6–22 years old. In addition, we aim to examine whether structural covariance correlates with aggression levels in the same sample. A conservative, whole-brain approach was used, hypothesizing that the volume of the amygdala will be most significantly associated with CTh of the mPFC, and that, in turn, this covariance will be associated with differences in aggression levels.

2. Material and methods

2.1. Sampling and recruitment

The National Institutes of Health (NIH) MRI Study of Normal Brain Development is a multi-site project that aimed to provide a normative database to characterize healthy brain maturation. Subjects were recruited across the United States with a population-based sampling method seeking to achieve a representative sample in terms of income level, race and ethnicity (Evans, 2006). All experiments on human subjects were conducted in accordance with the Declaration of Helsinki. All procedures were carried out with the adequate understanding and written parental consent, as well as assent of the subjects (or consent, if ≥18 years old). Subjects underwent repeated magnetic resonance brain imaging (MRI) every 2 years, with a maximum of 3 scans over 4 years. The sample was limited to developmentally healthy children with rigorous exclusion criteria, described in detail elsewhere (Evans, 2006). After strict quality control of MRI data (see Section 2.3) and the exclusion of scans without paired hormonal measurements or behavioral parameters, 216 subjects were used for hormonal-related analyses and 207 subjects for behavioral analyses (see Tables 1 and 2).

2.2. MRI protocol

A three-dimensional T1-weighted (T1W) Spoiled Gradient Recalled (SPGR) echo sequence from 1.5 Tesla scanners was obtained on each participant, with 1 mm isotropic data acquired sagittally from the entire head for most scanners. In addition, T2-weighted (T2W) and proton density-weighted (PDW) images were acquired using a two-dimensional (2D) multi-slice (2 mm) dual echo fast spin echo (FSE) sequence.

2.3. Cortical thickness: image processing

All quality-controlled MR images were processed through the CIVET pipeline (version 1.1.9) developed at the MNI for fully automated structural image analysis. The pipeline processing steps have been described at length in other publications (Nguyen et al., 2013a,b). In addition to the CIVET pipeline, a multistage quality control (QC) process was implemented, as described previously (Nguyen et al., 2013a,b), excluding subjects with white or gray matter artifacts.

2.4. Amygdala volume: image processing

Volumetric measures were obtained using a validated, fully automatic segmentation method for the amygdala in human subjects from MRI data (Collins and Pruessner, 2010). The method utilizes a large, manually labeled MRI dataset ($n = 80$) of young healthy adults that serves as a template library (Pruessner et al., 2001). The manual segmentation was done by four different raters, and intra-class intra-rater and inter-rater reliability varied between $r = 0.83$ for the right and $r = 0.95$ for the left amygdala (Pruessner et al., 2000). The segmentation method is characterized by label fusion techniques that combine segmentations from a subset of 'n' most similar templates. Specifically, each template is used to produce an independent segmentation of the subject using the ANIMAL procedure (Collins and Evans, 1997) followed by a thresholding step to eliminate CSF, which results in 'n' different segmentations. To fuse the segmentations at each voxel, a voting strategy is used; the label with the most votes from the 'n' templates is assigned to the voxel. The rationale for combining multiple segmentations is to minimize errors and maximize consistency between segmentations. When using $n = 11$ templates, the label fusion technique has been shown to yield an optimal median Dice Kappa of 0.826 and Jaccard similarity of 0.703 for the amygdala (Collins and Pruessner, 2010).

2.5. Hormonal measurement

During each MRI visit, children provided two separate 1–3 cm^3 samples of saliva, collected on the day of the scan, which were assayed by enzyme-linked immunosorbent assay (ELISA) methods, and the average results used as a measure of testosterone levels. The intra-assay coefficient was 6.1%, and the inter-assay coefficient was 13.5% for testosterone. Intra-assay coefficients of less than 10% and inter-assay coefficients of less than 15% are considered acceptable by the manufacturer (Salimetrics Salivary DHEA, ELISA, State College, PA; Salimetrics Salivary Testosterone ELISA Kit, State College, PA). At the next MRI, a similar procedure was followed and the child again provided two separate saliva samples for hormonal measurement. Each child was scheduled for three repeat MRI scans separated by two year-intervals. Each scan was paired with the hormonal sample collected at the time of the scan, thereby covering all relevant developmental ages. All steroid hormones easily cross the blood brain barrier when not bound to proteins such as the sex hormone-binding globulin or alpha-fetoprotein, and salivary sampling measures the free, biologically active portions of