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Associations between adrenarcheal hormones, amygdala functional connectivity and anxiety symptoms in children

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ABSTRACT

Objective: The transition from childhood to adolescence is a vulnerable period for the development of anxiety symptoms. There is some evidence that hormonal changes occurring during adrenarche, an early pubertal phase, might play a role in this increased vulnerability. Little is known about underlying brain mechanisms. Given the role of the amygdala-based fear circuit in anxiety, the current study aimed to investigate whether children's adrenarcheal hormone levels were associated with functional connectivity of the amygdala while processing fearful facial expressions, and how this in turn related to anxiety symptoms.

Method: Participants were 83 children (*M* age 9.53 years) who completed two morning saliva collections to measure levels of dehydroepiandrosterone (DHEA), its sulphate (DHEAS), and testosterone. They also completed the Spence Children's Anxiety Scale (SCAS), and viewed fearful and calm facial expressions while undergoing a functional MRI scan. Psychophysiological interaction (PPI) analyses were performed to examine amygdala connectivity and significant clusters were fed into a bootstrapping mediation model.

Results: In boys, mediation analyses showed an indirect positive effect of testosterone on anxiety symptoms, which was mediated by amygdala-secondary visual cortex connectivity as well as amygdala-anterior cingulate connectivity. In girls, DHEAS showed a negative indirect association with anxiety symptoms mediated by amygdala connectivity to the fusiform face area and insula.

Conclusion: The results indicate unique roles for adrenarcheal hormones in anxiety and suggest that amygdala connectivity may represent an important neural mechanism in these associations. Importantly, results reveal prominent sex differences in the biological mechanisms associated with anxiety in children undergoing adrenarche.

1. Introduction

Anxiety disorders are common in childhood and adolescence (Costello et al., 2003). They have an early median age of onset compared to other forms of psychopathology, such as depression (Kessler et al., 2005), and often persist through adolescence (Costello et al., 2003). Further, elevated anxiety symptoms during childhood are associated with increased risk for later anxiety and depressive disorders (Keenan et al., 2009). The transition from childhood to adolescence seems to be a particularly vulnerable period for the development of anxiety symptoms and disorders. There are suggestions that hormonal changes play a role in this increased vulnerability (Reardon et al., 2009). These hormonal changes start in mid to late childhood with adrenarche, an early pubertal phase that takes place prior to gonadarche. Adrenarche is marked by the activation of the hypothalamo–pituitary–adrenal (HPA) axis that leads to increases of circulating androgens, including dehydroepiandrosterone (DHEA), dehydroepiandrosterone-sulfate (DHEAS), and testosterone (Styne and Grumbach, 2011).

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Children with 'premature' adrenarche (a clinical condition marked by high adrenal hormone levels) show more anxiety problems than their later developing peers (Dorn et al., 2008; Sontag-Padilla et al., 2012). When examining the associations between levels of adrenarcheal hormones and anxiety in non-clinical samples, the findings are somewhat mixed. For DHEA, a majority of studies have found a positive link with anxiety or internalizing symptoms in general, although some showed this result only in boys or only in girls (reviewed in (Byrne et al., 2017)). Studies measuring testosterone levels have inconsistent results, with positive (Mundy et al., 2015; Susman et al., 1991), negative (Granger et al., 2003) and no (Susman et al., 1991, 1987) associations with anxiety (or more broadly internalizing) symptoms being observed. A reason that the findings are more mixed for testosterone could be that this is both an adrenal and a gonadal hormone and study samples often include participants that have entered gonadarche. Where gonadarche has commenced, gonadal testosterone might be dominating, especially in boys, and the levels and source might have a different effect on anxiety symptoms compared to adrenal testosterone in children.

Thus, one explanation of inconsistencies in the literature might be the age at data collection in a rapidly changing hormonal transition period. Further explanation might also come from a deeper understanding of the underlying brain functional mechanisms, especially in relation to the processing of affective stimuli particularly relevant for anxiety. Indeed, we have previously found that affective brain function statistically mediates, and thus potentially explains, the link between adrenarcheal hormones and mental health problems in children (Whittle et al., 2015). One of the main networks in the brain that is thought to underlie fear processing and anxious behaviour ("the fear circuit") includes limbic regions, such as the amygdala and insula, and medial frontal regions, such as the anterior cingulate and medial prefrontal cortex (Etkin, 2010). The amygdala is a key brain region in this network; it plays a crucial role in the processing of fear, including fearful faces (Fusar-Poli et al., 2009), and increased activation of this area has been linked to anxiety disorders and symptoms (Brühl et al., 2014; Etkin and Wager, 2007; Stein et al., 2007). Alterations in amygdala connectivity with medial frontal areas as well as with more basic emotion processing areas, such as other limbic regions and the visual cortex, have repeatedly been found in adults and children with anxiety disorders and symptoms (Brühl et al., 2014; Kim et al., 2011; Roy et al., 2013). Although amygdala hyperactivation is a common characteristic of anxiety disorders, a clear pattern of altered amygdala *connectivity* hasn't been established yet, and could be different for different types of anxiety (Etkin, 2010).

Importantly, this network is likely to be influenced by adrenarcheal hormones. The amygdala and other regions implicated in emotional processing contain many androgen receptors (Sar et al., 1990; Simerly et al., 1990), which bind all of the adrenarcheal hormones. In addition, these areas and their connections are influenced by adrenarcheal hormones in adults (Sripada et al., 2014, 2013; van Wingen et al., 2010). However, the associations between adrenarcheal hormones and functional connectivity in the "fear circuit" has never been studied in children (to our knowledge).

The purpose of this study was to examine the relationship between adrenarcheal hormone levels and amygdala functional connectivity while processing fearful facial expressions, and investigate how this in turn is related to anxiety symptoms in a community sample of children. While we have previously published work from this sample linking individual differences in adrenarcheal hormones and brain function (Whittle et al., 2015), white matter structure (Klauser et al., 2015) and gray matter structure (Murray et al., 2016), this is the first study to investigate hormonal associations with brain functional *connectivity*. Because anxiety is heterogeneous, and amygdala connectivity could be differentially related to different anxiety disorders/symptoms (Etkin, 2010), we examined these associations for different types of anxiety symptoms (general, social, and separation anxiety, obsessive-compulsive symptoms, panic-agoraphobia, and specific phobias). We explored sex-specific effects in these associations because previous studies have found sex differences in the associations between adrenal hormones and both mental health outcomes and brain function (Mundy et al., 2015; Reardon et al., 2009; Whittle et al., 2015) in this age group.

2. Methods

2.1. Participants

Full details of the recruitment strategy and data acquisition can be found in the imaging in the Childhood to Adolescence Transition Study (iCATS) protocol (Simmons et al., 2014). To summarize, participants were invited to participate in iCATS if they were in the lowest or highest tertile of DHEA and testosterone levels as measured in a larger parent project (CATS; Mundy et al., 2013). This was done to maximize variance in hormone levels in the sample. Hormone levels at participation in iCATS (see below for details) correlated significantly with levels at participation in the parent project (DHEA: $r = 0.749$, DHEAS: $r = 0.627$, testosterone: $r = 0.445$, all $p < 0.001$, which was on average 6.8 months prior. Continuous measures of the hormones at the CATS assessment were used here, instead of the tertile groups, to be able to examine the hormones separately and get a more refined picture of the association of each with functional connectivity. Children were excluded if they had a history of developmental or intellectual disorder, claustrophobia, non-removable ferrous metals in their body, and if there was amphetamine-based medication use at the time of hormone collection (since this has been associated with alterations in brain structure and function (de Luis-García et al., 2015)). None of the participants used other types of psychotropic medications, and none were diagnosed with premature adrenarche or precocious puberty, based on parent report. Written consent was obtained from the parent/guardian and verbal consent from the child. Ethics approval was granted by the Royal Children's Hospital Human Research Ethics Committee (#32171).

From the initial sample of 128 iCATS children, 96 participants agreed to and completed a functional MRI scan and saliva collections. Eight participants were removed due to MRI acquisition problems, and five due to movement artifacts, resulting in a final sample of 83 participants (40 boys, 43 girls) with a mean age of 9.53 years (SD = 0.34). Children in the final sample did not differ from those not in the final sample on age, sex, anxiety scores or hormone levels (all $p > 0.05$).

2.2. Saliva collection and processing

Saliva samples were collected at waking on the day prior to, and on the day of MRI scanning. Children collected 2.5 ml of saliva in a test tube, via passive drooling, and they recorded the time it took them to collect the saliva using a stopwatch. Samples were initially frozen at −30 °C, and prior to analysis, defrosted and centrifuged, with the supernatant assayed in duplicate for levels of DHEA, DHEAS and testosterone using Salimetrics ELISA kits. The inter-assay coefficients of variation (CVs) for DHEA, DHEAS and testosterone were 5.45%, 7.53%, and 13.54% respectively and intra-assay CVs were 8.56%, 9.38% and 7.32%, respectively. For more details on collection and processing of the saliva, see (Simmons et al., 2014). An average measure from the two days was used for analyses (correlations between the two samples were high: $r = 0.86$ (DHEA), $r = 0.87$ (DHEAS) and $r = 0.75$ (testosterone)).

2.3. Anxiety measure

Children completed the Spence Children's Anxiety Scale self-report (SCAS; Spence, 1998) as a measure of anxiety symptoms. The SCAS is a commonly used screening instrument for anxiety, with good reliability and validity (Spence, 1998). Total scores and subscale scores were calculated and adjusted for any missing items (1% of all data) by