HHS Public Access

Author manuscript

Front Neuroendocrinol. Author manuscript; available in PMC 2018 January 01.

Published in final edited form as:

Front Neuroendocrinol. 2017 January ; 44: 122–137. doi:10.1016/j.yfrne.2016.12.003.

Puberty and structural brain development in humans

Megan M. Herting[1] and **Elizabeth R. Sowell**[2]

[1]University of Southern California, Keck School of Medicine, Department of Preventive Medicine, Los Angeles, CA 90089

[2]Children's Hospital Los Angeles, Division of Research on Children, Youth, and Families, Los Angeles, CA 90027

Abstract

Adolescence is a transitional period of physical and behavioral development between childhood and adulthood. Puberty is a distinct period of sexual maturation that occurs during adolescence. Since the advent of magnetic resonance imaging (MRI), human studies have largely examined neurodevelopment in the context of age. A breadth of animal findings suggest that sex hormones continue to influence the brain beyond the prenatal period, with both organizational and activational effects occurring during puberty. Given the animal evidence, human MRI research has also set out to determine how puberty may influence otherwise known patterns of age-related neurodevelopment. Here we review structural-based MRI studies and show that pubertal maturation is a key variable to consider in elucidating sex- and individual-based differences in patterns of human brain development. We also highlight the continuing challenges faced, as well as future considerations, for this vital avenue of research.

Keywords

puberty; adolescence; magnetic resonance imaging; diffusion tensor imaging; hormones; neurodevelopment

I. Introduction

Puberty is an important period of development that occurs during adolescence. However, only recently has the notion been accepted that hormonal changes during puberty may continue to remodel and facilitate sexual differentiation of the brain. As outlined in a recent review (Juraska, Sisk et al. 2013), sexual differentiation in mammals was originally thought to occur during a relative finite period of prenatal and early postnatal development, with sex-specific increases in testosterone leading to masculinization along with defeminization of the male brain. In recent years, however, animal studies on the impact of pubertal hormones have revealed that the brain continues to be remodeled and is even further sexually differentiated by sex steroids during pubertal development (see (Juraska, Sisk et al. 2013))

for extensive review of the animal literature). Similarly, the field of neuroimaging has also begun to explore the role of puberty in human brain development. Here, we review the most up to date findings regarding pubertal maturation and typical brain development using magnetic resonance imaging (MRI). While it is important to note there are a number of physical growth and endocrine disorders that lead to early onset or delayed patterns of pubertal maturation (e.g. precocious puberty, Turner's Syndrome, Klinefelter Syndrome, etc.)(Bramswig and Dubbers 2009), these conditions are not reported on below as the current review aims to highlight the role puberty may have on brain maturation, above and beyond age, in typically developing adolescents.

II. Puberty

Puberty is complex set of neuroendocrine processes that occurs between childhood and adulthood to produce internal and external physical changes to primary and secondary sexual characteristics allowing for sexual reproduction. Puberty is initiated by reactivation of the hypothalamic-pituitary-gonadal (HPG) axis. During pre and early post-natal development, the HPG axis is responsible for sexual differentiation and organization of the central nervous system through its production of high levels of gonadal steroids, including testosterone and estradiol. After the first year of postnatal life, the HPG-axis lays dormant until resurgence of gonadotropin releasing hormone (GnRH) is secreted from neurons in the median eminence of the hypothalamus to facilitate pubertal onset (Knobil 1988, Grumbach and Styne 2003). Pulsatile GnRH release stimulates the pituitary gland to produce gonadotropins (luteinizing hormone (LH) and follicle-stimulating hormone (FSH)) into the circulatory system. While early production of LH and FSH occur during sleep (Boyar, Finkelstein et al. 1972), the amplitudes of LH and FSH release increases over time and eventually acting on the ovaries and testes to produce gonadal sex steroids of estradiol and testosterone, respectively. The gonadal sex steroids result in the development of breast and uterine tissue as well as testes and penile size and structure. These processes, from the reactivation of GnRH release to the first signs of physical maturation, are thought to take up to a year for pubertal onset to be put in motion and are together referred to as "gonadarche" (Grumbach and Styne 2003).

A separate endocrine function, known as "adrenarche", is the maturation of the adrenal glands, and is complementary to gonadarche in terms of its contribution to additional notable physical changes that occur during puberty. As the adrenal glands mature from approximately ages 6 to 8 in girls and 7 to 9 in boys, they produce an increase in adrenal androgens, including dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), and androstenedione (Cutler and Loriaux 1980, Parker 1991, Grumbach and Styne 2003). Increases in these androgens continue during gonadarche as well as into young adulthood (Saenger and Dimartino-Nardi 2001), and are responsible for the development of under-arm and pubic hair.

While separate processes that lead to different external physical characteristics, both gonadarche and adrenarche are relevant in our quest to measure puberty and to further our understanding of how puberty may contribute to brain and behavioral development.

a. Measuring puberty in humans

Physical Markers—Physical measurements may be used to estimate stages of gonadarche and adrenarche. Physical changes to secondary sexual characteristics are often captured by non-invasive techniques including self-report or clinical inspection by a trained medical expert. The most well recognized system for physical staging of pubertal development is based on Tanner (Marshall and Tanner 1969, Marshall and Tanner 1970). Breast (in females) or genital development (in males) (gonadarche) as well as pubic hair (adrenarche) are given ratings, including Stage 1 – prepubertal; Stage 2 – breast and genital development have begun; and up to Stage 5 – full maturity. The gold standard for Tanner staging continues to be physical examination by a trained medical expert using these criteria. However, alternative methods of self-report have become widely popular and also use Tanner staging to benchmark physical stages of pubertal development. For example, tanner stage pictorial representations (Dorn, Susman et al. 1990) or line schematic drawings (Morris and Udry 1980, Taylor, Whincup et al. 2001) have been created for each of the 5 stages of breast development and pubic hair for females and testis growth and pubic hair for boys. In addition, the Pubertal Development Scale (PDS) is a self-report verbal questionnaire that has been shown to be reliable and valid in assessing physical stages of pubertal maturation (Petersen, Crockett et al. 1988). The PDS asks 5 questions for each individual, with items 1 thru 3 relating to growth in height, growth in body hair, and changes in skin (i.e. pimples), whereas items 4 and 5 include deepening of voice and facial hair growth for boys and breast growth, menstruation (yes/no; age of menstruation) for girls. PDS scores range from 1 to 4 (“not yet started” to “seems complete”, with the ability to calculate 1 of 5 Puberty Category Scores (“Prepubertal”, “Early puberty”, “Midpubertal”, “Late pubertal”, “Postpubertal”) (Carskadon and Acebo 1993). A more recent advancement is a new automated audio computer-assisted self-interview (ACASI) to help aid children and adolescents in completing a self-report of sexual maturation (Lamb, Beers et al. 2011); although note the ACASI version is not widely accepted for use (Dorn and Susman 2011).

Hormonal Markers—Hormone levels can be assessed to allow for an objective measure to estimate pubertal development. Testosterone and estradiol levels increase in both males and females during puberty, but the magnitude is greater for testosterone in boys and estradiol in girls. Testosterone has been shown to be 45 times higher in adult males as compared to prepubertal boys (Biro, Lucky et al. 1995), whereas estradiol levels have been shown to be 4 to 9 times higher in later adolescence as compared to childhood in girls (Ikegami, Moriwake et al. 2001). Adrenal and gonadal sex steroids, such as DHEA, DHEA-S, testosterone, and estradiol, may be obtained from various biological samples, including urine, saliva, and blood; although the latter two methods are more common in the literature. The total amount of testosterone and estradiol circulating in the bloodstream is either “bound” or “unbound”. The majority of the bound testosterone or estradiol is attached during transport to sex hormone binding globulin (SHBG)(Selby 1990). Only a very small amount (~1–2%) of the total is actually unbound, or “free”, meaning it is biologically active and able to enter a cell and bind to a receptor. Saliva is relatively non-invasive, and it measures the “unbound” biologically active hormone levels in the body (Hofman 2001). The more invasive methods of blood serum measures total hormone levels (i.e. both bound and free levels); however, quantification of both total hormone levels and sex hormone binding globulin (SHBG)

allows for the calculation of the estimated percentage of bound versus free levels of testosterone and estradiol (Anderson 1974). While each method has strengths and weaknesses, blood serum levels may be better than saliva assays for estimating low levels of pubertal hormones seen early in puberty, especially for estradiol sampling (Shirtcliff, Granger et al. 2000). That is, in boys ages 8 to 9 years and non-menstruating girls ages 10 to 12 years, correlations of estradiol were high (r=.73 for boys, r =.96 for girls) for serum and blood spot, whereas saliva and blood spot were much lower (r=-.18 for boys and r=.72 for girls)(Shirtcliff, Granger et al. 2000). Serum to saliva estimates are highly correlated for DHEA in adults (r=0.85)(Shirtcliff, Granger et al. 2001). For testosterone, the serum to saliva correlation was found to be high (r=0.83) in 11 to 23 year-old boys; although serum testosterone was more effective at distinguishing between various stages of pubertal development (Rilling, Worthman et al. 1996). As for women, serum to saliva correlations for total testosterone has been found to be relatively low (r=.37) (Granger, Shirtcliff et al. 2004).

Regardless of the method, collecting hormonal data at the same time every morning (8 AM to 10 AM) is imperative because of circadian rhythms (Ankarberg and Norjavaara 1999, Ankarberg-Lindgren and Norjavaara 2004), with levels peaking earlier in the morning and declining across the day. Menstruation is also an important variable in pubertal development in females. For women with regular cycles, estradiol levels are low at the beginning of the cycle (follicular phases; ~1–14 days) and begin to rise through mid-cycle. LH and FSH are then released mid-cycle to trigger ovulation (Aedo, Landgren et al. 1981). During the second half of the cycle (luteal phase; ~14–28 days), estradiol levels reach two peaks, with the first peak being the largest and the second smaller peak occurring ~5 days later. During the luteal phase, progesterone levels also steadily increase until later in the cycle when they will drop if the egg does not become fertilized. Beyond estradiol and progesterone, testosterone also varies across the cycle. Testosterone secretion is highest just after ovulation (periovulatory phase) and follows a diurnal pattern during the follicular phase of the menstrual cycle (but not during the periovulatory period or luteal phases)(Aedo, Landgren et al. 1981, Rothman, Carlson et al. 2011). Although other findings suggest variability across the cycle may be less important than the individual differences seen in daily fluctuations between women (Bui, Sluss et al. 2013). It is also important to note that low testosterone and estradiol concentrations in pre- and early pubertal levels in girls and boys may be difficult to detect as they may fall below detection level using radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) methods (Ankarberg and Norjavaara 1999). However, a more recent liquid chromatography-mass spectrometry (LC-MS) technique (Herold and Fitzgerald 2003, Albrecht and Styne 2007) may be more reliable and able to capture low concentration in pre- and early pubertal levels in girls and boys (Buttler, Peper et al. 2016).

Limitations of physical and hormonal measures—Despite the advantages, there are limitations to each of the presented methods for both physical and hormonal measures of puberty (See also Table 1 in Berenbaum, Beltz et al. 2015). For non-invasive pictorial or verbal questionnaires of physical development, individual and sex differences have been seen in terms of reliability between self-report as compared to physician ratings of pubertal maturation. For example, Marshall and Tanner note that early stages of pubic hair development, particularly among fair-haired participants, were difficult to see from

photographs and resulted in participants being classified into this stage too late (Marshall and Tanner 1969, Marshall and Tanner 1970). Similarly, self-report accuracy can also be affected by child's weight status (obesity/over-weight)(Bonat, Pathomvanich et al. 2002). Accuracy of Tanner stage self-reporting also varies between sexes, and does so as a function of pubertal stage and gonads/breast and pubic hair measurements (Dorn, Susman et al. 1990). These sex differences in self-report accuracy have been seen for both paper (Carskadon and Acebo 1993) and computerized versions (Lamb, Beers et al. 2011) of sexual maturation. For these reasons, self-reports can show less than ideal reliability and accuracy, resulting in Brook-Gunn et al. (Brooks-Gunn, Warren et al. 1987) and Petersen et al. (Petersen, Crockett et al. 1988) to both conclude that PDS indicators of maturation may be adequate only for rough estimates or for use in longitudinal studies. Both studies suggest verbal PDS questionnaires should only be used when parental- or self-reports of development using the Tanner stage drawings are not acceptable.

Beyond physical staging, hormone specimen collection is limited by participant burden and feasibility. In the literature, hormone levels are typically representative of a single time point and often taken during a single phase of the menstrual cycle (i.e. follicular or luteal phase) to reduce variability. More than 1 sample (2 adjacent days or 2 in month) may be more reliable and help deal with monthly variability (of both estradiol and testosterone) due to menstruation in girls (Aedo, Landgren et al. 1981, Rothman, Carlson et al. 2011). Moreover, recent research suggests that to capture inter-individual differences with a single sample, that sample timing is vital (Ahmad, Pollard et al. 2002). Based on the start of the previous menses and using serum estimates, sampling was found to be consistent (correlated) when measured between 9 to 11 days for estradiol, 17 to 21 days for progesterone, and 12 to 15 days for free androgen levels (Ahmad, Pollard et al. 2002). Others have reported higher intraclass correlation coefficents for serum and urinary levels of estradiol if measured 4 to 10 days before the estimated start date (Michaud, Manson et al. 1999). However, despite efforts to capture hormone levels during a specific portion of the menstrual cycle, the length of the cycle is greatest 1–2 years following menarche (Treloar, Boynton et al. 1967), and approximately 80% of girls are often anovulatory in the first year after menarche (Apter, Viinikka et al. 1978, Apter 1980). Thus, daily diaries or electronic mobile devices may also be needed to capture additional variability in early post-menarche girl hormone levels across erratic menstrual cycles.

b. Physical and Hormonal Time-courses

It is important to note that neither the physical or hormonal changes occur in isolation during puberty. A growth spurt is also seen to co-occur with both sexual markers of maturation as well as the increase in hormones during puberty. In girls, a linear growth spurt typically begins during tanner stage 2 in girls (~ 9.5 and 14.5 years), with the peak velocity seen approximately 6 to 12 months prior to menarche (Marshall and Tanner 1969). In boys, peak velocity of linear growth occurs later, usually beginning during tanner stage 4 (~14.4 years) and closely coincides with testicular and facial hair development (Marshall and Tanner 1970). Moreover, in girls, height velocity has been associated with growth hormone, estradiol, and other androgens (i.e. androstenedione), whereas this peak height velocity in boys was found to relate to growth hormone, estradiol, and testosterone (Delemarre-van de

Waal, van Coeverden et al. 2001). Similarly, there is overlap between developmental patterns of pubertal hormones and tanner staging, with a wide range of steroid concentrations seen within each Tanner stage and in between the sexes (Nottelmann, Susman et al. 1987, Ankarberg and Norjavaara 1999). For this reason, some have argued that morning assessment of testosterone may be the only way to reliably distinguish “prepubertal versus pubertal” in boys (Wu, Brown et al. 1993). Moreover, while hormone values increase with pubertal maturation, a specific hormone value probably cannot be directly matched to a specific Tanner stage, and individual differences may exist in the concentration needed to advance secondarily sexual characteristics of pubertal maturation (Shirtcliff and colleagues 2009). For example, a study by Shirtcliff and colleagues (Shirtcliff, Dahl et al. 2009) examined how different physical characteristics based on physician exam may relate to basal hormone levels. Results showed that estradiol related to breast development, whereas testosterone and DHEA related to pubic hair development in girls. In boys, testosterone was found to relate to both genital development and pubic hair, whereas DHEA was found only to relate to pubic hair. However, self-reports of maturation of secondary sexual characteristics were similar, or in some cases were more closely related, to hormone levels compared to physician reports (Shirtcliff, Dahl et al. 2009). Thus, both physical markers as well as hormone levels may be useful and reflect different processes related to puberty (Dorn and Biro 2011).

III. Common Measurements of Brain Development using MRI

Advances in magnetic resonance imaging (MRI) have allowed for further understanding of how the brain continues to rapidly develop between childhood and adulthood. Using the same piece of equipment, various MRI sequences can collect images that allow for estimates of brain size, shape, and structural connectivity between distal regions. That is, structural MRI (T1-weighted imaging) can be used to quantify size and shape of gray and white matter areas. More specific to white matter, diffusion-weighted MRI, which includes diffusion tensor imaging (DTI), can be used to make inferences about microstructural properties of tissues (Alexander, Eun Lee et al. 2007). DTI has been widely used to assess white matter diffusion that may be influenced by organization of axons, myelination, axon caliber, and other intra and extra cellular processes. Another common approach to assess white matter composition has been magnetic transfer ratio (MTR). MTR assesses macromolecular content and structural integrity to estimate differences in myelination (McGowan 1999).

Overall, these structural MRI methods have allowed for a better understanding of *in vivo* brain development, with common patterns of age and sex-specific changes seen between childhood and adulthood. Grey matter volume development is curvilinear, generally peaking in late childhood and decreasing throughout adolescence. Dividing gray matter volume into two morphometric components known as cortical thickness and surface area (Winkler, Sabuncu et al. 2012), has shown total cortical thickness and surface area show distinct trajectories across childhood and adolescence (Raznahan, Shaw et al. 2011). For example, while both cortical thickness and surface area show inverted U-shape curves, sex differences are seen in the shapes and trajectories of surface area (girls peak at 8.1 years, boys peak at 9.7), whereas cortical thickness patterns are similar between the sexes (8.4 and 8.6, respectively)(Raznahan, Shaw et al. 2011). Similar to the cortex, subcortical regions undergo

significant changes in morphology across childhood and adolescence (Giedd, Vaituzis et al. 1996, Sowell, Trauner et al. 2002, Toga, Thompson et al. 2006, Koolschijn and Crone 2013). These include areas important for sensorimotor processing, such as the thalamus and caudate (Haber 2003), as well as limbic regions that are essential for emotion and memory, such as the amygdala and hippocampus (Richter-Levin and Akirav 2000, Phelps 2004). Several studies have also shown subcortical development may differ between boys and girls (Giedd, Vaituzis et al. 1996, Sowell, Trauner et al. 2002, Toga, Thompson et al. 2006, Lenroot, Gogtay et al. 2007, Koolschijn and Crone 2013).

In contrast to gray matter volumes, total white matter volumes have been found to demonstrate a more linear pattern, increasing over time (Jernigan, Trauner et al. 1991, Giedd 1999, Sowell, Peterson et al. 2003). In addition, during adolescence boys show a more robust increase in global white matter volume with age (Giedd, Blumenthal et al. 1999, De Bellis, Keshavan et al. 2001, Lenroot, Gogtay et al. 2007). Child and adolescent longitudinal DTI studies have also reported decreases in mean diffusivity (MD), suggesting increases in the size/density of axon bundles, myelin lipids, number of cells and/or their cell processes, or some combination, as well as increases in fractional anisotropy (FA) with age (Bava, Thayer et al. 2010, Lebel, Gee et al. 2012, Wang, Adamson et al. 2012, Simmonds, Hallquist et al. 2014), suggesting greater myelin and/or fiber organization. However, sex-differences in white matter microstructure have been largely inconsistent. Schmithorst, Holland et al. (2008) found in a sample of 5 to 18 year-olds that girls had higher FA in regions of the corpus callosum, whereas boys had higher FA in more associative brain regions including the frontal lobes. Alternatively, in a separate study of adolescents ages 10 to 16 years, boys were found to have higher FA in corticospinal, long-range association, and cortico-subcortical white matter regions compared with girls (Herting, Maxwell et al. 2012). In a more recent mixed cross-sectional and longitudinal study, Simmonds and colleagues (2014) examined FA in 8 to 29 years old and found that boys (but not girls) showed significant increases in FA in cerebellar and limbic white matter pathways during late childhood (8.2–12.8 years) and late adolescence (16.5–19.2 years). However, both sexes showed increases in FA in callosal and association white matter regions.

In summary, the adolescent brain continues to develop with volumetric, shape, and microstructural changes seen in both grey and white matter. Below, we review studies that have aimed to examine the contribution of pubertal-related processes on these aforementioned age and sex related changes in brain structure across adolescence.

IV. Puberty and Brain Development

a. Associations between pubertal development and brain volume

A number of cross-sectional studies have examined cortical and subcortical brain volumes based on grouping a narrowly defined age-range of individuals based on physical maturation scores, or correlating various puberty markers (including hormone levels and physical maturation scores) with brain volumes, while statistically controlling for age. A summary of these studies, including the age range and sample size of each study, can be found in Table 1. Below, we highlight some of the key findings in the field.

i. Physical Markers

Gray Matter: Physical markers of maturation have been linked with gray matter volumes in both cortical and subcortical regions. In a large group of 9 year-old twins, girls that showed any sign of physical maturation (Tanner stage ≥ 2) had smaller gray matter density in frontal and parietal areas compared to their peers that had not begun to show signs of maturation (Tanner stage ≤ 1)(Peper, Schnack et al. 2009). In another slightly older sample, gray matter was examined in early (Tanner stage ≤ 2) versus mid- to-late staged (Tanner stage ≥ 3) 11 to 14 year old boys and 10 to 13 year old girls (Bramen, Hranilovich et al. 2011). After accounting for age, mid-to-late pubertal staged girls showed smaller total gray matter volumes (Bramen, Hranilovich et al. 2011).

Beyond total gray matter, a number of studies have examined how physical maturation relates to subcortical volumes. For example, Bramen and colleagues also found mid-to-late staged girls had smaller left amygdala volumes as compared to their earlier staged peers (Bramen, Hranilovich et al. 2011). Similarly, a cross-sectional study that dichotomized 10–22 year-old males and females based on self-report of pubic hair stage into early puberty (Tanner stage ≤ 2) and post-pubertal (Tanner stage = 5) and controlled for age, found that post-pubertal individuals had smaller hippocampal volumes as compared to those in early pubertal stages, with larger effects seen in males as compared to females (Satterthwaite, Vandekar et al. 2014). However, no significant effects were detected in the amygdala (Satterthwaite, Vandekar et al. 2014). Research by Blanton and colleagues (Blanton, Cooney et al. 2012) also examined physical markers and subcortical volumes in 54 girls, 9 to 15 years of age. Using separate Tanner stage scores for breast development and pubic hair, while also controlling for age, they found that advancement of pubic hair was related to smaller right hippocampal volumes, whereas breast size related to smaller amygdala volumes bilaterally. In contrast, a more recent study by Hu and colleagues (Hu, Pruessner et al. 2013) found pubertal scores correlated positively with the right hippocampus and left parahippocampus in both boys and girls. Furthermore, post-hoc analyses also found that various physical markers of puberty (e.g. height, breast, facial hair) had unique associations with amygdala, hippocampus, and parahippocampus volumes (Hu, Pruessner et al. 2013).

White Matter: In addition to gray matter, white matter volumes and microstructure have also been found to correlate with physical markers of pubertal development. Perrin and colleagues (Perrin, Leonard et al. 2009) assessed puberty in its relation to lobar (e.g. frontal, parietal, temporal, and occipital) white matter volumes and microstructural properties via MTR. Physical maturation (PDS scores) predicted larger white matter volumes, with larger effects seen in boys as compared to girls in the parietal and occipital lobes. In addition, although no significant relationship was seen in girls, higher PDS scores predicted smaller MTR values, thought to reflect more myelination, in the parietal and occipital lobes in boys. Lastly, these puberty models were then compared to identically structured age-based models, to show that puberty and age account for a similar amount of variance in white matter outcomes. However, age was not controlled for in any of the PDS analyses. Similarly, only one study has specifically examined PDS scores and corpus callosum white matter thickness in a sample of 5 to 18 year olds (Chavarria, Sanchez et al. 2014); although, again age was not controlled for in these analyses. Because neither Chavarria et al. nor Perrin et al chose to

include age as a covariate, it is unclear if the outcomes seen are uniquely linked to puberty, driven by age, or due to a combination of both age and puberty. In contrast, using DTI, Asato, Terwilliger et al. (2010) examined how white matter microstructure varied as a function of puberty in children (8–12 years), adolescents (13–17 years), and adults (18–28 years), while controlling for age. The results showed that for the inferior frontal occipital fasciculus and inferior longitudinal fasciculus maturation seemed complete for individuals by mid-puberty. However, for all other white matter regions of interest, maturation remained incomplete for individuals in mid-puberty, but rather reached adult-like microstructural patterns only in those that had completed pubertal maturation (i.e. post-pubertal stage).

Together, these initial cross-sectional studies have been vital in highlighting that physical markers of puberty relate to gray and white matter in adolescent girls and boys. Moreover, unique associations between various physical characteristics (e.g. pubic hair, breast development) and brain structure have suggested the importance of understanding how distinct hormones (e.g. androgens, estradiol) may be related to cortical, subcortical, and white matter volumes.

ii. Androgens (Testosterone and DHEA)

Gray Matter: Using a large cross-sectional cohort, associations have been examined between testosterone levels and various gray matter metrics in boys ages 12 to 18 years, including volume, density, and the ratio between white and gray matter (white:gray) (Paus, Nawaz-Khan et al. 2010). Higher testosterone levels were found to relate to smaller total grey matter volumes as well as larger total white:gray and less gray matter density in multiple distinct cortical regions. Furthermore, a common androgen receptor gene variant was found to modify this correlation. Gray matter findings and white:gray correlations were larger for boys with the short AR genotype (resulting in more transcriptional activity) versus their peers with the long AR allele. However, age was not accounted for in this study, making it unclear if testosterone accounts for a unique and separate proportion of variance in these metrics of brain development. Two additional studies that have controlled for age found sex-specific associations between testosterone and gray matter. After statistically controlling for age, Peper and colleagues (Peper, Brouwer et al. 2009) found higher testosterone levels were related to larger, rather than smaller, global gray matter volumes in 10 to 15 year-old boys. Whereas Bramen and colleagues (Bramen, Hranilovich et al. 2011) found testosterone levels correlated with smaller total gray matter volumes in 10–13 year-old girls, but not 11–14 year-old boys.

Beyond these initial volumetric studies, a number of additional studies have examined testosterone levels and cortical thickness and volumes in more distinct brain regions. For example, Bramen and colleagues reported that higher testosterone was related to a thicker occipital cortex in boys (ages 12–14 years), but a thinner occipital and superior temporal cortex in girls (ages 10–11 years) (Bramen, Hranilovich et al. 2012). In addition, higher testosterone levels were also related to thinner cortex values in the inferior parietal lobule and the medial temporal gyrus in both sexes. More recently, after controlling for age, higher testosterone levels were also found to relate to smaller anterior cingulate cortex (Koolschijn, Peper et al. 2014) and orbital frontal volumes (Peper, Koolschijn et al. 2013). Together these

data suggest that the relationship between testosterone and gray matter volumes and thickness varies based on brain region and an individual's sex.

There has also been great interest in understanding how testosterone relates to subcortical brain volumes. The first study on this topic was published by Neufang and colleagues (Neufang, Specht et al. 2009) and examined correlations between testosterone and brain volumes in 15 boys and 15 girls (ages 8 to 15 years-old) using a whole brain voxel based morphometry approach, while controlling for age. In both boys and girls, higher testosterone levels were related to larger amygdala volumes but smaller hippocampus volumes. In addition, a positive correlation was detected between testosterone and hypothalamus, mammillary bodies, and thalamus gray matter, but a negative association with gray matter volumes in the parietal cortex in boys (but not girls). Since then, it is also important to note that a number of additional studies have also studied testosterone and subcortical volumes, but have been unable to find similar correlations. For example, the correlation between testosterone and amygdala did not pass age and multiple comparison correction in a medium size sample of 10–14 yearolds (Bramen, Hranilovich et al. 2011), whereas no correlation was detected between the amygdala or hippocampus in a large group of 8 to 25 year-olds (Koolschijn, Peper et al. 2014). Thus, more research is needed to clarify consistency and establish reliability of these relationships.

White Matter: The first study to examine the association between testosterone levels and cortical white matter volumes was in 2008 by Perrin and colleagues (Perrin, Herve et al. 2008). After controlling for age in a large sample of 12 to 18 year-olds, higher bioavailable testosterone levels were found to correlate with larger whole brain white matter volumes, but lower MTR values, in boys. The authors concluded that these findings suggest testosterone is related to increases in axonal caliber, rather than myelin, in adolescent boys. Boys with a short AR allele genotype (resulting in more transcriptional activity) showed a stronger association between testosterone and white matter growth variables as compared to those with a long AR allele genotype. In fact, testosterone only remained a significant predictor for boys with the short AR allele (but not those with the long AR allele) when age was also put in the same model. More recently, additional analyses have been performed to examine the unique contributions of testosterone and physical markers in puberty on corticospinal tract and corpus callosum white matter in boys (Pangelinan, Leonard et al. 2014). Both PDS and testosterone were found to negatively relate to corticospinal tract and corpus callosum MTR and T1-weighted intensities, with age in the model. Moreover, testosterone and PDS uniquely contributed to T1-weighted intensity of the corticospinal tract (Pangelinan, Leonard et al. 2014).

Besides these studies in boys, additional cross-sectional studies have examined how androgens relate to white matter microstructure development in both adolescent boys and girls using DTI. In 10 to 16 year-olds, after controlling for age, testosterone was found to positively predict voxelwise FA values in wide-spread white matter regions where boys had higher FA values as compared to girls (Herting, Maxwell et al. 2012). Two more recent studies have examined similar relationships between testosterone and DTI outcomes, while controlling for age. One study of 61 adolescent boys (ages 12 to 16 years) did not detect a significant testosterone and FA relationship using a similar voxelwise technique. Rather,

testosterone was found to relate to lower levels of MD in adolescent boys (Menzies, Goddings et al. 2015). The other DTI study examined testosterone and FA, MD, AD, and RD in a large sample of both girls and boys (ages 8–25 years) for a number of designated regions of interest as defined by tractography (Peper, de Reus et al. 2015). This study found positive correlations between testosterone and MD in most regions, although only the subcortico-temporal tract remained significant after multiple comparison corrections. In addition, a negative correlation was found between testosterone and FA in the subcortico-frontal tract. These discrepancies may arise from the various DTI techniques utilized, such as voxel-based versus tractography, as well as the ability to disentangle testosterone versus age in wide versus narrow aged samples. Nonetheless, together, these studies are important because they suggest that testosterone may be especially important in various aspects of white matter maturation that is seen across adolescence. However, given that FA, MD, and MTR are indirect estimates of white matter microstructure, more research is needed to understand the biological mechanisms, including axon caliber, organization, and myelination, that are at play (Paus 2010).

Pituitary: Two studies have examined androgen levels and pituitary volumes. In 9 year-old boys and girls, higher DHEA levels (but not testosterone levels) were found to be associated with larger pituitary volumes (Murray, Simmons et al. 2016). Alternatively, testosterone was found to be positively associated with larger pituitary volumes in 12–18 year old boys, even after controlling for age (Wong, Pipitone et al. 2014). Together, these findings might suggest that different androgens may relate to pituitary volumes at unique periods of pubertal development during adolescence.

In summary, these cross-sectional studies have provided a strong rationale for further clarifying how variation in androgen levels due to puberty, sex, as well as genetic AR polymorphisms may contribute to developmental patterns of cortical versus subcortical brain structure in adolescents.

iii. Estradiol and gonadotropins (FSH and LH)

Gray Matter: Given the challenges of accurately capturing hormonal variability in girls, it is not surprising only a few studies have examined estradiol and brain structure. The first study to assess estradiol levels during puberty was by Neufang and colleagues (Neufang, Specht et al. 2009). This study included measurements of estradiol in 15 boys and 15 girls (ages 8 to 15 years-old). After controlling for age, a positive correlation was seen between estradiol and parahippocampal and uncus gray matter in girls (but not boys). In a separate study of 10 to 15 year-olds, after controlling for age, estradiol levels related to lower grey matter density in regions of the inferior, superior, middle and orbitofrontal gyri, the supramarginal and angular gyri and the middle temporal gyus, but higher density in the middle frontal, inferior temporal, and middle occipital gyrus (Peper, Brouwer et al. 2009).

White Matter: One cross-sectional study has been published on LH and white matter. Peper and colleagues found that higher concentrations of LH in pre-pubertal individuals (age 9) were found to positively associated with greater global white matter volume, and regional white matter density in the cingulum, middle temporal gyrus, and splenium (Peper, Brouwer

et al. 2008). In addition, the previously mentioned DTI study by Herting et al. also found that estradiol was negatively related to FA values in girls; which was a rather stark contrast to the opposite, positive relationship seen in this study between testosterone and FA values in boys (Herting, Maxwell et al. 2012). Although replication is needed, these studies provided the field with initial evidence that in addition to testosterone, estradiol and gonadotropins may also influence white matter development.

Pituitary: Two studies have examined estradiol and gonadotropin levels in relation to pituitary volumes. In one study, higher estradiol levels were found to predict larger volumes, while controlling for age, in premenstrual girls (Wong, Pipitone et al. 2014). Another study examined estradiol as well as other various hormones (LH, FSH, estradiol, and testosterone levels)(Peper, Brouwer et al. 2010). After controlling for age, only FSH levels positively related to larger pituitary volumes in girls; with no other significant relationships detected between hormone levels and pituitary volumes in this wider age range of adolescents (10 to 15 year-olds)(Peper, Brouwer et al. 2010).

These studies suggest the importance of not only measuring androgens, but also examining estradiol and gonadotropins in order to better understand adolescent brain development. Thus, future research should aim to determine the unique influence of each pubertal hormone as well as the possible synergistic effects on brain volumes.

b. Within- and between- subjects changes in puberty and brain development

The cross-sectional studies outlined above have been instrumental towards highlighting the need to consider the role of puberty in brain development. However, it also is clear from this emerging body of literature that, regardless of physical or hormonal measurements, the associations between puberty and brain structure have reported mixed findings. These discrepancies are likely related to the various age ranges that have been studied as well as smaller sample sizes that may result in limited power. Moreover, as detailed by a recent timely review (Crone and Elzinga 2015), cross-sectional studies are confounded in their ability to disentangle age-related differences with true change; even when controlling for age statistically. While age and puberty are highly correlated, individuals mature at different ages and progress through puberty at various rates. As such, cross-sectional studies are limited in their ability to capture *individual differences* in age, puberty, and brain maturation. That is, key aspects of pubertal maturation are markedly different not only between males and females, but also between individuals of the same sex. Longitudinal studies allow for more complex models to assess how much people differ between each other (inter-individual differences), as well as how much an individual changes (intra-individual change) over time (Singer and Willet 2003). To date, eight longitudinal studies have started to shed additional light on how physical and hormonal changes predict brain structure across adolescence (Table 2). Below, we discuss each of these studies in detail.

i. Physical Markers—Pubertal development, as assessed by self-reported Tanner stage, was shown to predict changes in subcortical volume, including the hippocampus, amygdala, and caudate, between the ages of 7 and 22 years (Goddings, Mills et al. 2013). Importantly, age and tanner stage had unique and interacting effects on changes in volumes for these

structures. Examined in both sexes separately, the amygdala and hippocampus were both found to continue to increase, whereas the other subcortical structures decreased, with pubertal maturation. However, when estimating volume changes based on both age and pubertal development, girls who were more mature than their peers showed larger volumes, with an earlier peak, and smaller volumes towards late maturation as compared to their less mature age-matched peers. However, larger structural volumes were seen in boys at later stages of puberty as compared to those at a similar age that were still in early phases of pubertal development (Goddings, Mills et al. 2013). This study was the first to suggest that both age and pubertal-changes lead to sex specific changes in subcortical development.

Two follow-up studies using the cohort of children published by Bramen and colleagues (Bramen, Hranilovich et al. 2011, Bramen, Hranilovich et al. 2012) also employed growth curve modeling to assess changes in subcortical brain volumes from a subset of 126 adolescents, ages 10–14 years, that were successfully restudied ~2 years later (Herting, Gautam et al. 2014, Herting, Gautam et al. 2015). Independent of age, changes in Tanner stage predicted total white matter volumes and right amygdala growth across adolescence in boys and girls, as well as decreases in total gray matter and caudate volumes (Herting, Gautam et al. 2014). Moreover, greater physical maturation across a 2-year period predicted decreased superior temporal cortex in girls, as well as a greater thinning in the right bank of the superior temporal sulcus surface area in boys as compared to more modest thinning seen in girls (Herting, Gautam et al. 2015). Because the studies differed in the age ranges included, the number of time points assessed, the physical maturation assessment procedure (self-report versus physician ratings), as well as using raw (Goddings, Mills et al. 2013) versus statistically controlling for intracranial volume (Herting, Gautam et al. 2014), more research is needed to understand some of the inconsistencies in directionality of results between these studies. Nonetheless, both studies suggest an important role of pubertal development in understanding brain volume development over time.

ii. Androgens (Testosterone and DHEA)—Two studies by Nguyen and colleagues have examined how testosterone and DHEA relate to cortical thickness using a longitudinal sample between the ages of 4 to 22 years (Nguyen, McCracken et al. 2013, Nguyen, McCracken et al. 2013). In these studies, participants were followed up to 3 times at ~2 years between MRI assessments. In girls, higher testosterone levels predicted thickening of somatosensory cortices during childhood, but predicted thinning in early adulthood. In post pubertal boys, higher levels of testosterone predicted smaller cortical thickness in the posterior cingulate and the dorsal lateral prefrontal cortex (Nguyen, McCracken et al. 2013). For DHEA, higher levels predicted increases in cortical thickness of the left dorsal lateral prefrontal cortex, the right tempro-parietal and premotor regions, and right entorhinal cortex at younger, pre-pubertal ages (4 to 13 year-olds) in both sexes (Nguyen, McCracken et al. 2013). Moreover, an interaction was seen between testosterone and DHEA, with high levels of testosterone, but low levels of DHEA, related to decreases in cortical thickness of the cingulate and occipital cortex (Nguyen, McCracken et al. 2013). Nguyen and colleagues also showed in the same cohort of children that higher testosterone levels related to a negative relationship between left amygdala growth and cortical thickness in the anterior cingulate and orbital frontal cortex in both sexes after controlling for age (Nguyen, McCracken et al.

2016). Similarly, after controlling for age, higher DHEA levels were also found to predict negative correlations in the growth of the amygdala and cortical thickness of the left occipital pole, right somatosensory cortex, and the right sub-genual anterior cingulate cortex in both boys and girls (Nguyen, Gower et al. 2016). Moreover, androgen levels and amygdala and cortical thickness co-development were linked to aggression levels and visual attention measurements in these samples.

Beyond these two studies, the previously mentioned studies examining longitudinal changes in Tanner stage and brain structure by Herting and colleagues (Herting, Gautam et al. 2014, Herting, Gautam et al. 2015) also found significant effects of testosterone levels on brain volumes and cortical thickness, independent of age. Similar to Tanner stage, higher testosterone levels predicted larger white matter volumes and smaller caudate volumes in both boys and girls. Testosterone was found to predict decreases in amygdala volumes in boys, but increases followed by a plateau in girls (Herting, Gautam et al. 2014). Within-subject changes in testosterone were also found to relate to decreases in surface area for both boys and girls in the precuneus, whereas testosterone related to decreases in the middle superior frontal surface area in boys, but an increase in surface area for this region in girls (Herting, Gautam et al. 2015). Although these findings have only been observed in two cohorts of children, these studies highlight that within-subject changes in androgens are linked to structural brain development, including amygdala, caudate, and white matter volumes, as well as cortical thickness and surface area of boys and girls.

iii. Estradiol and gonadotropins (FSH and LH)—In 10 to 13 year old girls, larger increases in estradiol across a 2-year follow-up period resulted in a greater decrease in left middle temporal gyrus thickness (Herting, Gautam et al. 2015). In addition, at low levels of estradiol, increases in white matter volumes, but decreases in total gray matter and right amygdala volume, were seen. At higher levels of estradiol, these patterns were seen to reverse and/or plateau in this cohort of girls (Herting, Gautam et al. 2014). In a more recent longitudinal analysis of twins between the ages of 9 and 12 years of age, a negative correlation was also seen between estradiol levels and grey matter density in the left frontal and parietal regions at age 12 (Brouwer, Koenis et al. 2015), with the majority of the variance due to the shared environment among the twin pairs. However, within-subject changes in estradiol levels were not found to predict brain outcomes. Interestingly, other studies have not been able to detect significant within-subject effects of estradiol on cortical thickness outcomes (Nguyen, Gower et al. 2016, Nguyen, McCracken et al. 2016).

To our knowledge, Brouwer and colleagues (Brouwer, Koenis et al. 2015) twin study is the only published research on longitudinal changes in gonadotropins and brain structure in adolescents. While no effects were seen in boys, increases in FSH were related to increases in grey matter density in the left hippocampus, left prefrontal cortex, right cerebellum, and left anterior cingulate and precuneus. Given the twin study approach, it was also found that 58% of the significantly detected voxels showing a relationship between FSH and density were driven by the non-shared environment of the individual, rather than the shared environment among the twin pair. Thus, these findings suggest that future studies should also consider understanding both the genetic and environmental factors that may account for

the individual differences seen between hormones and brain development during adolescence.

V. Summary and Future Directions

Review of the current literature on puberty and structural MRI highlights that both physical and hormonal changes during puberty are closely linked with changes in gray and white matter development. The noted inconsistencies among the correlational studies are likely multifactorial, including differences in age range, imaging methodology and analyses, as well as the limited ability to statistically disentangle the highly collinear factors of age and pubertal development. While still preliminary, the emerging longitudinal studies hold great promise to help dissect between- and within-subject variance as well as capture the individual variability seen in the age of onset and the timing or progression of puberty across adolescence. In fact, the longitudinal studies suggest that age and puberty are distinct and yet integrated processes in how they influence neurodevelopmental trajectories. That is, the longitudinal studies suggest that physical and hormonal changes during puberty may be directly involved with the gray matter decreases and white matter increases seen to occur from childhood to adulthood. Moreover, some of the longitudinal findings also suggest that puberty may slow down, stop, or even reverse patterns of age-related processes in gray and white matter. For example, Goddings et al. (Goddings, Mills et al. 2013), Herting et al. (Herting, Gautam et al. 2014), and Nguyen et al.(Nguyen, McCracken et al. 2013) all found that gray matter trajectories of change were steeper during early pubertal maturation (as indexed by lower hormone levels), followed by a plateau or even reversals in growth, by late puberty (i.e. when hormone levels were high). This was seen for subcortical volumes (Goddings, Mills et al. 2013), gray, white, and amygdala volumes (Herting, Gautam et al. 2014), and cortical thickness (Nguyen, McCracken et al. 2013). In addition, of the 8 longitudinal studies published, 4 of them found a number of sex-specific effects of pubertal development on trajectories of brain maturation (Table 2), suggesting pubertal development may have both similar as well as distinct effects on neurodevelopment in boys and girls. However, there are no longitudinal studies on pituitary and white matter microstructure (DTI or MTR), thus, much less is known about how within-subject *changes* in pubertal development may influence *changes* in pituitary volumes and white matter microstructural properties in adolescent boys and girls.

Together, each of the above studies has provided tremendous insight on the importance of pubertal maturation and sex steroids on structural brain maturation across adolescence. It is clear that while age can be used as a proxy for general developmental changes, pubertal maturation has unique and additive influences on structural neurodevelopmental trajectories. Moving forward, replication, refinement, and expansion of this body of research is needed to further our understanding of how puberty does, and does not, influence the developing adolescent brain. Within this realm, we highlight some of the more pressing challenges and questions that are needed in the next decade regarding our knowledge of pubertal maturation and structural brain development.

a. Direct versus Indirect Mechanisms of Action

The above studies suggest that measuring pubertal development and understanding its contribution towards neurodevelopment patterns are important aspects to understanding brain maturation during the second decade of life. However, the manner in which puberty is influencing brain outcomes as measured by MRI in humans remains unknown. Moreover, most human studies to date have been restricted by their spatial resolution to only assess large global changes among very small subcortical structures, such as the amygdala, and have been unable to meaningfully delineate the hypothalamus. This is unfortunate as some of the most interesting animal studies on sex steroids and adolescent brain development highlight the importance of changes in the hypothalamus as well as show sex-specific neuronal changes in sub-regions of these subcortical structures as a function of sex steroid changes associated with puberty (De Lorme, Schulz et al. 2012). In this regard, the interpretations of the human studies have relied heavily on a large body of supporting evidence from animal research that have shown sex hormones to act directly on neurons and supporting neural processes (see (Juraska, Sisk et al. 2013). For example, sex steroids and pubertal development have been shown to affect synapse number, dendritic branching and outgrowth, as well as pre-myelination events and myelination (Kashon and Sisk 1994, Jordan and Williams 2001, Melcangi, Magnaghi et al. 2001, Melcangi, Magnaghi et al. 2001, Melcangi, Magnaghi et al. 2001, Romeo 2003, Cooke and Woolley 2005, Cooke and Woolley 2005, Garcia-Segura and Melcangi 2006, Zehr, Todd et al. 2006, Ahmed, Zehr et al. 2008, Zehr, Nichols et al. 2008). Similar to humans, the effects of hormones are diverse and region- and sex- specific. That is, male rodents show linear increases in myelination, whereas estradiol during adolescence has been found to inhibit these myelination processes in females (Juraska and Markham 2004, Yates and Juraska 2008). Moreover, estradiol has been linked to greater neuronal and glia loss in the medial prefrontal cortex (Koss, Lloyd et al. 2015) as well as reduction of dendritic spines in the visual cortex (Munoz-Cueto, Garcia-Segura et al. 1990) in females, but not males. Alternatively, pubertal increases in testosterone have been shown to influence the number of neurons and androgen receptors within various subnuclei within larger subcortical regions, including the amygdala (De Lorme, Schulz et al. 2012) and the hypothalamus (Meek, Romeo et al. 1997).

Beyond direct mechanisms, others have also stated the importance of possible indirect mechanisms that may account for these results (Berenbaum, Beltz et al. 2015). That is, sex steroid and physical changes may lead to altered behaviors by the individuals themselves or those around them (e.g. peers, parents), which may drive neurodevelopmental changes (Blakemore, Burnett et al. 2010). While animal studies will remain invaluable in estimating the possible biological mechanisms, future studies should aim to better capture social and behavioral factors to help decipher how these environmental factors may contribute to the previous and future research examining puberty's effects on brain structure across adolescence. Improvements in, and utilization of, high-resolution MRI methods and adolescent-based subcortical atlases may also help improve our understanding of regional specificity within subcortical and hypothalamic changes due to pubertal maturation.

b. Individual differences in timing and progression of puberty on brain development

While known trajectories of brain development and puberty occur in parallel, it has become increasing clear that widespread individual differences exist in these patterns. Although the longitudinal studies have attempted to account for individual differences, more research is needed to truly understand how individual differences in pubertal onset and tempo may influence developmental outcomes. In fact, the influence of individual's progression through puberty and its impact on brain development remains an understudied area (Piekarski, Johnson et al. 2016). To study both onset and progression, studies would need to begin studying children as early as ages 6 or 7, before the onset of adrenarche and the re-activation of the HPG-axis. The same study would then also have to successfully follow the same participants until adult maturation was complete, which could be as late as young adulthood for some. However, if successful, the relative timing of different pubertal hormonal processes on underlying brain trajectories could be especially insightful. For example, a few hormonal studies have found small or even inverse correlations between onset of puberty and menarche, with those with a later pubertal onset experiencing menarche sooner in time than those that enter puberty at an earlier age (MartiHenneberg and Vizmanos 1997, Biro, Huang et al. 2006, Pantsiotou, Papadimitriou et al. 2008). As such, the time in which an individual begins puberty, and/or how quickly he or she progresses to adult maturation may have vital implications for behavior and brain development. For example, there is some data to suggest that a faster tempo of pubertal maturation may be linked with poor psychological outcomes in high-risk girls (Mendle, Leve et al. 2014). Moreover, recent twin-based genetic studies also highlight the importance of individual differences, as pubertal onset was found to be largely driven by genetic factors for both sexes, whereas non-shared environment had a larger impact on some models of progression or tempo (Corley, Beltz et al. 2015).

The needed future longitudinal studies examining onset and progression should also consider the number of repeated-measurements and the time between measurement occasions. A minimum of three-occasion data is needed to examine non-linearity in the rate of change. This seems to be extremely important given the previous findings that puberty's effects on brain structure may be curve-linear (i.e. quadratic or cubic as seen (Goddings, Mills et al. 2013). For instance, if puberty onset leads to an accelerated change between Time 1 and 2 and then shows a gradual decrease by Time 3, having collected only two occasions will provide a misleading estimate of change. Moreover, it is important for MRI-based studies to think about the progression of puberty during study design. All of the longitudinal studies have used a relatively arbitrary interval of ~1.5 to 2 years between measurements. Future studies should consider if hormonal and physical markers should be measured more regularly (~6 to 12 months) to better capture individual differences in pubertal onset and progression and its impact on brain structure.

Lastly, although not specific to puberty per say, future studies need to consider the impact of controlling for intracranial volume when examining structural brain maturation. Using four individual developmental MRI dataset, we have recently replicated that intracranial volume is not stable, but rather changes across adolescent development (Mills, Goddings et al. 2016). More importantly, we also found that the manner by which intracranial volume is considered will affect the perceived influence of sex in models of brain development (Mills,

Goddings et al. 2016). Given there is no consensus in the field; analyses with and without statistically controlling for intracranial volume would be useful to report in future studies, as the results may not only impact sex differences, but also the associations between brain volumes and pubertal development.

c. Hormonal variation and associated factors

A current and remaining challenge in the field is trying to capture and interpret hormone levels during adolescence. In MRI-based research this includes minimizing effects of circadian rhythms as well as monthly variations, making it difficult to capture within- and between- subject differences, especially in girls with irregular cycles following menarche. Many have selected to estimate hormone levels during the early morning (8 to 10 AM) and during the follicular phase of the menstrual cycle, and a few studies were able to collect samples on two consecutive days to have a better estimate of levels. These techniques are useful, but estrogen and progesterone levels are low in females during this time, perhaps truncating the variability of the independent variable of interest (Michaud, Manson et al. 1999, Ahmad, Pollard et al. 2002). Morning measurements are often thought of as basal levels, but it is feasible that variability within the hormone levels across the day and/or cycle could be the most influential factor on brain and behavior outcomes (Granger, Shirtcliff et al. 2003). More research using multiple samples across the day and month and latent-trait modeling approaches (Kirschbaum, Steyer et al. 1990, Stroud, Chen et al. 2016) may help us to better understand when and how hormonal variation may play a role in brain development trajectories.

A number of additional factors that can influence hormone availability should also be considered in future study design (Berenbaum, Beltz et al. 2015). As previously mentioned, different techniques assess free levels of hormones (e.g. those unbound to receptors) versus total levels of hormones (e.g. both bound and unbound levels). Saliva measures total levels, whereas quantification of sex hormone binding globulin (SHBG), which binds sex steroids, allows for a calculation of free testosterone estimates (Anderson 1974, Hofman 2001). However, critical questions remain on understanding how periphery levels relate to active levels within the central nervous system and individual differences in these relationships. The importance of integrating these ideas into future studies are seen by the mediation effects reported between testosterone and brain volumes based on AR genotyping (Perrin, Herve et al. 2008, Paus, Nawaz-Khan et al. 2010, Raznahan, Lee et al. 2010). However, basic research is also needed to better understand how other factors may influence the bioavailability of peripheral hormone levels, such as individual differences in aromatase activity (which converts testosterone to estradiol)(Lephart, Simpson et al. 1992), *de novo* steroid synthesis in brain tissues (Pelletier 2010, Rossetti, Cambiasso et al. 2016), and variation in the composition and neural location of sex steroid receptors as a function of pubertal development (Kashon and Sisk 1994).

d. Behavioral Implications

Amidst all of these hormonal and neurodevelopmental changes, adolescence is also a time when psychopathology begins to emerge. Moreover, sex differences are seen in mental health problems that arise during this time, with disproportionate increases in rates of

anxiety and depression seen in girls (Angold, Costello et al. 1998, Angold, Costello et al. 1999) and an increased prevalence of substance abuse and externalizing disorders in boys (Statistics 2009). In addition, both adaptive and maladaptive changes in risk taking, impulsivity, and reward processing are also seen during adolescence (van Duijvenvoorde, Peters et al. 2016). Thus, there is great interest in understanding how sex hormones and puberty influence typical brain development as a point of reference in identifying differences in biomarkers of risk for, or resilience against, adolescent psychopathology in boys versus girls (Blakemore, Burnett et al. 2010, Naninck, Lucassen et al. 2011, Ladouceur 2012, Ladouceur, Peper et al. 2012). Thus, pubertal-related timing of region specific brain changes may contribute to sex-specific differences in the rapid and disproportionate increases in rates of psychopathology seen between girls and boys (Berenbaum, Beltz et al. 2015, Piekarski, Johnson et al. 2016). As previously alluded to, timing and tempo of pubertal maturation has been linked with various psychological outcomes, including internalizing behaviors (Mendle, Leve et al. 2014) and depression (Angold, Costello et al. 1998, Angold, Costello et al. 1999). Additional evidence for this idea stems from functional MRI studies linking pubertal development and sex steroids to emotional and social processing (Pfeifer, Kahn et al. 2013, Spielberg, Olino et al. 2014, Pagliaccio, Luby et al. 2015, Spielberg, Forbes et al. 2015), emotional-cognitive interactions (Cservenka, Stroup et al. 2015, Tyborowska, Volman et al. 2016), and risk and reward processing (Op de Macks, Gunther Moor et al. 2011, Braams, van Duijvenvoorde et al. 2015, LeMoult, Colich et al. 2015). While recent studies have begun to link pubertal-related changes in brain structure with behavioral correlates (Nguyen, Gower et al. 2016, Nguyen, McCracken et al. 2016), future structural MRI and behavioral studies may help to elucidate if sex, regional patterns of brain growth, and timing of hormonal action on brain structure may interact to impart risk towards maladaptive risk-taking and reward processing, as well as other mental health problems, in adolescent boys and girls.

VI. Conclusions

The existing studies suggest that physical and hormonal changes during puberty are linked with unique patterns of structural brain maturation in humans. Furthermore, pubertal related changes seem to have differing effects on cortical versus subcortical limbic regions. The emerging evidence also suggests that future studies can help us to improve our understanding by designing studies aimed at better capturing hormone variance and individual differences in timing and progression of both physical and hormonal changes across adolescence.

Acknowledgments

This work was supported by the National Institutes of Health (NIMH: R01 MH087563 - PI: Sowell; NICHD: F32 HD078084 - PI: Herting).

References

Aedo AR, Landgren BM, Diczfalusy E. Studies on ovarian and adrenal studies at different phases of the menstrual cycle. IV. The effect of dexamethasone suppression and subsequent ACTH stimulation at different phases of the menstrual cycle and following the administration of 150 mg of

depot-medroxy-progesterone acetate (DMPA). Contraception. 1981; 24(5):543–558. [PubMed: 6274585]

Ahmad N, Pollard TM, Unwin N. The optimal timing of blood collection during the menstrual cycle for the assessment of endogenous sex hormones: can interindividual differences in levels over the whole cycle be assessed on a single day? Cancer Epidemiol Biomarkers Prev. 2002; 11(1):147–151. [PubMed: 11815414]

Ahmed EI, Zehr JL, Schulz KM, Lorenz BH, DonCarlos LL, Sisk CL. Pubertal hormones modulate the addition of new cells to sexually dimorphic brain regions. Nat Neurosci. 2008; 11(9):995–997. [PubMed: 19160494]

Albrecht L, Styne D. Laboratory testing of gonadal steroids in children. Pediatr Endocrinol Rev. 2007; 5(Suppl 1):599–607. [PubMed: 18167469]

Alexander AL, Eun Lee J, Lazar M, Field AS. Diffusion Tensor Imaging of the Brain. Neurotherapeutics. 2007; 4(3):316–329. [PubMed: 17599699]

Anderson DC. Sex-hormone-binding globulin. Clin Endocrinol (Oxf). 1974; 3(1):69–96. [PubMed: 4134992]

Angold A, Costello EJ, Erkanli A, Worthman CM. Pubertal changes in hormone levels and depression in girls. Psychological Medicine. 1999; 29(5):1043–1053. [PubMed: 10576297]

Angold A, Costello EJ, Worthman CM. Puberty and depression: the roles of age, pubertal status and pubertal timing. Psychol Med. 1998; 28(1):51–61. [PubMed: 9483683]

Ankarberg C, Norjavaara E. Diurnal rhythm of testosterone secretion before and throughout puberty in healthy girls: correlation with 17beta-estradiol and dehydroepiandrosterone sulfate. Journal of Clinical Endocrinology & Metabolism. 1999; 84(3):975–984. [PubMed: 10084582]

Ankarberg-Lindgren C, Norjavaara E. Changes of diurnal rhythm and levels of total and free testosterone secretion from pre to late puberty in boys: testis size of 3 ml is a transition stage to puberty. European Journal of Endocrinology. 2004; 151(6):747–757. [PubMed: 15588242]

Apter D. Serum steroids and pituitary hormones in female puberty: a partly longitudinal study. Clinical endocrinology. 1980; 12(2):107–120. [PubMed: 6249519]

Apter D, Viinikka L, Vihko R. Hormonal pattern of adolescent menstrual cycles. The Journal of clinical endocrinology and metabolism. 1978; 47(5):944–954. [PubMed: 122422]

Asato MR, Terwilliger R, Woo J, Luna B. White Matter Development in Adolescence: A DTI Study. Cereb Cortex. 2010

Bava S, Thayer R, Jacobus J, Ward M, Jernigan L, Tapert SF. Longitudinal characterization of white matter maturation during adolescence. Brain Res. 2010; 1327:38–46. [PubMed: 20206151]

Berenbaum, SA., Beltz, AM., Corley, R. Advances in Child Development and Behavior. Vol. 48. St Louis: Academic Press; 2015. The Importance of Puberty for Adolescent Development: Conceptualization and Measurement; p. 53-85.

Berenbaum SA, Beltz AM, Corley R. The importance of puberty for adolescent development: conceptualization and measurement. Adv Child Dev Behav. 2015; 48:53–92. [PubMed: 25735941]

Biro FM, Huang B, Crawford B, Lucky AW, Striegel-Moore R, Barton BA, Daniels S. Pubertal correlates in black and white girls. J Pediatr. 2006; 148(2):234–240. [PubMed: 16492435]

Biro FM, Lucky AW, Huster GA, Morrison JA. Pubertal staging in boys. J Pediatr. 1995; 127(1):100–102. [PubMed: 7608791]

Blakemore SJ, Burnett S, Dahl RE. The role of puberty in the developing adolescent brain. Hum Brain Mapp. 2010; 31(6):926–933. [PubMed: 20496383]

Blanton RE, Cooney RE, Joormann J, Eugene F, Glover H, Gotlib IH. Pubertal stage and brain anatomy in girls. Neuroscience. 2012; 217:105–112. [PubMed: 22569152]

Bonat S, Pathomvanich A, Keil F, Field AE, Yanovski JA. Self-assessment of pubertal stage in overweight children. Pediatrics. 2002; 110(4):743–747. [PubMed: 12359788]

Boyar R, Finkelstein J, Roffwarg H, Kapen S, Weitzman E, Hellman L. Synchronization of augmented luteinizing hormone secretion with sleep during puberty. N Engl J Med. 1972; 287(12):582–586. [PubMed: 4341276]

Braams BR, van Duijvenvoorde AC, Peper JS, Crone EA. Longitudinal changes in adolescent risk-taking: a comprehensive study of neural responses to rewards, pubertal development, and risk-taking behavior. J Neurosci. 2015; 35(18):7226–7238. [PubMed: 25948271]

Bramen JE, Hranilovich JA, Dahl RE, Chen J, Rosso C, Forbes EE, Dinov ID, Worthman CM, Sowell ER. Sex matters during adolescence: testosterone-related cortical thickness maturation differs between boys and girls. PloS one. 2012; 7(3):e33850. [PubMed: 22479458]

Bramen JE, Hranilovich JA, Dahl RE, Forbes EE, Chen J, Toga AW, Dinov ID, Worthman CM, Sowell ER. Puberty influences medial temporal lobe and cortical gray matter maturation differently in boys than girls matched for sexual maturity. Cereb Cortex. 2011; 21(3):636–646. [PubMed: 20713504]

Bramswig J, Dubbers A. Disorders of pubertal development. Dtsch Arztebl Int. 2009; 106(17):295–303. quiz 304. [PubMed: 19547638]

Brooks-Gunn J, Warren MP, Rosso J, Gargiulo J. Validity of self-report measures of girls' pubertal status. Child Development. 1987; 58(3):829–841. [PubMed: 3608653]

Brouwer RM, Koenis MM, Schnack HG, van Baal GC, van Soelen IL, Boomsma DI, Hulshoff Pol HE. Longitudinal development of hormone levels and grey matter density in 9 and 12-year-old twins. Behav Genet. 2015; 45(3):313–323. [PubMed: 25656383]

Bui HN, Sluss PM, Blincko S, Knol DL, Blankenstein MA, Heijboer AC. Dynamics of serum testosterone during the menstrual cycle evaluated by daily measurements with an ID-LC-MS/MS method and a 2nd generation automated immunoassay. Steroids. 2013; 78(1):96–101. [PubMed: 23127814]

Buttler RM, Peper JS, Crone EA, Lentjes EG, Blankenstein MA, Heijboer AC. Reference values for salivary testosterone in adolescent boys and girls determined using Isotope-Dilution Liquid-Chromatography Tandem Mass Spectrometry (ID-LC-MS/MS). Clin Chim Acta. 2016; 456:15–18. [PubMed: 26920638]

Carskadon MA, Acebo C. A self-administered rating scale for pubertal development. J Adolesc Health. 1993; 14(3):190–195. [PubMed: 8323929]

Chavarria MC, Sanchez FJ, Chou YY, Thompson PM, Luders E. Puberty in the corpus callosum. Neuroscience. 2014; 265:1–8. [PubMed: 24468104]

Cooke BM, Woolley CS. Gonadal hormone modulation of dendrites in the mammalian CNS. J Neurobiol. 2005; 64(1):34–46. [PubMed: 15884004]

Cooke BM, Woolley CS. Sexually dimorphic synaptic organization of the medial amygdala. J Neurosci. 2005; 25(46):10759–10767. [PubMed: 16291949]

Corley RP, Beltz AM, Wadsworth SJ, Berenbaum SA. Genetic influences on pubertal development and links to behavior problems. Behav Genet. 2015; 45(3):294–312. [PubMed: 25903988]

Crone EA, Elzinga BM. Changing brains: how longitudinal functional magnetic resonance imaging studies can inform us about cognitive and social-affective growth trajectories. Wiley Interdiscip Rev Cogn Sci. 2015; 6(1):53–63. [PubMed: 26262928]

Cservenka A, Stroup ML, Etkin A, Nagel BJ. The effects of age, sex, and hormones on emotional conflict-related brain response during adolescence. Brain Cogn. 2015; 99:135–150. [PubMed: 26175008]

Cutler GB Jr, Loriaux DL. Andrenarche and its relationship to the onset of puberty. Fed Proc. 1980; 39(7):2384–2390. [PubMed: 6445284]

De Bellis MD, Keshavan MS, Beers SR, Hall J, Frustaci K, Masalehdan A, Noll J, Boring AM. Sex differences in brain maturation during childhood and adolescence. Cerebral Cortex. 2001; 11(6): 552–557. [PubMed: 11375916]

De Lorme KC, Schulz KM, Salas-Ramirez KY, Sisk CL. Pubertal testosterone organizes regional volume and neuronal number within the medial amygdala of adult male Syrian hamsters. Brain Res. 2012; 1460:33–40. [PubMed: 22578470]

Delemarre-van de Waal HA, van Coeverden SC, Rotteveel J. Hormonal determinants of pubertal growth. J Pediatr Endocrinol Metab. 2001; 14(Suppl 6):1521–1526. [PubMed: 11837509]

Dorn LD, Biro FM. Puberty and Its Measurement: A Decade in Review. Journal of Research on Adolescence. 2011; 21(1):180–195.

Dorn LD, Susman EJ. The Audio Computer-Assisted Self-Interview method for self-report of puberty: is it ready for prime time? J Adolesc Health. 2011; 48(4):323–324. [PubMed: 21402258]

Dorn LD, Susman EJ, Nottelmann ED, Inoff-Germain G, Chrousos GP. Perceptions of Puberty: Adolescent, Parent, and Health Care Personnel. Developmental Psychology. 1990; 26(2):322–329.

Garcia-Segura LM, Melcangi RC. Steroids and glial cell function. Glia. 2006; 54(6):485–498. [PubMed: 16906540]

Giedd J. Brain development, IX: human brain growth. Am J Psychiatry. 1999; 156(1):4. [PubMed: 9892290]

Giedd JN, Blumenthal J, Jeffries O, Castellanos FX, Liu H, Zijdenbos A, Paus T, Evans AC, Rapoport JL. Brain development during childhood and adolescence: a longitudinal MRI study. Nature Neuroscience. 1999; 2(10):861–863. [PubMed: 10491603]

Giedd JN, Vaituzis AC, Hamburger SD, Lange N, Rajapakse C, Kaysen D, Vauss C, Rapoport JL. Quantitative MRI of the temporal lobe, amygdala, and hippocampus in normal human development: ages 4–18 years. Journal of Comparitive Neurology. 1996; 366(2):223–230.

Goddings AL, Mills KL, Clasen LS, Giedd JN, Viner RM, Blakemore SJ. The influence of puberty on subcortical brain development. NeuroImage. 2013

Granger DA, Shirtcliff EA, Booth A, Kivlighan KT, Schwartz EB. The “trouble” with salivary testosterone. Psychoneuroendocrinology. 2004; 29(10):1229–1240. [PubMed: 15288702]

Granger DA, Shirtcliff EA, Zahn-Waxler C, Usher B, Klimes-Dougan B, Hastings P. Salivary testosterone diurnal variation and psychopathology in adolescent males and females: individual differences and developmental effects. Dev Psychopathol. 2003; 15(2):431–449. [PubMed: 12931836]

Grumbach, MM., Styne, DM. Puberty: Ontogeny, neuroendocrinology, physiology, and disorders. In: Larsen, PR.Kronenberg, HM.Melmed, S., Polonsky, KS., editors. Williams textbook of endocrinology. Philadelphia: W. B. Saunders; 2003. p. 1115-1286.

Haber SN. The primate basal ganglia: parallel and integrative networks. J Chem Neuroanat. 2003; 26(4):317–330. [PubMed: 14729134]

Herold DA, Fitzgerald RL. Immunoassays for testosterone in women: better than a guess? Clin Chem. 2003; 49(8):1250–1251. [PubMed: 12881438]

Herting MM, Gautam P, Spielberg M, Dahl RE, Sowell ER. A longitudinal study: changes in cortical thickness and surface area during pubertal maturation. PLoS One. 2015; 10(3):e0119774. [PubMed: 25793383]

Herting MM, Gautam P, Spielberg M, Kan E, Dahl E, Sowell ER. The role of testosterone and estradiol in brain volume changes across adolescence: a longitudinal structural MRI study. Hum Brain Mapp. 2014; 35(11):5633–5645. [PubMed: 24977395]

Herting MM, Maxwell EC, Irvine C, Nagel BJ. The impact of sex, puberty, and hormones on white matter microstructure in adolescents. Cerebral Cortex. 2012; 22(9):1979–1992. [PubMed: 22002939]

Hofman LF. Human saliva as a diagnostic specimen. Journal of Nutrition. 2001; 131(5):1621s–1625s. [PubMed: 11340128]

Hu S, Pruessner JC, Coupe P, Collins DL. Volumetric analysis of medial temporal lobe structures in brain development from childhood to adolescence. NeuroImage. 2013; 74:276–287. [PubMed: 23485848]

Ikegami S, Moriwake T, Tanaka H, Inoue M, Kubo T, Suzuki S, Kanzakili S, Seino Y. An ultrasensitive assay revealed age-related changes in serum oestradiol at low concentrations in both sexes from infancy to puberty. Clin Endocrinol (Oxf). 2001; 55(6):789–795. [PubMed: 11895221]

Jernigan TL, Trauner DA, Hesselink JR, Tallal PA. Maturation of human cerebrum observed in vivo during adolescence. Brain. 1991; 114(Pt 5):2037–2049. [PubMed: 1933232]

Jordan CL, Williams TJ. Testosterone regulates terminal Schwann cell number and junctional size during developmental synapse elimination. Dev Neurosci. 2001; 23(6):441–451. [PubMed: 11872945]

Juraska JM, Markham JA. The cellular basis for volume changes in the rat cortex during puberty: white and gray matter. Ann N Y Acad Sci. 2004; 1021:431–435. [PubMed: 15251923]

Juraska JM, Sisk CL, DonCarlos LL. Sexual differentiation of the adolescent rodent brain: hormonal influences and developmental mechanisms. Hormones and behavior. 2013; 64(2):203–210. [PubMed: 23998664]

Kashon ML, Sisk CL. Pubertal maturation is associated with an increase in the number of androgen receptor-immunoreactive cells in the brains of male ferrets. Brain Res Dev Brain Res. 1994; 78(2): 237–242. [PubMed: 8026077]

Kirschbaum C, Steyer R, Eid M, Patalla U, Schwenkmezger P, Hellhammer DH. Cortisol and behavior: 2. Application of a latent state-trait model to salivary cortisol. Psychoneuroendocrinology. 1990; 15(4):297–307. [PubMed: 2087536]

Knobil E. The neuroendocrine control of ovulation. Hum Reprod. 1988; 3(4):469–472. [PubMed: 3292570]

Koolschijn PC, Crone EA. Sex differences and structural brain maturation from childhood to early adulthood. Developmental cognitive neuroscience. 2013; 5:106–118. [PubMed: 23500670]

Koolschijn PC, Peper JS, Crone EA. The influence of sex steroids on structural brain maturation in adolescence. PloS one. 2014; 9(1):e83929. [PubMed: 24416184]

Koss WA, Lloyd MM, Sadowski RN, Wise LM, Juraska JM. Gonadectomy before puberty increases the number of neurons and glia in the medial prefrontal cortex of female, but not male, rats. Dev Psychobiol. 2015; 57(3):305–312. [PubMed: 25782706]

Ladouceur CD. Neural systems supporting cognitive-affective interactions in adolescence: the role of puberty and implications for affective disorders. Front Integr Neurosci. 2012; 6:65. [PubMed: 22969712]

Ladouceur CD, Peper JS, Crone EA, Dahl RE. White matter development in adolescence: the influence of puberty and implications for affective disorders. Dev Cogn Neurosci. 2012; 2(1):36–54. [PubMed: 22247751]

Lamb MM, Beers L, Reed-Gillette D, McDowell MA. Feasibility of an Audio Computer-Assisted Self-Interview method to self-assess sexual maturation. J Adolesc Health. 2011; 48(4):325–330. [PubMed: 21402259]

Lebel C, Gee M, Camicioli R, Wieler M, Martin W, Beaulieu C. Diffusion tensor imaging of white matter tract evolution over the lifespan. Neuroimage. 2012; 60(1):340–352. [PubMed: 22178809]

LeMoult J, Colich NL, Sherdell L, Hamilton JP, Gotlib IH. Influence of menarche on the relation between diurnal cortisol production and ventral striatum activity during reward anticipation. Soc Cogn Affect Neurosci. 2015; 10(9):1244–1250. [PubMed: 25678549]

Lenroot RK, Gogtay N, Greenstein DK, Wells EM, Wallace GL, Clasen LS, Blumenthal JD, Lerch J, Zijdenbos AP, Evans AC, Thompson PM, Giedd JN. Sexual dimorphism of brain developmental trajectories during childhood and adolescence. Neuroimage. 2007; 36(4):1065–1073. [PubMed: 17513132]

Lephart ED, Simpson ER, McPhaul MJ, Kilgore MW, Wilson JD, Ojeda SR. Brain aromatase cytochrome P-450 messenger RNA levels and enzyme activity during prenatal and perinatal development in the rat. Brain Res Mol Brain Res. 1992; 16(3–4):187–192. [PubMed: 1337928]

Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Archives of disease in childhood. 1969; 44(235):291–303. [PubMed: 5785179]

Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child. 1970; 45(239):13–23. [PubMed: 5440182]

MartiHenneberg C, Vizmanos B. The duration of puberty in girls is related to the timing of its onset. Journal of Pediatrics. 1997; 131(4):618–621. [PubMed: 9386670]

McGowan JC. The physical basis of magnetization transfer imaging. Neurology. 1999; 53(5 Suppl 3):S3–7.

Meek LR, Romeo RD, Novak CM, Sisk CL. Actions of testosterone in prepubertal and postpubertal male hamsters: dissociation of effects on reproductive behavior and brain androgen receptor immunoreactivity. Horm Behav. 1997; 31(1):75–88. [PubMed: 9109601]

Melcangi RC, Magnaghi V, Galbiati M, Martini L. Formation and effects of neuroactive steroids in the central and peripheral nervous system. Int Rev Neurobiol. 2001; 46:145–176. [PubMed: 11599299]

Melcangi RC, Magnaghi V, Galbiati M, Martini L. Glial cells: a target for steroid hormones. Prog Brain Res. 2001; 132:31–40. [PubMed: 11544999]

Melcangi RC, Magnaghi V, Galbiati M, Martini L. Steroid effects on the gene expression of peripheral myelin proteins. Horm Behav. 2001; 40(2):210–214. [PubMed: 11534984]

Mendle J, Leve LD, Van Ryzin M, Natsuaki MN. Linking Childhood Maltreatment with Girls' Internalizing Symptoms: Early Puberty as a Tipping Point. J Res Adolesc. 2014; 24(4):689–702. [PubMed: 25419091]

Menzies L, Goddings AL, Whitaker KJ, Blakemore SJ, Viner RM. The effects of puberty on white matter development in boys. Dev Cogn Neurosci. 2015; 11:116–128. [PubMed: 25454416]

Michaud DS, Manson JE, Spiegelman D, Barbieri RL, Sepkovic DW, Bradlow HL, Hankinson SE. Reproducibility of plasma and urinary sex hormone levels in premenopausal women over a one-year period. Cancer Epidemiol Biomarkers Prev. 1999; 8(12):1059–1064. [PubMed: 10613337]

Mills KL, Goddings AL, Herting MM, Meuwese R, Blakemore J, Crone EA, Dahl RE, Guroglu B, Raznahan A, Sowell ER, Tamnes CK. Structural brain development between childhood and adulthood: Convergence across four longitudinal samples. Neuroimage. 2016; 141:273–281. [PubMed: 27453157]

Morris NM, Udry JR. Validation of a self-administered instrument to assess stage of adolescent development. J Youth Adolesc. 1980; 9(3):271–280. [PubMed: 24318082]

Munoz-Cueto JA, Garcia-Segura LM, Ruiz-Marcos A. Developmental sex differences and effect of ovariectomy on the number of cortical pyramidal cell dendritic spines. Brain research. 1990; 515(1–2):64–68. [PubMed: 2357578]

Murray CR, Simmons JG, Allen NB, Byrne ML, Mundy LK, Seal ML, Patton GC, Olsson CA, Whittle S. Associations between dehydroepiandrosterone (DHEA) levels, pituitary volume, and social anxiety in children. Psychoneuroendocrinology. 2016; 64:31–39. [PubMed: 26600008]

Naninck EF, Lucassen PJ, Bakker J. Sex differences in adolescent depression: do sex hormones determine vulnerability? J Neuroendocrinol. 2011; 23(5):383–392. [PubMed: 21418338]

Neufang S, Specht K, Hausmann M, Gunturkun O, Herpertz-Dahlmann B, Fink GR, Konrad K. Sex differences and the impact of steroid hormones on the developing human brain. Cereb Cortex. 2009; 19(2):464–473. [PubMed: 18550597]

Nguyen TV, Gower P, Albaugh D, Botteron KN, Hudziak JJ, Fonov VS, Collins L, Ducharme S, McCracken JT. The developmental relationship between DHEA and visual attention is mediated by structural plasticity of cortico-amygdalar networks. Psychoneuroendocrinology. 2016; 70:122–133. [PubMed: 27236606]

Nguyen TV, McCracken J, Ducharme S, Botteron KN, Mahabir M, Johnson W, Israel M, Evans AC, Karama S. Testosterone-Related Cortical Maturation Across Childhood and Adolescence. Cerebral cortex. 2013; 23(6):1424–1432. [PubMed: 22617851]

Nguyen TV, McCracken JT, Albaugh MD, Botteron KN, Hudziak JJ, Ducharme S. A testosterone-related structural brain phenotype predicts aggressive behavior from childhood to adulthood. Psychoneuroendocrinology. 2016; 63:109–118. [PubMed: 26431805]

Nguyen TV, McCracken JT, Ducharme S, Cropp BF, Botteron KN, Evans AC, Karama S. Interactive effects of dehydroepiandrosterone and testosterone on cortical thickness during early brain development. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2013; 33(26):10840–10848. [PubMed: 23804104]

Nottelmann ED, Susman EJ, Dorn LD, Inoff-Germain G, Loriaux L, Cutler GB Jr, Chrousos GP. Developmental processes in early adolescence. Relations among chronologic age, pubertal stage, height, weight, and serum levels of gonadotropins, sex steroids, and adrenal androgens. J Adolesc Health Care. 1987; 8(3):246–260. [PubMed: 3583875]

Op de Macks ZA, Gunther Moor B, Overgaauw S, Guroglu B, Dahl E, Crone EA. Testosterone levels correspond with increased ventral striatum activation in response to monetary rewards in adolescents. Dev Cogn Neurosci. 2011; 1(4):506–516. [PubMed: 22436568]

Pagliaccio D, Luby JL, Bogdan R, Agrawal A, Gaffrey S, Belden AC, Botteron KN, Harms MP, Barch DM. Amygdala functional connectivity, HPA axis genetic variation, and life stress in children and relations to anxiety and emotion regulation. J Abnorm Psychol. 2015; 124(4):817–833. [PubMed: 26595470]

Pangelinan MM, Leonard G, Perron M, Pike GB, Richer L, Veillette S, Pausova Z, Paus T. Puberty and testosterone shape the corticospinal tract during male adolescence. Brain structure & function. 2014

Pantsiotou S, Papadimitriou A, Douros K, Priftis K, Nicolaidou P, Fretzayas A. Maturational tempo differences in relation to the timing of the onset of puberty in girls. Acta Paediatr. 2008; 97(2): 217–220. [PubMed: 18177444]

Parker LN. Adrenarche. Endocrinol Metab Clin North Am. 1991; 20(1):71–83. [PubMed: 2029889]

Paus T. Growth of white matter in the adolescent brain: myelin or axon? Brain Cogn. 2010; 72(1):26–35. [PubMed: 19595493]

Paus T, Nawaz-Khan I, Leonard G, Perron M, Pike B, Pitiot A, Richer L, Susman E, Veillette S, Pausova Z. Sexual dimorphism in the adolescent brain: Role of testosterone and androgen receptor in global and local volumes of grey and white matter. Horm Behav. 2010; 57(1):63–75. [PubMed: 19703457]

Pelletier G. Steroidogenic enzymes in the brain: morphological aspects. Prog Brain Res. 2010; 181:193–207. [PubMed: 20478439]

Peper JS, Brouwer RM, Schnack HG, van Baal GC, van Leeuwen M, van den Berg SM, Delemarre-Van de Waal HA, Boomsma DI, Kahn RS, Hulshoff Pol HE. Sex steroids and brain structure in pubertal boys and girls. Psychoneuroendocrinology. 2009; 34(3):332–342. [PubMed: 18980810]

Peper JS, Brouwer RM, Schnack HG, van Baal GC, van Leeuwen M, van den Berg SM, Delemarre-Van de Waal HA, Janke AL, Collins DL, Evans AC, Boomsma DI, Kahn RS, Hulshoff Pol HE. Cerebral white matter in early puberty is associated with luteinizing hormone concentrations. Psychoneuroendocrinology. 2008; 33(7):909–915. [PubMed: 18640784]

Peper JS, Brouwer RM, van Leeuwen M, Schnack HG, Boomsma DI, Kahn RS, Hulshoff Pol HE. HPG-axis hormones during puberty: a study on the association with hypothalamic and pituitary volumes. Psychoneuroendocrinology. 2010; 35(1):133–140. [PubMed: 19570613]

Peper JS, de Reus MA, van den Heuvel MP, Schutter DJ. Short fused? associations between white matter connections, sex steroids, and aggression across adolescence. Hum Brain Mapp. 2015; 36(3):1043–1052. [PubMed: 25359710]

Peper JS, Koolschijn PC, Crone EA. Development of risk taking: contributions from adolescent testosterone and the orbito-frontal cortex. J Cogn Neurosci. 2013; 25(12):2141–2150. [PubMed: 23859649]

Peper JS, Schnack HG, Brouwer RM, Van Baal GC, Pjetri E, Szekely E, van Leeuwen M, van den Berg SM, Collins DL, Evans AC, Boomsma DI, Kahn RS, Hulshoff Pol HE. Heritability of regional and global brain structure at the onset of puberty: a magnetic resonance imaging study in 9-year-old twin pairs. Hum Brain Mapp. 2009; 30(7):2184–2196. [PubMed: 19294640]

Perrin JS, Herve PY, Leonard G, Perron M, Pike B, Pitiot A, Richer S, Veillette S, Pausova Z, Paus T. Growth of white matter in the adolescent brain: role of testosterone and androgen receptor. J Neurosci. 2008; 28(38):9519–9524. [PubMed: 18799683]

Perrin JS, Leonard G, Perron M, Pike GB, Pitiot A, Richer L, Veillette S, Pausova Z, Paus T. Sex differences in the growth of white matter during adolescence. Neuroimage. 2009; 45(4):1055–1066. [PubMed: 19349224]

Petersen A, Crockett L, Richards M, Boxer A. A self-report measure of pubertal status: Reliability, validity, and initial norms. Journal of Youth and Adolescence. 1988; 17:117–133. [PubMed: 24277579]

Pfeifer JH, Kahn LE, Merchant JS, Peake SJ, Veroude K, Masten CL, Lieberman MD, Mazziotta JC, Dapretto M. Longitudinal change in the neural bases of adolescent social self-evaluations: effects of age and pubertal development. J Neurosci. 2013; 33(17):7415–7419. [PubMed: 23616547]

Phelps EA. Human emotion and memory: interactions of the amygdala and hippocampal complex. Curr Opin Neurobiol. 2004; 14(2):198–202. [PubMed: 15082325]

Piekarski DJ, Johnson CM, Boivin JR, Thomas AW, Lin WC, Delevich K, E MG, Wilbrecht L. Does puberty mark a transition in sensitive periods for plasticity in the associative neocortex? Brain Res. 2016

Raznahan A, Lee Y, Stidd R, Long R, Greenstein D, Clasen L, Addington A, Gogtay N, Rapoport L, Giedd JN. Longitudinally mapping the influence of sex and androgen signaling on the dynamics

of human cortical maturation in adolescence. Proc Natl Acad Sci U S A. 2010; 107(39):16988–16993. [PubMed: 20841422]

Raznahan A, Shaw P, Lalonde F, Stockman M, Wallace L, Greenstein D, Clasen L, Gogtay N, Giedd JN. How does your cortex grow? The Journal of neuroscience : the official journal of the Society for Neuroscience. 2011; 31(19):7174–7177. [PubMed: 21562281]

Richter-Levin G, Akirav I. Amygdala-hippocampus dynamic interaction in relation to memory. Mol Neurobiol. 2000; 22(1–3):11–20. [PubMed: 11414274]

Rilling JK, Worthman CM, Campbell BC, Stallings JF, Mbizva M. Ratios of plasma and salivary testosterone throughout puberty: production versus bioavailability. Steroids. 1996; 61(6):374–378. [PubMed: 8776800]

Romeo RD. Puberty: a period of both organizational and activational effects of steroid hormones on neurobehavioural development. J Neuroendocrinol. 2003; 15(12):1185–1192. [PubMed: 14636181]

Rossetti MF, Cambiasso MJ, Holschbach MA, Cabrera R. Oestrogens and Progestagens: Synthesis and Action in the Brain. J Neuroendocrinol. 2016; 28(7)

Rothman MS, Carlson NE, Xu M, Wang C, Swerdloff R, Lee P, Goh H, Ridgway EC, Wierman ME. Reexamination of testosterone, dihydrotestosterone, estradiol and estrone levels across the menstrual cycle and in postmenopausal women measured by liquid chromatography-tandem mass spectrometry. Steroids. 2011; 76(1–2):177–182. [PubMed: 21070796]

Saenger P, Dimartino-Nardi J. Premature adrenarche. J Endocrinol Invest. 2001; 24(9):724–733. [PubMed: 11716159]

Satterthwaite TD, Vandekar S, Wolf H, Ruparel K, Roalf R, Jackson C, Elliott A, Bilker WB, Calkins ME, Prabhakaran K, Davatzikos C, Hakonarson H, Gur E, Gur RC. Sex differences in the effect of puberty on hippocampal morphology. J Am Acad Child Adolesc Psychiatry. 2014; 53(3):341–350 e341. [PubMed: 24565361]

Schmithorst VJ, Holland SK, Dardzinski BJ. Developmental differences in white matter architecture between boys and girls. Hum Brain Mapp. 2008; 29(6):696–710. [PubMed: 17598163]

Selby C. Sex hormone binding globulin: origin, function and clinical significance. Ann Clin Biochem. 1990; 27(Pt 6):532–541. [PubMed: 2080856]

Shirtcliff EA, Dahl RE, Pollak SD. Pubertal development: correspondence between hormonal and physical development. Child development. 2009; 80(2):327–337. [PubMed: 19466995]

Shirtcliff EA, Granger DA, Schwartz E, Curran MJ. Use of salivary biomarkers in biobehavioral research: cotton-based sample collection methods can interfere with salivary immunoassay results. Psychoneuroendocrinology. 2001; 26(2):165–173. [PubMed: 11087962]

Shirtcliff EA, Granger DA, Schwartz EB, Curran MJ, Booth A, Overman WH. Assessing estradiol in biobehavioral studies using saliva and blood spots: simple radioimmunoassay protocols, reliability, and comparative validity. Hormones and behavior. 2000; 38(2):137–147. [PubMed: 10964528]

Simmonds DJ, Hallquist MN, Asato M, Luna B. Developmental stages and sex differences of white matter and behavioral development through adolescence: a longitudinal diffusion tensor imaging (DTI) study. NeuroImage. 2014; 92:356–368. [PubMed: 24384150]

Singer, JD., Willet, JB. Applied Longitudinal Data Analysis: Modeling Change and Event Occurrence. New York: Oxford University Press, Inc; 2003.

Sowell ER, Peterson BS, Thompson PM, Welcome SE, Henkenius AL, Toga AW. Mapping cortical change across the human life span. Nat Neurosci. 2003; 6(3):309–315. [PubMed: 12548289]

Sowell ER, Trauner DA, Gamst A, Jernigan TL. Development of cortical and subcortical brain structures in childhood and adolescence: a structural MRI study. Developmental Medicine and Child Neurology. 2002; 44(1):4–16. [PubMed: 11811649]

Spielberg JM, Forbes EE, Ladouceur CD, Worthman CM, Olino TM, Ryan ND, Dahl RE. Pubertal testosterone influences threat-related amygdala-orbitofrontal cortex coupling. Soc Cogn Affect Neurosci. 2015; 10(3):408–415. [PubMed: 24795438]

Spielberg JM, Olino TM, Forbes EE, Dahl RE. Exciting fear in adolescence: does pubertal development alter threat processing? Dev Cogn Neurosci. 2014; 8:86–95. [PubMed: 24548554]

Statistics, FoCaF. American's Children: Key National Indicators of Well-Being. US Government Printing Office; Washington, DC: 2009.

Stroud CB, Chen FR, Doane LD, Granger DA. Individual differences in early adolescents' latent trait cortisol (LTC): Relation to recent acute and chronic stress. Psychoneuroendocrinology. 2016; 70:38–46. [PubMed: 27155256]

Taylor SJ, Whincup PH, Hindmarsh PC, Lampe F, Odoki K, Cook DG. Performance of a new pubertal self-assessment questionnaire: a preliminary study. Paediatr Perinat Epidemiol. 2001; 15(1):88–94. [PubMed: 11237120]

Toga AW, Thompson PM, Sowell ER. Mapping brain maturation. Trends in Neurosciences. 2006; 29(3):148–159. [PubMed: 16472876]

Treloar AE, Boynton RE, Behn BG, Brown BW. Variation of the human menstrual cycle through reproductive life. International journal of fertility. 1967; 12(1 Pt 2):77–126. [PubMed: 5419031]

Tyborowska A, Volman I, Smeekens S, Toni I, Roelofs K. Testosterone during Puberty Shifts Emotional Control from Pulvinar to Anterior Prefrontal Cortex. J Neurosci. 2016; 36(23):6156–6164. [PubMed: 27277794]

van Duijvenvoorde AC, Peters S, Braams BR, Crone EA. What motivates adolescents? Neural responses to rewards and their influence on adolescents' risk taking, learning, and cognitive control. Neurosci Biobehav Rev. 2016

Wang Y, Adamson C, Yuan W, Altaye M, Rajagopal A, Byars AW, Holl SK. Sex differences in white matter development during adolescence: a DTI study. Brain Res. 2012; 1478:1–15. [PubMed: 22954903]

Winkler AM, Sabuncu MR, Yeo BT, Fischl B, Greve N, Kochunov P, Nichols E, Blangero J, Glahn DC. Measuring and comparing brain cortical surface area and other areal quantities. NeuroImage. 2012; 61(4):1428–1443. [PubMed: 22446492]

Wong AP, Pipitone J, Park T, Dickie EW, Leonard G, Perron M, Pike G, Richer L, Veillette S, Chakravarty MM, Pausova Z, Paus T. Estimating volumes of the pituitary gland from T1-weighted magnetic-resonance images: effects of age, puberty, testosterone, and estradiol. Neuroimage. 2014; 94:216–221. [PubMed: 24632090]

Wu FC, Brown DC, Butler GE, Stirling HF, Kelnar CJ. Early morning plasma testosterone is an accurate predictor of imminent pubertal development in prepubertal boys. J Clin Endocrinol Metab. 1993; 76(1):26–31. [PubMed: 8421096]

Yates MA, Juraska JM. Pubertal ovarian hormone exposure reduces the number of myelinated axons in the splenium of the rat corpus callosum. Exp Neurol. 2008; 209(1):284–287. [PubMed: 17963756]

Zehr JL, Nichols LR, Schulz KM, Sisk CL. Adolescent development of neuron structure in dentate gyrus granule cells of male Syrian hamsters. Dev Neurobiol. 2008; 68(14):1517–1526. [PubMed: 18792070]

Zehr JL, Todd BJ, Schulz KM, McCarthy MM, Sisk CL. Dendritic pruning of the medial amygdala during pubertal development of the male Syrian hamster. J Neurobiol. 2006; 66(6):578–590. [PubMed: 16555234]

Highlights

- Physical and hormonal markers of puberty have been linked with brain structure
- Challenges are discussed, including capturing variability in hormone levels
- More research is needed on individual differences in pubertal onset and progression

Table 1

Cross-sectional studies examining puberty and brain structure

	First Author	Year	Physical Marker(s)	Hormone(s)	Ages	Sex	Brain Outcomes	Main Findings
1	Perrin ◆	2008	PDS	• Testosterone (blood)	12–18 yrs	204 M/204 F	• VBM white matter density • MTR per lobe (frontal, parietal, temporal, occipital)	• ♂ Positive correlation between testosterone & white matter • ♂ Negative correlation between testosterone & MTR • ♂ Larger effects with short vs. long AR genotype
2	Peper ^	2008	Expert TS	• LH (urine)	9 yrs; twin-pairs	57 M/47 F	• VBM global white matter • VBM gray matter density	• ♂♀ Positive correlation between LH & global, cingulum, medial temporal, & splenium white matter density
3	Perrin ◆	2009	PDS	• Testosterone (blood)	12–18 yrs	204 M/204 F	• VBM white matter volume & density • MTR	• ♂ PDS relates to larger parietal & occipital white matter volumes • ♂ PDS predicts smaller MTR values for parietal & occipital lobes *Note: did not control for age in same model
4	Neufang	2009	Expert TS	• Testosterone (blood) • Estradiol (blood) • LH (blood) • FSH (blood)	8–15 yrs	15 M/15 F	• VBM gray matter volume	• ♂♀ Positive correlation between testosterone & amygdala & hippocampus gray matter

	First Author	Year	Physical Marker(s)	Hormone(s)	Ages	Sex	Brain Outcomes	Main Findings
								• ♂ Larger negative correlation between testosterone & precunues & superior parietal gyrus compared to ♀ • ♂ Positive correlation between testosterone & hypothalamus & mammillary body gray matter • ♀ Positive correlation between estradiol & parahippocampus & uncus gray matter
5	Peper ■	2009	Expert TS	• Testosterone (saliva) • Estradiol (urine)	10–15 yrs	37 M/41 F	• VBM white matter & gray matter volume/ density	• ♀ Negative correlation between estradiol & gray matter density in prefrontal, parietal, & middle temporal • ♀ Positive correlation between estradiol & gray matter density in middle frontal, inferior temporal & middle occipital • ♂♀ Positive correlation with testosterone & absolute gray matter volume

	First Author	Year	Physical Marker(s)	Hormone(s)	Ages	Sex	Brain Outcomes	Main Findings
6	Peper ^	2009	Expert TS	NA	9 yrs; twin-pairs	96 M/99 F	• VBM white matter & gray matter volume/ density	• ♂ Smaller frontal & parietal gray matter density in pubertal vs. prepubertal groups
7	Paus ◆	2010	PDS	• Testosterone (blood) • Estradiol (blood)	12–18 yrs	204 M/215 F	• Gray matter & white matter volume & density	• ♂ Positive correlation between Testosterone & white matter volume, white:gray ratio; effects larger in short AR vs. long AR genotype • ♂ Negative correlation between Testosterone & gray matter
8	Asato	2010	Self-report TS	NA	8–28 yrs; child (8–12); adolescent (13–17); adult (18–28) groups	114 M+F	• DTI • Voxelwise TBSS (RD)	• ♂♀ RD values higher (“less mature”) in mid-puberty compared to post-puberty in all regions (except inferior frontal occipital/ inferior longitudinal fasciculus)
9	Peper ■	2010	Expert TS	• Testosterone (saliva) • LH (urine) • FSH (urine) • Estradiol (urine)	10–15 yrs	39 M/46 F	• Pituitary volume	• ♀ Positive correlation between FSH & pituitary volumes
10	Bramen □	2011	Expert TS	• Testosterone (finger stick)	10–14 yrs	32M/48 F	• Gray matter volume • Subcortical volumes (hippocampus,	• ♀ Smaller gray matter volume, right hippocampus & left amygdala

	First Author	Year	Physical Marker(s)	Hormone(s)	Ages	Sex	Brain Outcomes	Main Findings
							amygdala, thalamus, caudate)	volumes in mid-to-late vs. early pubertal group • ♂ Larger hippocampus & amygdala in mid-to-late vs. early pubertal group • ♂ Negative correlation between testosterone & total gray matter volume
11	Herting	2011	PDS	• Testosterone (blood) • Estradiol (blood)	10–16 yrs	38 M/39 F	• DTI • Voxelwise TBSS (FA, MD, RD, AD)	• ♂ Positive correlation between testosterone & FA in regions showing sex difference (FA: ♂ > ♀) • ♀ Negative correlation between estradiol & FA in regions showing sex difference (FA: ♂ > ♀)
12	Bramen □	2012	Expert TS	• Testosterone (finger stick)	10–13 yrs	36 M/49 F	• Cortical thickness	• ♂ Positive correlation between testosterone & thickness of occipital cortex • ♀ Negative correlation between testosterone & thickness of occipital & superior temporal cortex

	First Author	Year	Physical Marker(s)	Hormone(s)	Ages	Sex	Brain Outcomes	Main Findings
13	Blanton	2012	Expert TS	NA	9–15 yrs	54 F	• Global gray & white matter • Amygdala & hippocampus	• ♀ Negative correlation between pubic hair & right hippocampus volume • ♀ Negative correlation between breast development & bilateral amygdala volumes
14	Hu	2013	PDS	NA	4–18 yrs	139 M/167 F	• Subcortical volumes (hippocampus, amygdala, entorhinal & parahippocampal cortex)	• ♂♀ Positive relationship between PDS & right hippocampus volumes • ♂♀ Positive relationship between PDS & left parahippocampus volume • ♂♀ Early pubertal group shows large relationships between age & volumes, whereas these relationships are not seen in mid-to-late pubertal group
15	Satterthwaite	2013	Computer TS (pubic hair only)	NA	10–22 yrs	189 M/335 F	• Subcortical volumes & vertex shapes (hippocampus & amygdala)	• ♂♀ Hippocampus volumes smaller in post versus early pubertal groups; larger effect in ♂ vs. ♀ • ♂♀ Smaller lateral hippocampus areas & left

	First Author	Year	Physical Marker(s)	Hormone(s)	Ages	Sex	Brain Outcomes	Main Findings
								hippocampus tail in postpubertal ♂ vs. ♀
16	Peper ✗	2013	PDS	• Testosterone (saliva)	8–25 yrs	115 M/121 F	• Medial orbitofrontal gray matter volume, cortical thickness, & surface area	• ♂♀ Negative correlation between testosterone & orbitofrontal volume & surface area
17	Wong ◆	2014	PDS	• ♂ Testosterone (blood) • ♀ estradiol (blood)	12–18 yrs	467 M/495 F	• Pituitary volume	• ♂♀ Positive correlation between PDS & pituitary volumes • ♂ Positive correlation between testosterone & pituitary volumes • ♀ (premenarche only) Positive correlation between estradiol & pituitary volumes in premenarche
18	Koolschijn ✗	2014	PDS	• Testosterone (saliva) • Estradiol (saliva) • LH (urine)	8–25 yrs	103 M/112 F	• Cortical thickness, surface area, & brain volumes • ROIs (dorsolateral prefrontal cortex, anterior cingulate cortex, inferior frontal gyrus, orbitofrontal cortex)	• ♂♀ Age correlations were stronger than puberty correlations with brain • ♂ Negative correlation between testosterone & anterior cingulate cortex volumes • ♂♀ Other hormone & brain correlations did not pass multiple

	First Author	Year	Physical Marker(s)	Hormone(s)	Ages	Sex	Brain Outcomes	Main Findings
								comparison correction
19	Chavarria	2014	PDS	NA	5–18 yrs	62 M/62 F	• Callosal thickness	• ♂♀ Positive association between PDS & thickness of the anterior & posterior midbody & splenium • ♂♀ Mid- & late-puberty groups had thicker volumes in various portions of the corpus callosum as compared to early pubertal group *Note: did not control for age
20	Pangelinan ◆	2014	PDS	• Testosterone (blood)	12–18 yrs	461 M/480 F	• T1-weighted intensity of corticospinal tract • MTR of corticospinal tract	• ♂ Negative correlation between PDS & both T1 intensity & MTR • ♂ Negative correlation between testosterone & both T1 intensity & MTR • ♂ PDS & testosterone uniquely relate to T1 intensity, but account for similar amount of variance in MTR
21	Menzies	2015	Self-report TS	• Testosterone (saliva) • DHEA (saliva) • Estradiol (saliva)	12–16 yrs	61 M	• DTI • Voxelwise TBSS (FA, MD)	• ♂ Negative correlation between testosterone & MD across

	First Author	Year	Physical Marker(s)	Hormone(s)	Ages	Sex	Brain Outcomes	Main Findings
								multiple white matter pathways • ♂ Age & TS together best predicted RD values
22	Klauser □	2015	Self-report TS	• Testosterone (saliva) • DHEA (saliva)	8.5–9.5 yrs	37 M/48F	• Voxelwise VBM for gray & white matter volume/density	• ♂♀ Smaller white matter volumes in the left anterior corona radiata in the early vs. late pubertal onset groups (as defined by DHEA) • ♂♀ White matter volumes in the left anterior corona radiata negatively correlated with DHEA levels
23	Peper ✗	2015		• Testosterone (saliva) • Estradiol (saliva)	8–25 yrs	126 M/132 F	• DTI • Tractography regions of interest (FA, RD, MD, AD)	• ♂ Positive correlation between testosterone & MD (also RD & AD) in subcortico-subcortical & frono-frontal tracts (did not pass multiple comparisons) • ♀ Positive correlation between testosterone & MD (also RD & AD) in all 7 tracts of interest, but after correction only for subcortico-temporal tract

	First Author	Year	Physical Marker(s)	Hormone(s)	Ages	Sex	Brain Outcomes	Main Findings
								• ♀ Negative correlation between testosterone & FA in subcortico-frontal tract
24	Murray □	2016	Self-report TS	• Testosterone (saliva) • DHEA (saliva) • DHEA-S (saliva)	9 yrs	45 M/50 F	• Pituitary volume	• ♂♀ Positive correlations for DHEA & DHEA-S & pituitary volumes; although only DHEA-S remained significant after controlling for testosterone or TS

Matching symbols next to first author names reflect overlapping study design and samples.

Abbreviations:

PDS: Pubertal Development Scale

TS: Tanner staging

ROIs: Regions of Interest

AR: Androgen receptor

♂/M: Male

♀/F: Female

Front Neuroendocrinol. Author manuscript; available in PMC 2018 January 01.

Table 2

Longitudinal studies examining puberty and brain structure

	First Author	Year	Physical Marker(s)	Hormone(s)	Ages	Sex	Timepoints	Brain Outcomes	Main Findings
1	Nguyen ✚	2013a	PDS	• Testosterone (saliva)	4–18 yrs	281 M+F	• Up to 3; ~2 yr intervals • N=135 with 1 scan, N=104 with 2 scans, N=42 with 3 scans	• Cortical thickness	• ♀Increases in testosterone predict increases in somatosensory thickness across childhood, but decreases into adulthood • ♂(post-pubertal) Increase in testosterone predicts less cortical thickness in posterior cingulate & dorsolateral prefrontal cortex
2	Nguyen ✚	2013b	PDS	• Testosterone (saliva) • DHEA (saliva)	4–22 yrs	112 M/167 F	• Up to 3; ~2 yr intervals • N=137 with 1 scan, N=84 with 2 scans, N=34 with 3 scans	• Cortical thickness	• ♂♀(pre-pubertal; 4 to 13yrs) Increases in DHEA predict increases in thickness of dorsolateral prefrontal cortex, temproparietal, premotor, & entorhinal cortex • ♂♀(pre-pubertal; 4 to 13yrs) Increases in Testosterone but decreases in DHEA predict decreases in cortical thickness in cingulate & occipital pole
3	Goddings	2014	Self-report TS	NA	7–20 yrs	158 M/117 F	• Up to 3 scans; interval not reported • N=711 with 1 scan • 275 with 2+ scans	• Subcortical volumes (hippocampus, amygdala, nucleus accumbens, caudate, putamen, globus pallidus)	• ♂♀Increase in TS predicts increases in hippocampus & amygdala volumes; shape trajectories vary between ♂♀ • ♂♀Increases in TS predict decreases in globus pallidus, caudate, putamen & nucleus accumbens; shape trajectories vary between ♂♀ • ♂♀Age & TS together best predict amygdala, hippocampus, &

	First Author	Year	Physical Marker(s)	Hormone(s)	Ages	Sex	Timepoints	Brain Outcomes	Main Findings
									putamen volume changes
4	Herting ☐	2014	Expert TS	• Testosterone (finger stick) • ♀Estradiol (finger stick)	10–14 yrs	60 M/60 F	• Up to 2; ~2 yr interval	• Cortical gray & white matter volume • Subcortical volumes (amygdala, hippocampus, caudate, putamen, thalamus)	• ♂♀Increases in TS predicts decreases in gray matter & caudate volumes but increases in white matter volumes • ♂♀Increases in testosterone predicts decreases in caudate volumes • ♂Increases in estradiol predicts decreases in gray matter • ♂Increases lead to decreases in right amygdala volume; but an increase/plateau in ♀
5	Herting ☐	2015	Expert TS	• Testosterone (finger stick) • ♀Estradiol (finger stick)	10–14 yrs	33 M/38 F	• 2; ~2 yr interval • N=71 with 2 scans	• Cortical volume, thickness, & surface area	• ♀Increases in TS predicted superior temporal thinning • ♀Increases in estradiol predict middle temporal lobe thinning • ♂Increase in testosterone predicts decrease in middle superior frontal lobe surface area; but increase in this area for ♀
6	Brouwer ^	2015	Expert TS or oral description	• Testosterone (saliva) • LH (urine) • FSH (urine) • Estradiol (urine)	9–12 yrs; twin pairs	91 M/99 F	• 2; ~3 yr interval • N=113 with 2 scans	• Gray matter density	• ♀Negative correlation between estradiol & gray matter density in left frontal & parietal region at age 12 • ♀Increases in FSH predict gray matter decreases in prefrontal, hippocampus, cerebellum, & anterior cingulate; 58% of voxels better explained by non-

	First Author	Year	Physical Marker(s)	Hormone(s)	Ages	Sex	Timepoints	Brain Outcomes	Main Findings
									shared environment among twin pairs
7	Nguyen ✚	2016a	PDS	• Testosterone (saliva) • Estradiol (saliva)	6–22 yrs	216 M+F	• Up to 3; ~2 yr intervals • 129129 scans for time 1; 66132 for time 2 MRI; 2163 for time 3	• Amygdala volume & cortical thickness covariance	• ♂♀Increases in testosterone predict negative covariance between amygdala & cortical thickness of anterior cingulate & oribitofrontal cortex • ♂♀Covariance patterns linked to aggression
8	Nguyen ✚	2016b	PDS	• Testosterone (saliva) • DHEA (saliva) • Estradiol (saliva)	6–22 yrs	134 M/190 F	• Up to 3; ~2 yr intervals • N=129 with 1 scan, N=66 with 2 scans, N=21 for 3 scans	• Amygdala volume & cortical thickness covariance	• ♂♀Increases in DHEA predict negative covariance between amygdala & cortical thickness of occipital pole, somatosensory, & subgenual anterior cingulate cortex • ♂♀Covariance patterns linked to visual attention

Matching symbols next to first author names reflect overlapping study design and samples.

Abbreviations:

PDS: Pubertal Development Scale

TS: Tanner staging

♂/M: Male

♀/F: Female