THE IMPACT OF PUBERTY ON ADOLESCENT BRAIN DEVELOPMENT

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A thesis submitted for the degree of Doctor of Philosophy in Developmental Cognitive Neuroscience

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I, Anne-Lise Mary Goddings, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed: 

Date: **16th March 2015**

ACKNOWLEDGEMENTS

There are a great number of people without whom this thesis would never have started, progressed or finished. Firstly, to my two supervisors, Russell Viner and Sarah-Jayne Blakemore, thank you for introducing me to the possibility of a PhD, and for all your help and advice over the last 5 years. I'm certain I wouldn't have got to this point without your support, and your ability to keep me on track every time I head off on a tangent.

I would like to thank my many collaborators: Stephanie Burnett Heyes and Geoff Bird, who got me started in my very first fMRI project, as well as for the use of Stephanie's social emotion task; Iroise Dumontheil for all her MRI expertise and programming skills; Lara Menzies for exploring the possibilities of DTI and sharing all of her new-found knowledge; Eduard Klapwijk for all his PPI enthusiasm; and Emily Garrett and Ashok Sakharande, and the rest of the Blakemore lab, for helping with data collection and entertaining adolescents and their parents. Particular thanks also go to Jay Giedd, Armin Raznahan and Liv Clasen at the CPB of the NIH. Thank you for sharing your data, your methods and your time.

I have been fortunate to be part of two 'labs' for my PhD, and I have greatly appreciated all the people in both. At the ICH, special thanks for feedback and insights into both my PhD and my future career go to Terence Stephenson, Paul Winyard, and Alastair Sutcliffe. Thank you to Billy White, Sophie Khadr, Carrie Williams, Lee Hudson and Philippa Prentice for all your distractions and procrastinations, as well as your support and help, without which I would never have made it through the early days.

To all in the Blakemore lab, Emma Kilford, Lisa Knoll, Hauke Hillebrandt, Guillaume Barbalat, all the Masters students as well as Iroise, Ash and Emily (again), thank you for making the last 3 years such a wonderful experience, for your feedback and help, for making the office so welcoming, for the birthday cakes and Christmas teas, and for all the conference travels. An extra big thank you Laura Wolf and Kate Mills, my fellow PhD partners in crime. Laura, thank you for keeping me organised, up to date on gossip and well fed for 3 years. Kate, my fellow Brain Detective, office mate, programming guru and inspiration – I am eternally grateful that you made the trip from the US. I have benefitted in innumerable ways by your daily presence in my life, and I hope that this thesis reflects some of them. I miss you but can't wait for the next project.

To all of my friends and family, thank you for your support. I have been frequently reminded of how lucky I am to have you all, and hope that now this is finished, I will have a chance to show you.

Last, and most important of all, thank you to Adam for appearing in my life partway though this journey, and for your love and belief in me. There was a point when we first met when I thought your distracting presence might mean that this thesis would never be finished. How wrong I was - you are my rock and support, and I appreciate all that you have done to get me here. Thank you.

ABSTRACT

Research has demonstrated that the human brain undergoes significant change in both structure and function during adolescence, but little is known about the role of puberty in this developmental process. The aim of this thesis is to investigate the relationship between puberty and brain development during adolescence.

The first two chapters of this thesis summarise the current understanding of the behavioural and brain changes associated with both adolescence and puberty, and review the methods employed to assess puberty in research. ***Chapters 3 and 4*** *focus on the relationship between puberty and changes in brain structure. In* ***Chapter 3,*** *the influence of puberty on subcortical structural development is investigated in a large longitudinal MRI dataset, using a mixed effects modelling analysis method.* ***Chapter 4*** *investigates the relationship between pubertal status, as measured by physical pubertal stage and levels of salivary sex steroid hormones, and white matter structural development in a cross-sectional sample of 12-16 year old boys, using diffusion tensor imaging.*

In ***Chapters 5-7****, functional brain changes with puberty are explored. Chapters 5 and 6 focus on social emotion processing, where social emotions (e.g. embarrassment) are defined as emotions that require an awareness of other people's mental states, while basic emotions (e.g. fear) are those which do not. In* ***Chapter 5****, the neural correlates of social and basic emotion processing are investigated in relation to pubertal status. In* ***Chapter 6,*** *the fMRI data are reanalysed using psycho-physiological interaction (PPI) analysis to investigate puberty-related changes in functional connectivity during the same task.* ***Chapter 7*** *explores, in males, how developmental changes in brain function when performing a risk-taking task are related to puberty, independently of chronological age. Finally, in* ***Chapter 8****, the results of the empirical studies are summarised and the findings and implications of the thesis are discussed.*

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ABBREVIATIONS

ACC	anterior cingulate cortex
AD	axial diffusivity
AIC	Akaike information criterion
AP	anterior-posterior
AR	androgen receptor
ATC	anterior temporal cortex
AVPV	anteroventral periventricular nucleus of the hypothalamus
BART	Balloon-Analogue Risk Taking
BMI	body mass index
BOLD	blood oxygenation level-dependent
CPB	Child Psychiatry Branch at the National Instiute of Mental Health
CSF	cerebrospinal fluid
dACC	dorsal anterior cingulate cortex
DALY	disability-adjusted life year
DHEA	dehydroepiandrosterone
DHEA-S	dehydroepiandrosterone sulphate
DHT	dihydrotestosterone
dmPFC	dorsomedial prefrontal cortex
DTI	diffusion tensor imaging
EPI	echo planar imaging
ER	oestrogen receptor
FA	fractional anisotropy
fMRI	functional magnetic resonance imaging
FSH	follicle-stimulating hormone
FWE	family-wise error
GnRH	gonadotrophin-releasing hormone
GM	grey matter
GP	globus pallidus
HPA	hypothalamic-pituitary-adrenal

HPG	hypothalamic-pituitary gonadal
IQ	intelligence quotient
LH	luteinising hormone
LR	likelihood ratio
MD	mean diffusivity
MNI	Montreal Neuroimaging Institute
mPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
NA	nucleus accumbens
NIMH	National Institute of Mental Health, Bethesda, USA
OFC	orbitofrontal cortex
PDS	Pubertal Development Scale
PFC	prefrontal cortex
PPI	psychophysiological interaction
pSTS	posterior superior temporal sulcus
RD	radial diffusivity
ROI	region of interest
s	seconds
S.D.	standard deviation
SDN	sexually dimorphic nucleus of the preoptic area
SES	socio-economic status
SVC	small volume correction
TBSS	tract-based spatial statistics
TE	echo time
TPJ	temporo-parietal junction
TR	repetition time
vIQ	verbal IQ
vmPFC	ventromedial prefrontal cortex
VOI	Volume of interest
VS	ventral striatum
WM	white matter
YLD	years lost due to disability

RESEARCH QUESTIONS

1. What is the relationship between pubertal status and structural brain development?

2. How does the functional activation of the brain during tasks incorporating key adolescent behaviours differ in relation to pubertal status?

3. To what extent can chronological age and pubertal status be disentangled when looking at brain development in human adolescents?

PUBLISHED WORK

The work in this thesis is based on the following peer-reviewed papers:

* denotes joint first author

Chapter 3: **Goddings, A-L.,** Mills, K.L., Clasen, L.S., Giedd, J.N, Viner, R.M. and Blakemore, S-J. (2013) The influence of puberty on subcortical structural development. *Neuroimage.* 88; 242-251.
doi:10.1016/j.neuroimage.2013.09.073

Chapter 4: *Menzies,L., ***Goddings, A-L**., Whitaker, K.J., Blakemore, S-J., Viner R.M. (2014) The effects of puberty on white matter development in boys. *Developmental Cognitive Neuroscience.* 11; 116-128.
doi: 10.1016/j.dcn.2014.10.002

Chapter 5: **Goddings, A-L.,** Burnett-Heyes, S., Bird, G., Viner, R.M., Blakemore, S-J. (2012) The Relationship between Puberty and Social Emotion Processing. *Developmental science.* 15(6):801-11.
doi: 10.1111/j.1467-7687.2012.01174.x.

Chapter 6: Klapwijk, E.T., **Goddings, A-L.,** Burnett-Heyes, S., Bird, G., Viner, R.M. and Blakemore, S-J. (2013) Increased functional connectivity with puberty during a social emotion task. *Hormones and Behaviour.* 64(2):314-22.
doi: 10.1016/j.yhbeh.2013.03.012.

CHAPTER 1

Introduction

This chapter introduces the key concepts of adolescence and puberty and provides an overview of the current understanding of the behavioural and brain changes associated with both, in order to provide a background for the research questions addressed in this thesis. The chapter begins with a definition of adolescence, and outlines the importance of studying this critical period of the human lifespan. I summarise some of the key behavioural changes associated with adolescence and highlight published evidence for these changes. Following this, I define puberty and describe the different physiological processes incorporated within it, before discussing the evidence for the role of puberty in behavioural change during adolescence. In the latter part of the chapter, I summarise the current evidence for brain development during adolescence, related to both age and pubertal development, incorporating structural and functional findings. The chapter ends with a summary of each of the subsequent chapters, and how these relate to the research questions addressed in the thesis.

1.1 Defining adolescence

While adolescence is a commonly recognised concept in nearly all cultures, it can be challenging to provide a comprehensive definition for this developmental period that encompasses all of its facets and applies across different societies. Adolescence is often defined the period of human development between childhood and adulthood that incorporates physical, social, and psychological changes, and culminates in the attainment of a stable adult role (Lerner & Steinberg, 2004). This definition recognises adolescence as a biopsychosocial construct that can be flexibly applied and is open to diverse interpretation. However, it does not provide a chronological timeframe for this developmental period, so can lead to inconsistencies in research and wider society as a result of different interpretations of both the start and end points of adolescence.

The onset of adolescence is generally considered to be signalled by the overt physical signs related to the biological marker of pubertal onset (Steinberg, 2010). While the mean age for the onset of the physical changes associated with puberty is 11-12 years in females, and 12-13 years in males, there is substantial variation between individuals, with a 4-5 year range of age of onset (8-13 years in females, 9-14 years in males; Parent et al., 2003). The chronological timing of the end of adolescence is even more complex, since the concept of 'the attainment of a stable adult role' is subject to culturally diverse psychosocial and economic criteria leading to substantial variability between individuals and between societies (Caldwell, Caldwell, Caldwell, & Pieris, 1998). At the extremes, it is argued that some societies lack an adolescent period altogether, transitioning very rapidly from childhood to adulthood (Saraswathi, 1999; Schlegel & Barry, 1991), whilst in others adolescence is being extended well into the 3rd decade of life (Johnson, Blum, & Giedd, 2009).

Thus, both the beginning and end of adolescence can be conceptually challenging. This presents a problem when considering 'adolescents', since there can be many differing opinions as to who should be included in this definition in educational, legal, health and social frameworks, as well as for the purposes of targeted research. A

standardised, measurable definition of adolescence that is widely used by international organisations is the second decade of life, from 10 to 19 years (UNICEF, 2011; World Health Organisation [WHO], 2014). Providing this clear age range allows cross-cultural and interdisciplinary comparisons of adolescent data, but may do so at the expense of capturing the natural variability of this developmental phase. In this thesis, I have used this WHO definition to categorise adolescence to allow the data and interpretations incorporated within my thesis to be compared to other literature and research using the same definitions.

1.2 The importance of studying adolescence

Research into adolescent development has historically been relatively sparse compared to that published about infants and younger children (Lerner & Steinberg, 2004). Yet, an emerging research agenda focussing on adolescence has revealed that this is a period of extensive biopsychosocial change that is a critical part of the human life-course (Lerner & Steinberg, 2004). Ongoing scientific research is essential to better comprehend this influential life stage.

During adolescence, the lifestyle choices that are made and the qualifications that are acquired together expand or constrain an individual's social opportunities, and influence lifelong education, health, relationship and socioeconomic trajectories (Cotterell, 2007; Patton & Viner, 2007; Sawyer et al., 2012). Establishing an evidence base of basic and applied research knowledge that explores these transitions will result in improved understanding of how and why these behaviours are adopted, which can then provide a platform for developing valid interventions and improvements. Many of the behaviours that are exhibited in adult life originate or are intensified during adolescence e.g. cigarette smoking (Centers for Disease Control and Prevention [CDC], 2012), exercise levels and dietary habits (Patton et al., 2014), and therefore adolescent research promises to impact on our understanding not just of adolescence but also of many aspects of adult behaviour and health (Sawyer et al., 2012).

Globally, 15% of disability-adjusted life years (DALYs; a measure of overall disease burden which is expressed as the numbers of years lost due to ill-health, disability or early death) for all age groups are attributed to 10-24 year olds (Gore et al., 2011), a statistic that is at odds with general perception of adolescents as healthy. One of the main causes of this is the high level of years lost due to disability (YLDs) as a result of neuropsychiatric disease amongst this age group (45% of the total YLDs). WHO World Mental Health survey data have shown that 75% of mental health disorders have their onset before the age of 25 (Kessler et al., 2007). The mechanisms underlying this dramatic rise in pathology remain poorly understood, again emphasising the need for ongoing, multidisciplinary research into adolescent development.

1.3 Behavioural changes associated with adolescence

Adolescence has long been identified as a period with distinct behavioural characteristics that separates it from both earlier childhood and later adulthood. In this section, I summarise the evidence available for some of the key changes in behavioural development associated with adolescence that will be then discussed further throughout my thesis. This summary is not intended to represent a comprehensive or systematic review of the topics discussed, nor to cover all of the facets of adolescent behaviour in detail, but rather provides a summary of findings relevant to my thesis using selected high quality research papers to illustrate the main points.

1.3.1 Social behaviour and emotional development

One of the hallmarks of adolescent development is the dramatic change that occurs in social networks. During the second decade of life, there is an increasing importance of the role of peers. Individuals shift from spending the majority of their free time with their families to spending increasing time with peers and alone (Larson & Richards, 1991), although it is important to acknowledge that the existence and the extent of this shift varies between cultures (Larson, Richards, Sims, & Dworkin, 2001; Larson & Verma, 1999). This is followed by a later transition in early adulthood where there is a

resurgence in the amount of time spent with the family together with a related decrease in time spent with friends (Carstensen, 1992), although the role for the individual in this family unit is very different from their former one as a child or young adolescent. Peer relationships become more complex and hierarchical during adolescence (Brown, 2004), and individuals are significantly influenced by the opinions and actions of their peers (Berndt, 1979; Sumter, Bokhorst, Steinberg, & Westenberg, 2009). This has the potential for both positive and negative consequences (Mounts & Steinberg, 1995). Adolescence also marks the beginning of another form of relationship, with romantic bonds, sexual feelings and sexual relationships between individuals being initiated in this period (Meier & Allen, 2009).

Alongside changes in social structure, increases in social and self-awareness and greater emotional investment in social interactions have been described in adolescence, and are incorporated into our concept of 'teenagers' (Somerville et al., 2013; Steinberg & Morris, 2001). Adolescents are more susceptible to self-consciousness and embarrassment when being observed by peers than younger or older individuals (Rankin, Lane, Gibbons, & Gerrard, 2004; Somerville et al., 2013), and can experience strong emotional reactions in response to the actions of their peers. This is particularly apparent in examples of social ostracism and bullying, which provoke strong negative emotional reactions of anxiety and lowering of self-esteem in adolescence (Sebastian, Viding, Williams, & Blakemore, 2010). While the influence of peers can be either positive or negative, it appears to be largely driven by admiration and respect for the opinions of peers, not through coercive pressures (Susman et al., 1994). Thus, when considering the changing behaviour of adolescents, it is important to incorporate how their changing socio-emotional competence might influence the motivation behind behaviours.

Social competence and successful social interactions require a complex level of understanding of other people. The ability to 'read' another person through their actions, inflections, gestures and facial expressions, and from this to ascertain their thoughts, emotions and likely future actions, is known as 'theory of mind' or 'mentalising' (Frith & Frith, 2003). It has been hypothesised that the increasingly

complex social networks of adolescence rely on more sophisticated mentalising abilities than those of younger children (Blakemore, 2012). Development of theory of mind through infancy and early childhood is well documented (Barresi & Moore, 1996), using validated theory of mind tasks e.g. the Sally-Ann false belief task (Wimmer & Perner, 1983) and the 'Reading the Mind in the Eyes' task (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001). Mentalising tasks like these show behavioural ceiling effects in normally developing children (after the age of 4-5 years for the Sally-Ann Task, and by 8-10 years for the reading the mind in the eyes task (Gunther Moor et al., 2011)), which has limited their use when investigating mentalising capacity in older children and adolescents.

The recent development of more challenging theory of mind tasks is now enabling the study of more refined mentalising abilities in older children and adolescents. One such task is the 'Director' task, an online communication game (Dumontheil, Apperly, & Blakemore, 2010). In this game, participants see a set of shelves in front of them containing objects. On the other side of the shelves is a character known as the 'Director'. While the participant can see all the objects on the shelves, the Director is only able to see some of them, as a proportion of the shelves have opaque backing panels that obstruct his view. During the game, the participant is asked by the Director to move objects on the shelves. On some trials there is a conflict between the participant's knowledge and the Director's, and the participant must consider the Director's perspective and only move objects that he can see. In this task, adolescents were found to be more error-prone than adults in trials requiring them to take the Director's perspective. This provides evidence that the ability to take another's perspective into account to guide decisions may still be developing even in adolescence (Dumontheil et al., 2010).

1.3.2 Decision-making and risk-taking

During adolescence, individuals become more autonomous within society and begin to make decisions independently of adults. This drive for 'agency', the phenomenon of being an actor in one's own life rather than play a passive role in it, is a crucial aspect

of development (Sercombe, 2014). As adolescents develop agency, they have increasing opportunities to make choices for themselves, choices which may differ from those made on their behalf by their families and societies. The real-life decisions made by adolescents, and their consequential behaviours and actions, are characteristically perceived to be different from, and more risky than, those chosen by adults in the same situations (Blakemore & Robbins, 2012). Much of the evidence supporting this increased tendency for risk-taking in adolescence comes from epidemiological data (Dahl, 2004). In Western Europe and North America, the leading cause of death in adolescence is road traffic accidents, at least a proportion of which are associated with risky behaviours such as reckless driving and drug and alcohol consumption, followed by intentional and non-intentional injuries (Global Health Data Exchange [GHDx], 2014). These causes drive an overall rise in mortality during adolescence, from a nadir in mid-childhood (between ages 5 and 9 years; GHDx, 2014). At a global level, HIV/AIDs remains the greatest threat to mortality, and other infectious diseases feature more highly than in the US and Western Europe. Nevertheless, road traffic accidents and both intentional and unintentional injuries continue to be in the top 10 causes of mortality in the 10-24 age group. In both England and the US there are increases across the second decade of life in the numbers of admissions to hospital with unintentional injuries (Hargreaves & Viner, 2014; Willoughby, Good, Adachi, Hamza, & Tavernier, 2013).

These data describe a health paradox (Dahl, 2004; Patton & Viner, 2007): at a phase of life when humans are faster, stronger and relatively more resistant to disease than in childhood or much of adulthood, this increase in morbidity and mortality, driven by an increase in injuries, seems counterintuitive. It is important to emphasise that the absolute numbers of deaths and severe injuries in this age-group remains low, with survival rates of individuals through adolescence being over 99.95% in Western Europe and the US (GHDx, 2014; Willoughby et al., 2013). Nevertheless, more than 50% of the deaths that occur in adolescence in these regions are from causes that are widely considered to be preventable, making adolescence a particularly vulnerable period (Viner et al., 2011).

When adolescents are asked to make reasoned judgements about probability in controlled experimental decision-making contexts, a number of empirical studies have demonstrated that they perform equally well as adults at perceiving the riskiness of a choice by ascertaining the likelihood of different outcomes (Figner, Mackinlay, Wilkening, & Weber, 2009; Gardner & Steinberg, 2005; Harbaugh, Krause, & Vesterlund, 2002; Reyna & Farley, 2006; Van Leijenhorst, Westenberg, & Crone, 2008; Wolf, Wright, Kilford, Dolan, & Blakemore, 2013). There is also evidence concerning real life health risk perception that supports this view, finding that adolescents appear to be well-informed about health risks and able to make judgements when they have the appropriate information, in a manner equivalent to adults (Lundborg & Lindgren, 2004; Rodham, Brewer, Mistral, & Stallard, 2006).

This lends weight to the hypothesis that by adolescence, most individuals have developed the cognitive capacity to weigh up decisions and make a 'rational' choice, but also emphasises that the beliefs and information held by the young people are crucially important when weighing up these decisions (Gerking & Khaddaria, 2012). Work by Schlottmann (2001, 2005) suggests that this ability to judge probability and have a functional understanding of expected value can be seen in children as young as 8 or 9 years of age, or even younger (Schlottmann & Tring, 2005; Schlottmann, 2001). However the final choice made in a risky decision depends only partly on the ability to weigh up the probabilities of each potential outcome. The valence and value of the potential outcomes also have a significant impact on the eventual decision (Lundborg & Andersson, 2008; Schlottmann & Tring, 2005; Wolf et al., 2013).

While adolescents have the cognitive capacity to perceive and assess the risks associated with a decision similarly to adults, it has been hypothesised that the emotional or social value placed on the positive and negative outcomes of a decision may differentiate adolescents from adults or children, leading to different choices and resultant behaviours (Cauffman et al., 2010; Mills, Goddings, & Blakemore, 2014). Thus, in studies where there is little social or emotional context or consequence during decision-making, the age-related developmental trajectories for risk-taking tend to be linear, with adolescence representing an intermediary stage between childhood and

adulthood (Crone, Bullens, van der Plas, Kijkuit, & Zelazo, 2008; Paulsen, Platt, Huettel, & Brannon, 2011). In contrast, in decision-making studies incorporating an affective or social context, adolescents show a different pattern of behaviour from both children and adults.

One study used an adapted version of the Iowa Gambling Task (IGT) to look at affective decision-making in a large sample of 901 10-30 year olds (Cauffman et al., 2010). In this task, participants were shown four packs of cards, of which two would lead to an overall increase in points if played repeatedly (win decks) and two would lead to an overall decrease in points (loss decks). Each time a deck was presented to the participant, they had to choose whether to play or pass. Participants in mid-late adolescence tended to play the win decks more than adults or children, which the authors interpret as representative of high sensitivity to reward (Cauffman et al., 2010) In contrast, there was linear increase in the tendency to avoid the loss decks of cards, whereby adults outperformed adolescents, who in turn performed better than children. This result has been used as evidence that reward sensitivity and loss avoidance involve different cognitive processes, which may follow different developmental trajectories through adolescence (Cauffman et al., 2010).

A second study investigating decision-making in a risk context used emotional outcomes instead of material ones. In the 'wheels of fortune' task used, male participants (N=98; 9-35 years) viewed two wheels with different probabilities of winning or losing points (Burnett, Bault, Coricelli, & Blakemore, 2010). They had to choose one of two wheels, with the aim of maximising the number of points won. In a proportion of the trials, participants were shown the outcome of both wheels, although they only gained or lost the points on their chosen wheel. Unlike the previous study, therefore, this study combined reward-seeking and loss avoidance into a single decision. The authors found that there was an inverted U-shaped pattern of risk-taking, with young adolescents aged 12-15 years taking more risks than younger or older participants (Burnett et al., 2010). This correlated with an increase in the strength of counterfactually mediated emotions (i.e. relief when seeing the not chosen wheel, or at not losing a large number of points, or regret at not winning a large

number of points) from childhood to early adolescence, with the strongest relationship being with relief (Burnett et al., 2010).

These differences in adolescents' decision-making performance between controlled unemotional, or "cold', situations and more emotionally, socially or materially rewarding "hot" situations have been directly compared in experiments containing both conditions. In a study using the Columbia Card Task (Figner et al., 2009), adolescents and adults were shown a panel of facedown playing cards. The aim of the task is to turn over as many cards as possible without encountering a loss card, at which point the trial ends. There were two types of trial. In the hot trials, participants clicked on a specific card, watched it turn over, and gained immediate feedback. If the card was a win card, they could then choose another card. The trial continued until they chose to stop or turned over a loss card. The incremental decision-making and the immediate feedback were designed to trigger the affective processes involved in decision-making. In contrast, in the cold condition, participants were asked to pick a number of cards to turn over. The final outcome, win or loss, was then shown to them. The single decision-making step, and lack of progressive feedback, was designed to trigger a more deliberative decision-making process. In this study, adolescents and adults were found to exhibit the same levels of risk-taking in a deliberative, non-affective version of the task, but adolescents (aged 14-19 years) took significantly more risks than adults in a hot, affective version of the task (Figner et al., 2009).

A second study used a social variable to distinguish between hot and cold versions of a task (Gardner & Steinberg, 2005) by assessing the impact of the presence of peers on risky decision-making in adolescents and adults. The researchers used a task called the Stoplight task. In the task, participants controlled a car on a computer screen. As the car approached a series of traffic lights, they turned from green to amber. The participants had to decide whether to stop the car at the light, or to try to drive through the light before it turned red. Successfully getting through the light while amber earned the participants more points than stopping the car at the light, but they risked crashing if the light turned red (Gardner & Steinberg, 2005). In the original version of this task, there was an age-related effect on risk-taking performance when

alone, whereby younger participants took more risks than older ones (Gardner & Steinberg, 2005). This effect was not seen in a later, modified version of the task adapted for functional MRI, where adolescents and adults performed similarly when alone (Chein, Albert, O'Brien, Uckert, & Steinberg, 2011). In both versions of the task, adolescents were found to be more affected by the presence of their peers when performing the tasks, taking relatively more risks on the task than when alone (Chein et al., 2011; Gardner & Steinberg, 2005). This influential study demonstrates the importance of context on decision-making and suggests that the opinion of peers functions as a 'reward' in adolescence, comparable to emotional or material rewards.

The impact of hot and cold contexts on decision-making in adolescence has led to a proposed 'dual systems' model of adolescent risk-taking (Casey, Getz, & Galvan, 2008; Steinberg, 2008). This model hypothesises that the propensity for adolescents to make risky decisions results from a mismatch in the developmental timing of the neural systems involved in decision-making. In particular, this theory suggests that the brain regions responsible for *reward-processing* mature early and quickly in adolescence, around the time of puberty, while the *cognitive control* network, which allows self-regulation, develops gradually throughout adolescence and early adulthood (Casey et al., 2008; Steinberg, 2008). The differential timing of these two systems is hypothesised to result in a period of vulnerability during adolescence, when the maturity of the reward-processing system biases individuals towards reward-seeking choices (which are often associated with elevated risk) and there is a diminished level of self-regulation, as a result of a relatively immature cognitive control system.

The maturation of the reward processing system has been associated with the onset of puberty (Blakemore, Burnett, & Dahl, 2010; Forbes & Dahl, 2010; Steinberg, 2008). Before discussing the evidence for this association, I shall first define puberty and discuss the physiological processes included in this complex event.

1.4 Puberty

1.4.1 Definition

Puberty is the biological process of sexual maturation that culminates in reproductive competence (Sisk & Foster, 2004). In humans, it is often used as the defining event signifying the end of childhood and transition into adolescence (Steinberg, 2010). Puberty encompasses a combination of two distinct physiological processes, adrenarche (the activation of the hypothalamic-pituitary-adrenal axis) and gonadarche (the reactivation of the hypothalamic-pituitary-gonadal axis causing gonadal activation), which together lead to a dramatic rise in the circulating levels of sex steroid hormones including androgens e.g. testosterone and dehydroepiandrosterone (DHEA), oestrogens, particularly oestradiol, and progestagens, particularly progesterone. In addition to sexual maturation, puberty results in physical changes such as linear growth, maturation of body organ systems including the hepatic, renal and cardiovascular systems, and changes in body proportion and facial bone structure (Lee & Houk, 2006; Meindl, Windhager, Wallner, & Schaefer, 2012; Verdonck, Gaethofs, Carels, & de Zegher, 1999). Alongside these diverse physical changes, it has been proposed that changes in brain structure and function, increases in behaviours such as risk-taking and sensation-seeking, and the emergence of affective disorders, are also at least partly mediated by the process of puberty (Forbes & Dahl, 2010; Patton & Viner, 2007; Paus, Keshavan, & Giedd, 2008; Steinberg, 2008).

1.4.2 Adrenarche

The earliest chronological event of puberty is adrenarche, the activation of the hypothalamic-pituitary-adrenal (HPA) axis following a period of quiescence in childhood. Beginning between the ages of 6 and 8 years in humans, the maturation of the zona reticularis of the adrenal cortex results in a steady increase in serum levels of DHEA and its sulphated form, DHEA-S (De Peretti & Forest, 1976; Havelock, Auchus, & Rainey, 2004; Parker, 1991). Both of these adrenal androgens are produced during foetal life in high concentrations (Yuen & Mincey, 1987), and continue to be produced after birth. Serum levels of DHEA and DHEA-S drop during the first few months of life,

reaching a nadir at approximately one year of age, and remaining low until adrenarche begins (Rainey & Nakamura, 2008; see **Figure 1.1**). Testosterone is also produced in relatively small quantities by the adrenal cortex in both males and females, and increases in serum levels of other steroid hormones including oestrogens occur during adrenarche, since DHEA is a precursor to oestrogen and can therefore be converted by the enzyme aromatase (see **Figure 1.3**).

[Figure removed for copyright reasons; original available in Rege & Rainey, 2012]

Figure 1.1: Serum DHEA (in blue) and DHEA-S (in red) levels in childhood and adolescence*. Serum levels were measured in male and female participants including full term neonates, infants, children, adolescents and adults (adult data not shown). Levels of both DHEA and DHEA-S are high at birth, and fall sharply in the first month of life, continuing to decrease until the end of the first year. Levels remain low from 1-6 years, and begin to rise from the age 6 years, continuing to increase until age 16, and the end of puberty in both sexes. Post-pubertal increases were noted only in male subjects (data not shown). Figure from Rege & Rainey, 2012; original data from De Peretti & Forest, 1976.*

The trigger for the onset of adrenarche remains unknown (Havelock et al., 2004). Only a small number of higher primates are known to exhibit adrenarche, including chimpanzees and gorillas (Cutler, Glenn, Bush, Hodgen, & Loriaux, 1978), although a recent review argues that Old World primates may also experience adrenarche (Conley, Bernstein, & Nguyen, 2012). The evolutionary origin of adrenarche remains unexplained. One hypothesis proposes that the adrenarcheal hormone DHEA-S may play a role in the extended brain maturation seen in humans (Campbell, 2006), affecting both subcortical and cortical brain maturation, and associated behaviours.

The adrenarcheal rise in DHEA and DHEA-S usually precedes the rise in oestrogens or androgens associated with gonadarche (see **Section 1.4.3**), and the two processes appear to be independently controlled, despite their sequential timing (Ducharme et al., 1976). Thus, in individuals with premature adrenarche, premature gonadarche is

not associated, and in hypogonadotrophic states where gonadarche is absent, there may be no interruption of adrenarche (Sklar, Kaplan, & Grumbach, 1980). The chronological timing of adrenarche appears to be similar between the sexes, and levels of adrenal DHEA and DHEA-S appear to be equivalent until the onset of gonadarche, when male levels exceed females (see **Figure 1.1**).

Adrenal androgens like DHEA and DHEA-S are considered to be 'weak' androgens compared to the potent gonadal androgens. They contribute to the development of androgen-dependent body hair growth, particularly in the groin and axilla, as well as to adult body odour in both sexes. In males, the main driver for these changes becomes the gonadal steroids after the onset of gonadarche (see **Section 1.4.3**), while in women adrenal androgens continue to be the only source of androgens (Havelock et al., 2004).

1.4.3 Gonadarche

Gonadarche comprises the growth and maturation of the gonads and occurs as a result of the reactivation of the hypothalamic-pituitary-gonadal (HPG) axis following quiescence in childhood. The reactivation of the hypothalamus and associated hormonal changes begin at least a year before any external physical changes of puberty are noticeable (Biro et al., 2014; De Peretti & Forest, 1976). Gonadotrophin releasing hormone (GnRH), which had been produced at low tonic levels in childhood, begins to be produced in larger quantities and in a pulsatile manner. The exact mechanistic pathway to trigger this reactivation is not well-established, but likely includes signalling pathways and multiple hormones e.g. leptin, ghrelin and kisspeptin (Sanchez-Garrido & Tena-Sempere, 2013). The pulsatile GnRH signal stimulates the anterior pituitary gland to produce the gonadotrophins luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which are then released into the bloodstream (see **Figure 1.2**).

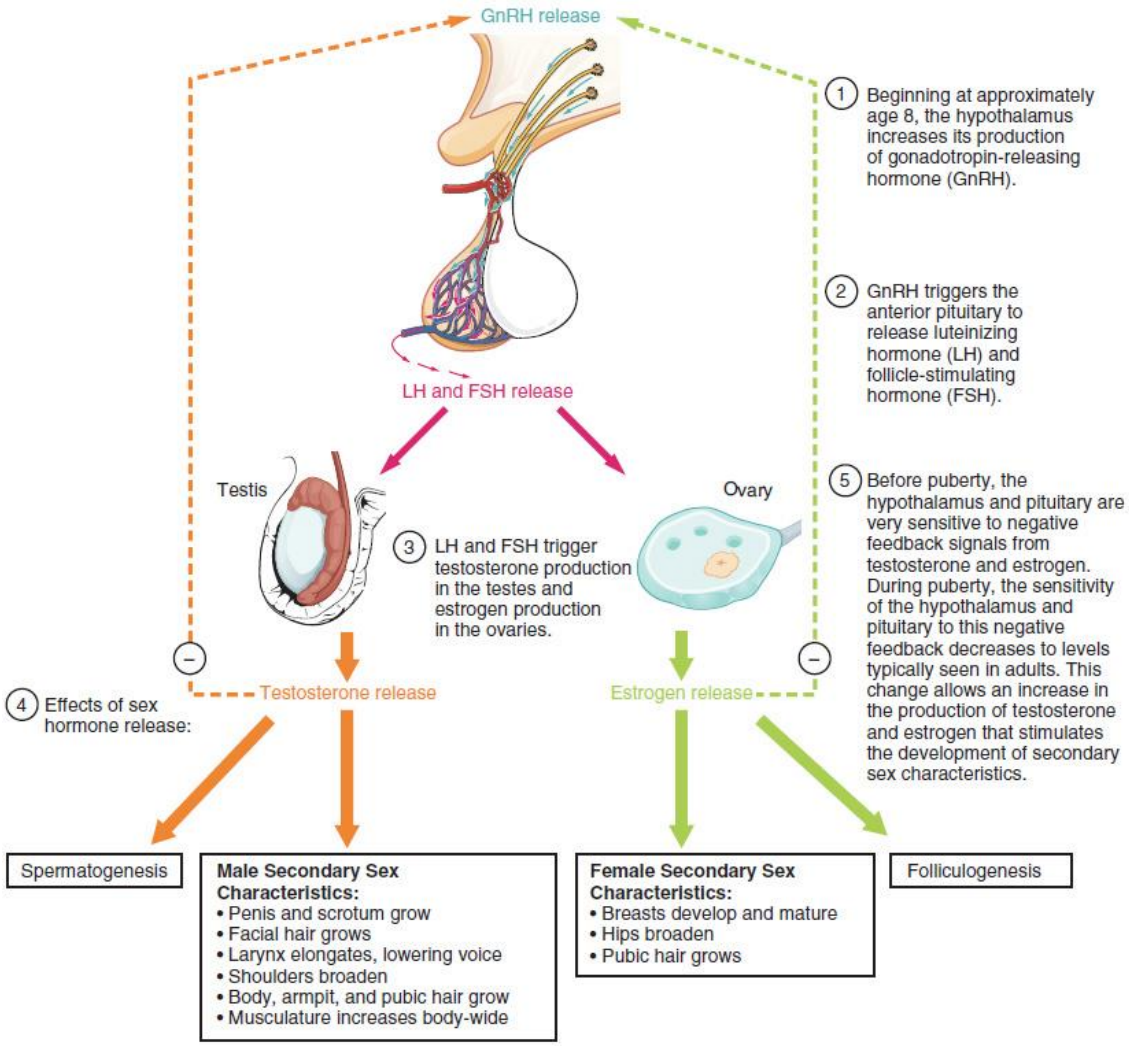


Figure 1.2: The cascade of the HPG axis. *The hypothalamus increases production of gonadotrophin releasing hormone (GnRH). This triggers production of gonadotrophins, luteinising hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. LH and FSH trigger testosterone production in the testes (male), and oestrogen production in the ovaries (female), leading to maturation of the gonads, and secondary sex characteristics. Figure from OpenStax College, 2014.*

This pulsatile release of LH and FSH increases in amplitude over time, eventually stimulating the gonads (testes in the male and ovaries in the female) to produce gonadal steroid hormones. Unlike adrenarche, which appears to be similar between the sexes, gonadarche produces different patterns of steroid hormone production between the sexes, and results in dramatically different secondary sexual characteristics. The majority of the pathway is similar between the sexes, with

cholesterol being converted into progestagens (see **Figure 1.3**; progestagens highlighted in yellow), and then into precursor androgens. The differences between the hormone levels by sex results from different final stages of this pathway.

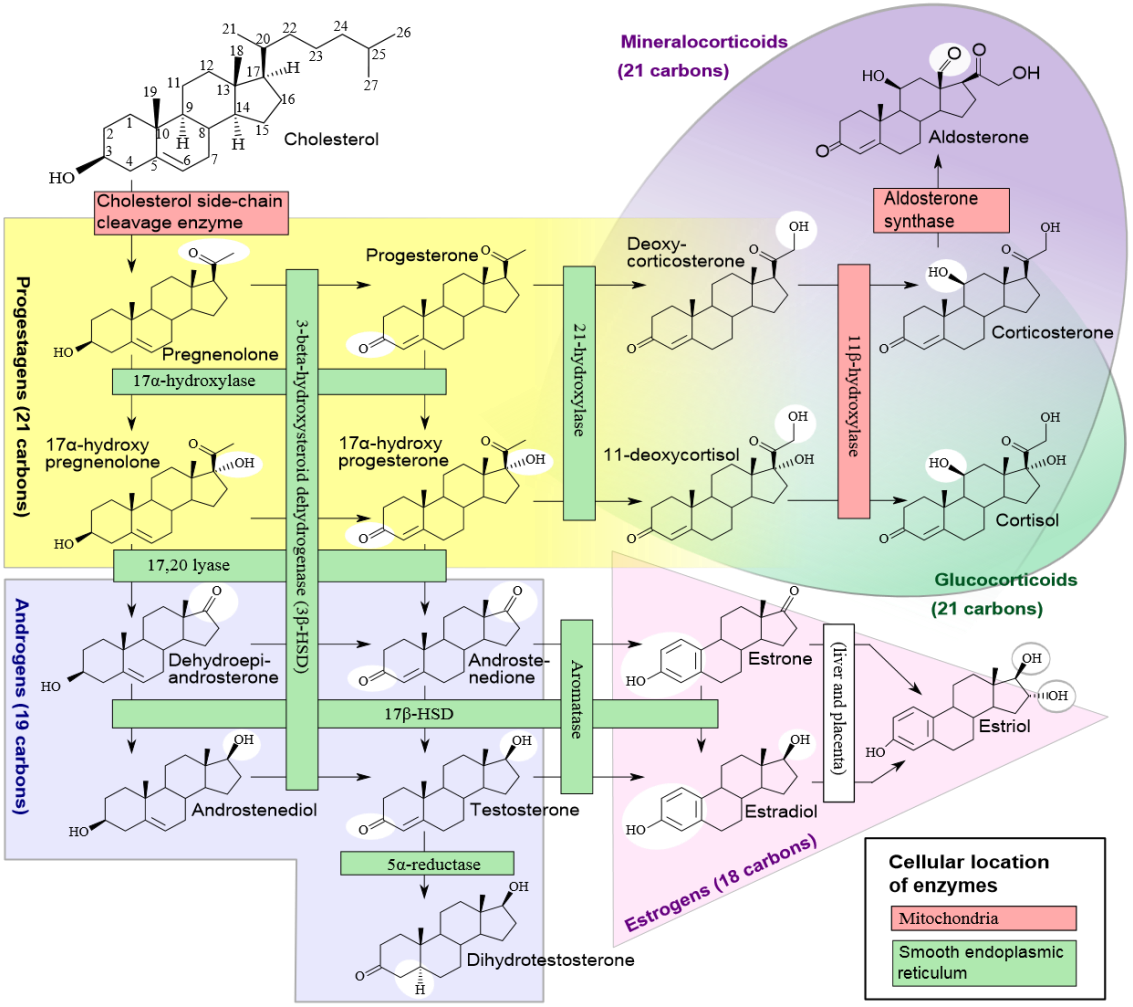


Figure 1.3: The sex steroid hormone cascade. *Cholesterol is the precursor for the biosynthesis of all the steroid hormones. This is converted to progestogens by mitochondrial enzymes. Enzymes in the testes (male) and ovaries (female) are then responsible for further conversion of progestogens (grouped in yellow) into androgens (in blue) and, in females, oestrogens (in pink). Note that DHEA-S (not shown) is the sulphated metabolite of DHEA, catalysed by the sulfotransferase enzyme SULT2A1 in the zona reticularis of the adrenal gland. From Häggström & Richfield, 2014.*

In males, Leydig cells in the testes under stimulation from LH convert these precursor androgens into testosterone, which is then secreted into the plasma. Some of the

testosterone is further converted into dihydrotestosterone (DHT), a highly potent androgen, in Sertoli cells of the testes before being secreted in the plasma. The testosterone and DHT are delivered to target tissues in the body, to signal secondary sexual characteristic development and associated body changes. Testosterone is also converted to DHT at a number of target sites, increasing the potency of the androgen signal. Many target tissues also have quantities of aromatase, an enzyme that converts testosterone to oestradiol. Therefore, whilst testosterone is the main sex steroid hormone circulating in plasma in males, some of the effects associated with the HPG axis in males may also result from oestrogenic effects at the site of the target tissue.

In females, conversion from precursor androgens to testosterone occurs in thecal cells in the ovaries (see **Figure 1.3**; androgens in blue). The vast majority of this testosterone is then converted into oestradiol in granulosa cells, as a result of the action of the enzyme aromatase (see **Figure 1.3**; oestrogens in pink). The final product, oestradiol, is then released into the plasma, where it is transported to target cells in the breast and uterus to develop secondary sex characteristics, and to further target tissues throughout the body including muscle, bone and potentially the brain. The ovaries also release large amounts of progestagens. The levels of both oestradiol and progesterone continue to increase throughout puberty. Cyclical release of the gonadotrophins LH and FSH prompt the release of increasing levels of these gonadal steroids as puberty progresses. These cycles are initially irregular, and are present before the onset of menarche (Hansen, Hoffman, & Ross, 1975). At the onset of menarche, these cycles are associated with ovulation and the release of an oocyte from the ovaries, but the cycles and ovulation may continue to be irregular for a number of months or years after menarche (Committee on American Academy of Paediatrics, 2006). Even when regular cycles have been established, levels of oestradiol and progesterone are known to show significant intra-individual and inter-individual variability (see **Figure 1.4**).

Whilst testosterone and oestradiol are the main products of this sex steroid hormone cascade in males and females respectively, it should be recognised that quantities of the other hormones in the pathways are also released, which can also have effects on

target tissues in the body. However, for the remainder of this thesis, I will focus on the main sex steroid hormones, testosterone and oestradiol, and the main adrenal steroid implicated in adolescent pubertal maturation, DHEA.

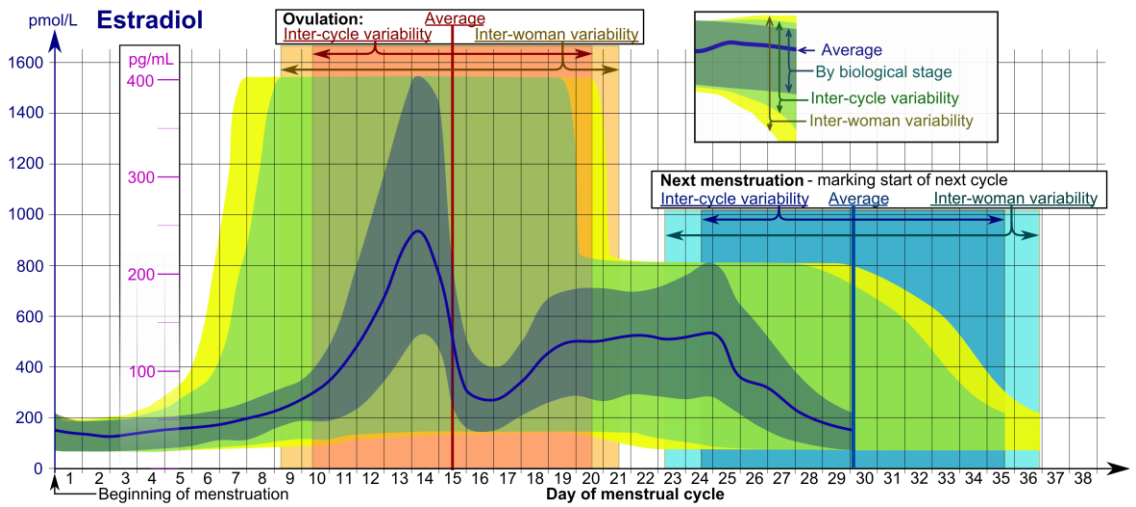


Figure 1.4: Reference ranges for blood content of oestradiol in female adults*. The time scale (x-axis) begins with the onset of the menstrual period. The graph shows the between cycle and between woman variability in time to ovulation (orange) and time to menstruation (blue). The dark blue line and green shading show the blood levels of oestradiol through the menstrual cycle. The dark blue line represents the average hormone level for each day of the cycle. The shades of green show how this varies with biological stage, inter-cycle variability and inter-woman variability. Figure from* Häggström, 2014*, using data from Chiazze L et al., 1968; Fehring, Schneider, & Raviele, 2006; Geirsson, 1991; Liu, Gold, Lasley, & Johnson, 2004; Pauerstein et al., 1978; Stricker et al., 2006.*

1.4.4 Menarche

Menarche is defined as the occurrence of the first menstrual period for a female (Tanner, 1962). It is a considered to be a key event in a young woman's life, and can represent the beginning of adulthood for them, with the expectation to marry, bear children, and leave their childhood home, depending on the cultural environment in which they live. At an individual level, the sudden onset of menarche can be associated with a wide variety of emotional reactions (Andrews, 1985; Golub & Catalano, 1983;

M. L. Marván & Molina-Abolnik, 2012; Morse & Doan, 1987). The psychological and emotional reaction to menarche is partly related to the preparation a girl experiences in advance, with negative emotional responses being related to negative or insufficient preparation for the event (Rierdan & Koff, 1990; Tang, Yeung, & Lee, 2003). These emotional responses can be complex, and can relate both to the social impact of menarche and its association with womanhood, but also to the practical consequences of menstruation and having to manage menstrual hygiene (Tegegne & Sisay, 2014).

The perceptions passed onto girls about the positive or negative impact of menarche are significantly influenced by her family and cultural environment (Marván & Molina-Abolnik, 2012). Globally, mothers are the typical source of information about menarche and menstruation, and how this information is related from mother to daughter affects their daughter's attitude (Lee, 2008; Marván & Molina-Abolnik, 2012). Menarche has significant cultural value, and its impact varies considerably across different societies. Again, the cultural perception of the positive or negative impact of menarche influences the experiences of girls living within that environment. Studies examining attitudes towards menarche in urban Karachi, Pakistan (Ali & Rizvi, 2010), rural Kenya (Mason et al., 2013; McMahon et al., 2011) and Taiwan (Chang, Chen, Hayter, & Lin, 2009) report predominantly negative emotions related to menarche, while in other countries, reported emotional reactions are more mixed (e.g. China and Mexico Marván, Morales, & Cortés-Iniestra, 2006). The emotional experiences related to menarche are subject to change with changing cultural attitudes and practices, with a recent US study suggesting that, compared to previous generations, the experience of menarche for young women in the US is now more positive (Lee, 2009).

Menarche occurs as a late pubertal event, and represents one of the key steps in the cascade of events that lead to reproductive capacity. Physiologically, menarche indicates that the endometrial lining of the uterus has developed sufficiently to result in a withdrawal bleed when shed, a process which requires sufficient quantities of both oestrogens and progesterone. It is therefore likely to be reflective of the combined functioning of the HPG axis within individuals. The timing of menarche in relation to the onset of ovulation varies substantially between individuals, and menarche does

not equate to the capability to reproduce (Zhang et al., 2008). Following menarche, there may be a period of months to years before gonadal hormones reach a stable cyclical pattern resulting in cyclical ovulation and menstruation (Lemarchand-Béraud, Zufferey, Reymond, & Rey, 1982). The age of menarche is highly correlated with breast development in puberty, and is often used as a retrospective surrogate marker of timing of pubertal onset. However, there has been shown to be some variability between timing of onset of breast development and of menarche, whereby the time lag between initial breast development and menarche is shorter in girls who undergo late puberty than those who experience puberty at a younger age (Martí-Henneberg & Vizmanos, 1997). This emphasises that the hormonal triggers for these two processes are not identical, and that they therefore represent different aspects of pubertal timing.

The relative ease of measuring menarche (see **Section 2.3.3**) has resulted in a rich repository of data of menarcheal timing across the last two centuries, and for multiple countries (Euling et al., 2008; Parent et al., 2003; Sørensen et al., 2012). These data show a clear decline in the age of menarche in most industrialised populations from approximately 17 years in the early 1800s to approximately 13 years by the mid-20th century (Sørensen et al., 2012; see **Figure 1.5**). This decline has been attributed to improvements in nutrition, sanitation and health provision (Euling et al., 2008). In general, age of menarche appears to have stabilised since the 1960s across these industrialised populations (Euling et al., 2008; Juul et al., 2006; Mul et al., 2001) although there is substantial variability between datasets, and some US samples, in particular, have suggested on-going modest declines of approximately 2.5 months in age of menarche in the latter half of the 20th century (Anderson, Dallal, & Must, 2003; Herman-Giddens, 2006). In other countries, for example Bulgaria, India and China, the decline in age of menarche is continuing, supporting the hypothesis that the declines in menarcheal age are related to nutritional, health and socioeconomic factors (Parent et al., 2003).

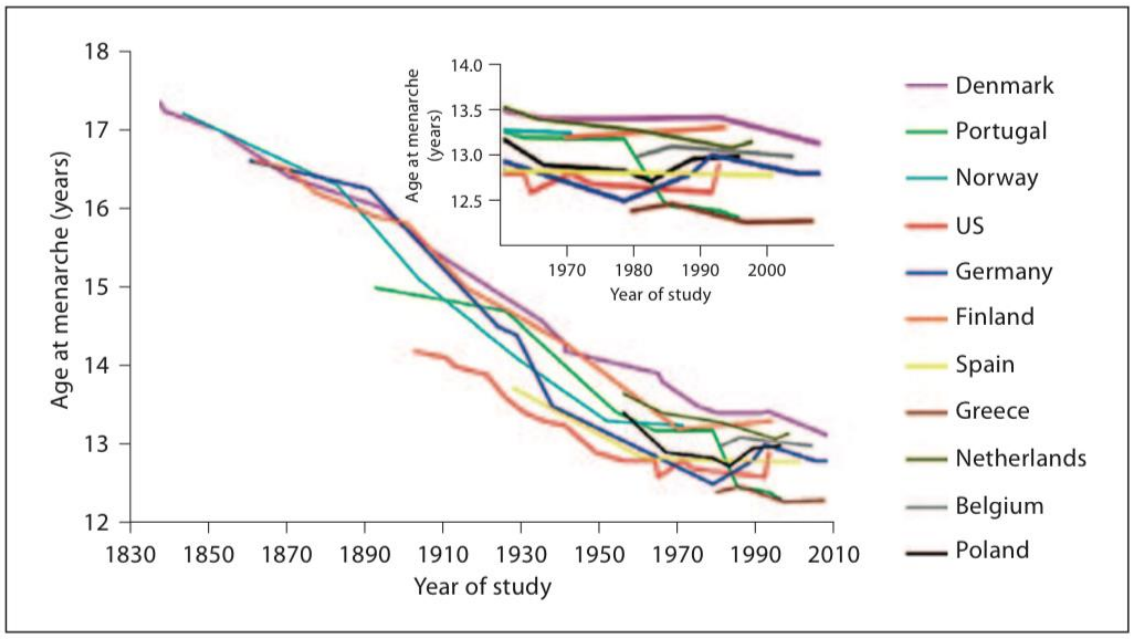


Figure 1.5: Secular trends in age at menarche *according to the first year of data collection for 11 countries. The inset shows the last 50 years in which the secular trend in age at menarche seems to have stabilised. Taken from* Sørensen et al., 2012*.*

In contrast to the age of menarche across Europe and the US in the last 50 years, which appears to have stabilised, there is some evidence of an on-going secular trend in the decreasing age of onset of breast development in girls from the US and Europe (Euling et al., 2008; Sørensen et al., 2012), although the data for this trend remain variable and conflicting, and there is no clear consensus on the presence or extent of such a decrease. This potential dissociation in timing between breast development and menarche again highlights that menarche and thelarche represent different aspects of pubertal development.

Having defined puberty, in the next section I will summarise the evidence linking human puberty to changes in behaviour that have been associated with typical adolescence.

1.5 Associating adolescent behavioural changes with puberty in humans

As discussed in **Section 1.2**, many behavioural changes occur during adolescence, and many of these have been understandably linked to puberty. However, the fact that both the behavioural changes discussed and pubertal development occur in adolescence does not mean that the behavioural changes in adolescence are directly correlated with puberty, and is certainly not evidence of a causal link. Puberty and chronological age are necessarily correlated. While puberty can have a varied time of onset (see **Section 2.3**), it spans a range from of 8-14 years in normal development. Furthermore it is a progressive process, and the tempo of pubertal development is measured in years. Thus, once puberty has started, it will inevitably progress as the individual simultaneously ages (assuming normal development and absence of pathology). Yet, while age and puberty are correlated, it is important to recognise that they are different measures, and that each is representing a combination of maturational events. To disentangle which effects may be driven by puberty, as opposed to other factors associated with changing chronological age, it is necessary to incorporate measures of pubertal maturation in developmental studies. However, it is important to note that studies incorporating measures of pubertal maturation and relating these to behavioural changes cannot be used to identify a causative relationship, and careful interpretation of such cross-sectional, observational studies, as well as further research using longitudinal methods, is needed.

1.5.1 Social behaviour and emotional development

There is a long history of linking sex steroid hormones to heightened emotional states and increased lability of mood. Since adolescents are stereotypically considered to be moody and emotional, it has been hypothesised that this behavioural change is associated with ‘raging hormones’ (Hall, 1904). Despite this long-standing hypothesis, there is limited literature to provide conclusive evidence for this theory. Studies investigating the link between pubertal status and ‘moodiness’, the intensity and lability of moods, have had variable results. One study in 9-10 year old girls (N=52)

found that girls who reported some pubertal development (using the Petersen Pubertal Development Scale [PDS]; see **Section 2.3.2.2** for further details) reported more negative moods and more nervousness, as well as greater variability in their negativity and nervousness, than their pre-pubertal peers (Buchanan, 1991). There was no difference in happiness, esteem, energy or restlessness. In contrast, a different study (N=253; US School grades 6-8), again using the self-report PDS scale to estimate pubertal status, found no relationship between pubertal status and moodiness in females, and found a positive correlation with emotional tone and impulse control in the older males in the sample, such that the pubertal boys had higher levels than their pre-pubertal peers (Crockett & Petersen, 1987). In both of these studies, the pubertal measure used relies on self-reported maturation, and is therefore a measure of perceived pubertal status, which may or may not reflect the extent of actual developmental changes (Buchanan, 1991). This could be subject to reporting bias, since the feeling of positive or negative moods might influence an individual's perception of their maturation and physical development.

The importance of peers in adolescence has already been discussed in **Section 1.2**, and there is evidence that more advanced pubertal development increases social stress, suggesting a greater sensitivity to the social environment and self-awareness. In a study of 9-15 year olds (N=82; 40 female), pubertal status was marginally correlated with cortisol responsiveness when performing the Trier Social Stress Test (Gunnar, Wewerka, Frenn, Long, & Griggs, 2009). A second, larger study (N=295; 9-17 years) replicated this effect, finding an increased cortisol response with advanced pubertal stage, and finding no difference between sexes. In this study, the authors noted that it was not possible to dissociate age and pubertal effects, due to the high correlation between the variables and the cross-sectional nature of the sample. (Sumter, Bokhorst, Miers, Van Pelt, & Westenberg, 2010). This association between increased social stress and advanced pubertal development has been hypothesised to jointly influence the evolution of symptoms of depression and anxiety in adolescent females (Ge, Conger, & Elder, 2001; Koenig & Gladstone, 1998). It is important to note that cross-sectional studies like these cannot be used to establish causality, and can only show associations between variables.

In adolescence, there is a dramatic increase in prevalence of a range of psychopathologies, with depression and anxiety disorders becoming particularly prevalent (Costello, Mustillo, Erkanli, Keeler, & Angold, 2003; Kessler et al., 2007). The literature is mixed regarding a link between pubertal status and depressive affect, with many early studies showing discrepant results (see Buchanan, 1991 for review). However, more recent large-scale, longitudinal studies have published evidence of a positive association between pubertal status and depressive symptoms in US and UK girls, where girls in later stages of puberty report greater levels of depressive symptoms than peers at earlier stages of puberty (Angold, Costello, & Worthman, 1998; Joinson et al., 2012). The Great Smoky Mountain Study (Angold et al., 1998) also commented on a decreasing level of depressive symptoms in boys as they progressed through puberty, which is consistent with some other literature (e.g. Crockett & Petersen, 1987), but again there is limited agreement between studies. Even using large sample sizes and longitudinal methods of investigations, these results are not sufficient to provide evidence of cause and effect i.e. that pubertal development causes or predisposes individuals to depressive symptoms. It is possible, for example, that increasing levels of depressive symptoms could drive pubertal maturation through changes in hormonal levels, or indeed that there is no direct link between these variables and the correlation reflects an indirect association.

Pubertal timing relative to peers may be another important risk factor in the development of depressive symptoms. There is broad consensus in girls that being relatively more pubertally mature compared to your peers is related to increased risk of depressive symptoms (see Negriff & Susman, 2011 for review), but a recent systematic review pointed out that the evidence for this association is still weak (Galvao et al., 2014). Less work has been published looking at pubertal timing in boys, and the results are more variable. Both early maturation compared to peers and late maturation have been shown to increase the risks of male depressive symptoms compared to ‘average’ timing of puberty (see Negriff & Susman, 2011 for review).

There is some physiological evidence that the processing of emotional stimuli changes during puberty. Using pupillometry, Silk and colleagues (2009) studied the affective reactivity of a group of children and adolescents when reading and processing emotional stimuli. This technique involves measuring pupil dilation when reading words with an emotional valence. Pupil dilation occurs when brain regions associated with cognitive and emotional processing are stimulated (Silk et al., 2009). The authors report increased peak pupillary reactivity to emotional word identification in the group of mid-late pubertal participants compared to the pre-early pubertal group, which they interpreted as evidence of puberty-specific changes in the neurobehavioural systems underpinning emotional reactivity (Silk et al., 2009). A second study used the eye-blink startle reflex to study fear/anxiety and the post-auricular to study reflex and reward processing in a sample of 12-13 year old participants who were divided into pre/early and mid/late pubertal groups (Quevedo, Benning, Gunnar, & Dahl, 2009). The mid-late puberty group showed an increase in startle magnitude compared to their less mature peers, and an appetitive potential of the post-auricular reflex. The increased startle magnitude may indicate increased sensitivity to threat stimuli in individuals in late puberty, while the changes in the post-auricular reflex may reflect underlying changes in reward neurobiology with puberty (Quevedo et al., 2009).

This sensitivity to fear and threat stimuli may become apparent as symptoms of anxiety during pubertal development. A comprehensive review of the evidence looking at puberty and the emergence of anxiety symptoms (Reardon, Leen-Feldner, & Hayward, 2009) concluded that there is an increased likelihood of symptoms with advancing puberty status in females, a trend that is not just explained by chronological age. In males, the data are more mixed, with some studies reporting similar trends to those seen in girls (Ge et al., 2001), while others find no significant effect after accounting for age (Patton et al., 1996), or even a decrease of symptoms with puberty (Laitinen-Krispijn, van der Ende, & Verhulst, 1999).

To date there is little work exploring the influence of puberty on the development of mentalising. In one study, the authors tested adolescents aged 13-19 on a mentalising task (Keulers, Evers, Stiers, & Jolles, 2010) and found that boys in more advanced

stages of puberty than their peers had increased mentalising speed, after controlling for age (Keulers et al., 2010). Girls were not included in the analysis due to insufficient variability in pubertal status. Further studies are needed in this area to establish whether this relationship is replicable and robust.

In summary, despite there being a strongly-held perception of the association between puberty and socio-emotional development, in most areas the empirical evidence is relatively weak, and has often not been replicated. There may be a number of reasons for the relative paucity of research in this area, but the field seems to be increasing in breadth and depth, and further evidence may be forthcoming in the future. Where associations between pubertal status and emotional behaviour have been shown in human adolescents, they are limited to correlations, and cannot be used as evidence for a causal pathway. Constructing studies that could examine causal links between puberty and social emotions is challenging, since they would be ethically difficult to design in humans, and non-human models can lack the complexity of pubertal development seen in humans, as well as equivalent socio-emotional behavioural indicators. Studies have been performed looking at surrogate markers for anxiety in animals (Brand & Slob, 1988; Primus & Kellogg, 1989).

In the first of these studies, performed in rats, activity when in an open field is used as an indicator of anxiety. In general, male rates move around less in an open field than female rats, suggesting greater levels of anxiety. Male rates deprived of testosterone during puberty (by castration) show reduced levels of anxiety, and a more female pattern of behaviour (Brand & Slob, 1988). A second study, this time using levels of social interaction in a novel compared to a familiar environment, again showed decreased levels of anxiety (and therefore increased levels of social interaction in the new environment) if male rats were castrated before puberty (Primus & Kellogg, 1989). This effect was not seen if the rats were castrated post-puberty, when no difference in social interaction was seen. These studies suggest a role of testosterone in the development of anxiety behaviour for male rats, but direct links to human behavioural patterns are unclear, and further research is needed.

1.5.2 Decision-making and risk-taking

As discussed in **Section 1.2**, during adolescence there is an increase in individual decision-making and autonomy, and this has been associated with the initiation of a number of 'risky' behaviours that have a significant potential for negative consequences. Early pubertal maturation in adolescence has been associated with increased rates of smoking (Harrell, Bangdiwala, Deng, Webb, & Bradley, 1998; Patton et al., 2004), alcohol use (Dick, Rose, Viken, & Kaprio, 2000) and marijuana use (Magnusson, Stattin, & Allen, 1985). Martin and colleagues (2002) linked the initiation of nicotine, alcohol and marijuana use with increases in sensation-seeking traits in adolescence, and further showed a correlation between pubertal development and sensation-seeking in the same population (Martin et al., 2002). They observed that sensation-seeking mediated the significant relationship between pubertal status and use of nicotine and alcohol, emphasising the potential importance of pubertal maturation on risk-taking behaviour. This correlation between sensation-seeking and health risk behaviours was one strand of evidence that led to the development of a 'dual systems' model of adolescent risk-taking (Casey et al., 2008; Steinberg, 2008), described in **Section 1.2.2**. This hypothesises that risky behaviours in adolescence result from a relatively earlier maturation of the reward-processing network of brain regions compared to the more prolonged maturation of the cognitive control system, which continues to develop into adulthood (Casey et al., 2008; Steinberg, 2008).

A behavioural study exploring this dual systems model evaluated self-reported sensation-seeking and impulsivity behaviours in 935 participants aged 10-30 (Steinberg et al., 2008). Measures of sensation-seeking are thought to reflect the maturation of the reward processing network, which is proposed to develop early in adolescence and to be associated with pubertal development (Martin et al., 2002). In contrast, measures of impulsivity are thought to reflect the maturity of the cognitive control network of brain regions, which allows us to make judgements, plan and inhibit actions (Steinberg et al., 2008). A subsample in the study by Steinberg and colleagues (N=417; 186 female) completed PDS questionnaires as a measure of self-assessed pubertal

status (see **Section 2.3.2.2** for further details of PDS). Regression analyses between pubertal status and the self-reported behavioural measures showed a relationship between pubertal status and sensation-seeking behaviour in boys, although this dropped to trend-level significance when controlled for age, and demonstrated no relationship in females (Steinberg et al., 2008). Using the Stoplight task (see **Section 1.2.2** for description) as a laboratory measure of sensation-seeking, pubertal development was related to performance in both males and females (Steinberg et al., 2008). Both males and females in early and mid-puberty were more likely to drive through the changing traffic light than those who reported being pre-pubertal or post-pubertal. Neither males nor females showed any significant relationship between pubertal status and impulsivity (Steinberg et al., 2008). These results provide some indirect evidence for a dual systems model, although the relationship between puberty and sensation-seeking appears complex.

In order to better understand the potential mechanisms underlying adolescent behaviours, and the validity of the dual systems model, research has focussed on the evolving field of adolescence neuroscience. In the next section, I will consider the physiological mechanisms by which pubertal hormones might affect the brain, and in doing so, influence behaviour. I will then go on to summarise our knowledge and understanding of the structural and functional changes of the adolescent brain, and discuss the limited evidence available of the role of puberty in this process.

1.6 Mechanisms underlying pubertal behavioural changes in adolescence

When considering the underlying reasons why puberty may relate to behavioural change in adolescence, it is important to acknowledge the mechanisms by which an association could occur. Puberty, as outlined above, is a complex, multi-faceted event, and therefore may have more than one way of impacting on the behaviour of adolescents. Broadly, the ways in which puberty can impact on adolescent behaviour can be divided into physiological mechanisms, where the changes in sex steroid

hormone levels can act directly on the brain to affect change, and psychosocial mechanisms, where the overt physical indicators of puberty can change social dynamics within a group having an impact on behaviour. Both of these potential mechanisms further interact with underlying genetic mechanisms associating puberty and behaviour, in a model of gene-environment interactions.

1.6.1 Mechanisms of sex steroid hormone action on the adolescent brain

The brain is known to be a target organ for sex steroid hormones (Sisk & Zehr, 2005), and the action of hormones on the brain are considered to be either organisational or activational. Organisational effects describe the direct effect of a hormone on the developing brain, causing a permanent change that persists beyond the period of hormonal exposure (Sisk & Zehr, 2005). Organisational effects may programme future activational effects in response to steroids later in the life course, often in adulthood. Activational effects refer to the ability of steroids to modify the activity of target cells in ways that facilitate behaviour in specific contexts. These effects are transient and are dependent on the presence of the relevant hormones (Sisk & Zehr, 2005). Understanding the link between organisational and activational effects of steroid hormones may aid in the understanding of the associated behavioural effects.

Organisational effects of steroid hormones on the brain have long been recognised during early development, leading to an understanding of sensitive periods of hormone-dependent developmental processes in fetal and early post-natal life (Resko & Roselli, 1997; Sisk & Zehr, 2005; Wallen, 2005). However, in addition to this critical perinatal period, organisational effects are also hypothesised to occur in other periods of development, and particularly during adolescence (Schulz et al., 2004; Schulz, Molenda-Figueira, & Sisk, 2009). Animal models have demonstrated that a number of social behaviours including sexual behaviours, aggression and flank-marking in males fail to develop fully if the animal is deprived of testosterone during puberty (see Schulz et al., 2009 for review), and these behaviours do not normalise if the testosterone is replaced in adulthood. The impact of female ovarian hormones during adolescence has been less well studied than that of testosterone in males, but has been associated with

the development of female-typical feeding behaviours and mating behaviours in animal studies (see Schulz et al., 2009 for review).

While there is good behavioural evidence in animals for sex steroid dependent organisational development in animals, and also evidence for an association between puberty and behavioural change, it is important to consider how puberty and pubertal hormones influence the structure and functional organisation of the brain in order to achieve these changes. In rats, particular structures of the brain are sexually dimorphic in adulthood, namely the anteroventral periventricular nucleus of the hypothalamus (AVPV), which is larger in females rats than males, and the sexually dimorphic nucleus of the preoptic area (SDN) and medial amygdala, which are larger in male rats than females (Ahmed et al., 2008). This dimorphism has been shown to develop during puberty as a result of new cells being born and proliferating following exposure to sex steroid hormones. Prepubertal gonadectomy prevents this process in the sex-specific structure. Therefore, in female rats who have had a prepubertal ovariectomy, no pubertally-born cells are seen in the AVPV of the adult female, and there is no dimorphism in structure, but normal new cell growth and dimorphism is seen in the SDN and medial amygdala (Ahmed et al., 2008). Contrastingly, male rats who have been castrated before puberty have no new cell proliferation in the SDN or medial amygdala, and do not exhibit the increased volumes expected for these structures, but normal AVPV development is seen (Ahmed et al., 2008). Similar effects in the medial amygdala have subsequently also been shown in the Syrian hamster (De Lorme, Schulz, Salas-Ramirez, & Sisk, 2012). This demonstrates the clear effect that sex steroid hormone exposure can have on the structural organisation of the brain by inducing new cell formation and proliferation, and highlights that the effects may be region-specific and differentially related to different hormones.

In addition to the production and proliferation of new cells, there is some evidence for pubertal influence on controlled cell death. In adult rats, there are sex differences in the volume of the primary visual cortex (Nuñez, Lauschke, & Juraska, 2001; Nuñez, Sodhi, & Juraska, 2002), where there are larger numbers of cells in males compared to females. This difference appears to be driven by greater cell death of female rats

during early puberty, which can be abolished by prepubertal ovariectomy, suggesting that ovarian hormones may promote cell death in the visual cortex during puberty (Nuñez et al., 2001; 2002).

A third way in which pubertal hormones have been shown to affect brain structure and organisation is by influencing the complexity and organisation of neural dendrites in the brain (Murphy & Segal, 1996; Zehr, Nichols, Schulz, & Sisk, 2008). Dendrites in the dentate gyrus in the hippocampus of Syrian hamsters have been shown to reduce in both length and the number of intersections during puberty (Zehr et al., 2008), evidence of dendritic pruning. This effect was only seen in selected areas and not throughout the dentate gyrus, highlighting the specificity of pubertal effects on the brain. *In vivo* and *in vitro* animal studies have directly related the presence of gonadal hormones, both testosterone and oestradiol, to region-specific changes in dendritic spine density (Meyer, Ferres-Torres, & Mas, 1978; Murphy & Segal, 1996).

Gonadal hormones act on the brain by binding to specific receptors. Within the brain, both androgen receptors (AR) and oestrogen receptors (ER) have been identified. There are different types of ER including classic nuclear receptors (α and β) and membrane receptors e.g. GPR30 and ER-X, and each are thought to have differing effects on the functioning of the brain (Cui, Shen, & Li, 2013). While oestrogens are thought to predominantly bind to ERs, androgens (including testosterone and DHEA) work both directly on ARs but also on ERs, after being converted into oestrogens locally via the enzyme aromatase (Kawata, 1995). Both ARs and ERs are found in multiple regions of the brain in varying concentrations, with high levels in subcortical regions, particularly the hippocampus and amygdala, both in animal species and in humans (Abdelgadir, Roselli, Choate, & Resko, 1999; Clark, MacLusky, & Goldman-Rakic, 1988; González et al., 2007; Sholl & Kim, 1989; Shughrue, Lane, & Merchenthaler, 1997). Whilst a small number of studies have demonstrated links between specific receptor expression and behaviour e.g. social decision-making in naked mole-rats (Holmes, Goldman, & Forger, 2008); object recognition and placement tasks in mice (Walf, Koonce, & Frye, 2008), overall our understanding of the actions of hormones via these receptors is still limited.

1.6.2 Psychosocial mechanisms for behavioural changes associated with puberty in adolescence

There is good evidence for the direct role of pubertal hormonal influences on the brain which is likely to cause changes in behaviour. However, it is important to recognise that there is a significant psychosocial impact of physical maturation at puberty, and that this can be influenced by, and in its turn can influence, other environmental and social factors, including peer perceptions and parental attitudes. For example, in a large longitudinal study of more than 4000 adolescents (<15 years of age), early maturation was associated with participating in risk behaviours, and this relationship was mediated by having a romantic partner, particularly if that partner was older (Halpern, Kaestle, & Hallfors, 2007). These results have been replicated across different large-scale longitudinal studies (Cance, Ennett, Morgan-Lopez, Foshee, & Talley, 2013; Kaltiala-Heino, Koivisto, Marttunen, & Fröjd, 2011). Timing of onset of puberty and on-going pubertal changes do not occur in isolation for an individual, and the impact of pubertal timing relative to one's peers is likely to have significant consequences on behaviour.

Early or late pubertal maturation may be directly related to behavioural change, but additional social consequences can arise from being out of synchrony with one's peers in terms of pubertal development. Being more physically mature than one's peers may result in socialising with older peer groups and therefore exposure to different behavioural patterns (Westling, Andrews, Hampson, & Peterson, 2008). Furthermore, any deviation from being 'on-time' compared to one's peers in terms of pubertal development can be socially stigmatising in adolescence, and may lead to an increased vulnerability to develop mood and behavioural problems (Negriff & Susman, 2011). The impact of the timing of pubertal maturation can extend further than the peer group to parents and teachers as well. Parental monitoring has been shown to moderate the relationship between early pubertal maturation and alcohol use in both boys and girls, suggesting that some stereotypical adolescent risky behaviours may be more prolific in early-maturing individuals perhaps because they are regarded as more mature and are monitored less closely (Westling et al., 2008).

In summary, there are numerous ways in which the pubertal development of an individual may affect parent-child relationships, and relationships with wider social contacts, and may therefore indirectly affect behaviour and mood (Paikoff & Brooks-Gunn, 1991). Dissociating the physiological and psychosocial mechanisms is complex, since the two necessarily co-occur. The empirical studies in this thesis focus on hypotheses of the physiological hormonal mechanisms as the explanation for brain and behavioural changes that are seen, but it is important to emphasise that the approaches used cannot exclude the possibility that the effects seen result from psychosocial consequences of puberty as opposed to direct physiological ones. These potentially confounding effects will be discussed further in the final discussion chapter of the thesis.

In order to investigate what impact puberty has on the developing adolescent brain, it is first necessary to review our current understanding of human brain development, both in terms of structure and function. The following sections summarise what is currently known using key studies to demonstrate the main findings.

1.7 Structural brain development in adolescence

The past 20 years have seen a major expansion in research on the structural development of the maturing human brain in childhood and adolescence, largely due to the advent of magnetic resonance imaging (MRI) technology. There is now substantial evidence for the on-going development of many regions of the brain into adulthood, both microscopically and macroscopically.

1.7.1 Histological changes in brain structure during adolescence

Since the late 1960s, studies have shown evidence of cellular age-related differences in particular regions of the human brain in childhood and adolescence (Huttenlocher, 1979, 1990; Huttenlocher & Dabholkar, 1997; Miller et al., 2012; Petanjek et al., 2011; Yakovlev & Lecours, 1967). These differences are likely related to on-going

developmental processes occurring in the white matter and the grey matter of the brain, and include changes in the myelination of axons and axonal calibre, and changes in the organization and density of synapses.

1.7.1.1 Changes in myelination

Myelination, the process of laying down myelin by oligodendrocytes, is important for the maturing connectivity within the developing brain, allowing the synchronization of information transfer within and between brain networks (Fields & Stevens-Graham, 2002). Myelination occurs as a result of reciprocal communication between neurons and oligodendrocytes (Simons & Trajkovic, 2006). Humans are born with relatively low levels of neocortical myelin compared with other nonhuman primates such as chimpanzees (Miller et al., 2012).

Human post-mortem studies have shown that myelination continues through the first and second decade of life (Benes, Turtle, Khan, & Farol, 1994; Yakovlev & Lecours, 1967). A recent study quantifying myelinated axon fibre length density in post-mortem samples (Miller et al., 2012) showed that myelination continues until at least 28 years of age, only reaching 60% of its adult levels by adolescence/early adulthood (age 11–23 years). Myelination does not occur uniformly across the brain according to these studies, but instead region-specific development occurs at different ages (Benes et al., 1994; Yakovlev & Lecours, 1967). Of the regions studied, those associated with primary sensory information e.g. visual cortex, show significant development early in life and plateau in late childhood. In contrast, regions involved in coordinating different sensory inputs, and in performing higher level complex functioning continue to show evidence of on-going myelination into the third decade of life.

1.7.1.2 Synaptic Changes

Synaptogenesis in humans begins in the third trimester of fetal life and continues for a variable time depending on the brain region being examined (Huttenlocher & Dabholkar, 1997). It has, for instance, been found that the auditory cortex shows a rapid burst of synaptogenesis postnatally, peaking at age 3 months. In contrast, the prefrontal cortex continues to demonstrate synaptogenesis until 3.5 years of age

(Huttenlocher & Dabholkar, 1997), and may continue for a longer period (Huttenlocher, 1990). Following this period of synaptogenesis, the cortex undergoes a period of synaptic pruning (Huttenlocher, 1979), which is again heterochronous, occurring earlier in the primary sensory cortices (e.g., visual cortex) than in the prefrontal cortex, where it continues well into the second decade of life (Huttenlocher, 1990).

This body of work by Huttenlocher and colleagues has been of great importance in our understanding of the on-going brain development past early childhood, and has been significantly expanded by a more recent study that investigated synaptic development in the prefrontal cortex of post-mortem brains from 32 individuals aged between 1 week and 91 years (Petanjek et al., 2011). This study also showed increasing dendritic spine density in prefrontal cortex throughout infancy and childhood, with a decrease in adolescence and into adulthood (see **Figure 1.6**). The peak dendritic spine density in late childhood was approximately twice that of adult levels, and remained significantly higher than adult levels throughout adolescence. These data suggest that there is protracted pruning of synaptic spines throughout adolescence and into early adulthood (see **Figure 1.6**; Petanjek et al., 2011).

While histological studies provide invaluable information regarding the basic processes underlying brain development, they are subject to a number of limitations. As post-mortem techniques, they are necessarily cross-sectional, and are therefore unable to inform us about how individuals change over time, how structures vary in their development both within and between individuals, and how these structural changes relate to functional correlates and behavioural patterns. Such histological studies in humans are scarce, and involve a limited number of participants, as a consequence of the high level of skill and experience, time, money and resources required to conduct them, and the limited availability of samples. Newer technologies, particularly MRI, can be used to supplement this histological evidence in order to better describe the major macroscopic developmental patterns of the human brain in adolescence, and to link these to the likely microscopic changes that underlie them.

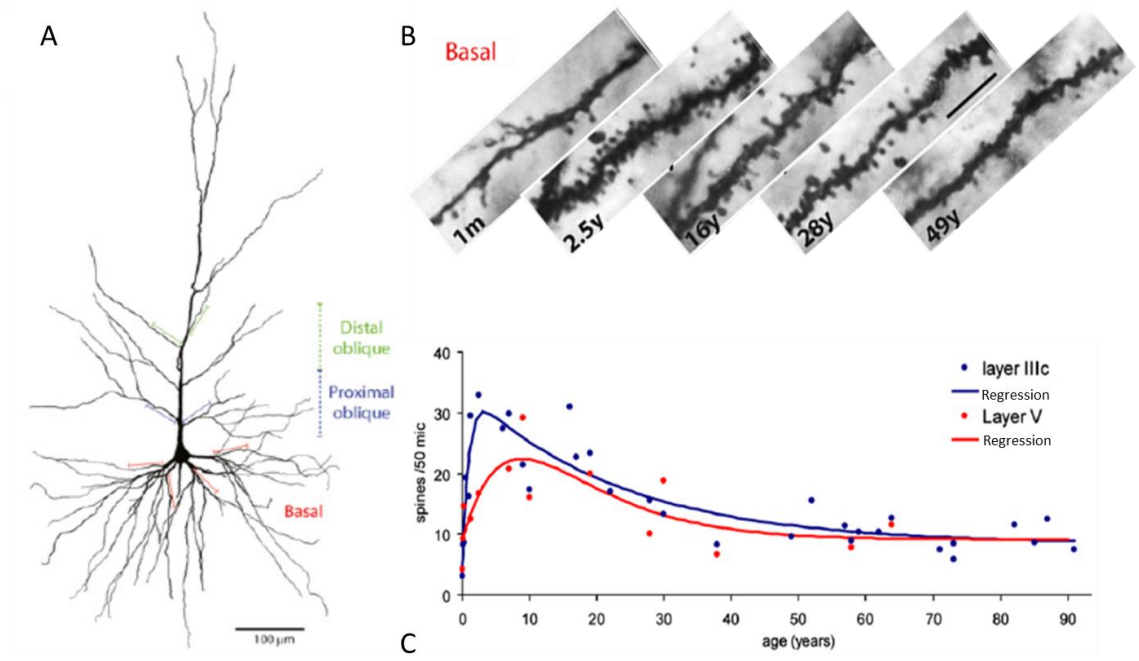


Figure 1.6: Synaptic changes with age showing a peak in dendritic spine density in the prefrontal cortex in late childhood. *(A) shows a reconstruction of a pyramidal neuron showing basal (red), proximal oblique (blue) and distal oblique (green) dendrites. (B) shows representative high-power magnification images of layer IIIc pyramidal neurons from the dorsolateral PFC showing basal dendrites in subjects of different ages; 1 month, 2.5 years, 16 years, 28 years and 49 years. (C) shows age on the x-axis and dendritic spine density (measured in spines/50 micrometres) on the y-axis for each subject. The blue line represents layer IIIc dendrites, and the red line represents layer V dendrites. The lines represent regression curves of best-fit for the data. Reproduced from Petanjek et al., 2011.*

1.7.2 Cortical structural changes using MRI

First used in paediatric neuroimaging studies of typical development in the late 1980s, MRI allows the collection of high-resolution anatomical brain images of large numbers of healthy children and adolescents without the use of ionizing radiation. A major benefit of MRI as an investigative technique over other forms of imaging or post-mortem studies is that it enables the collection of repeated scans of the whole brain in the same individuals over time. Given the large inter-individual variability in structural volumes, longitudinal data have proved invaluable for documenting developmental trajectories (i.e., change in volume over time) within individuals, and variability of

development between individuals. With the increased availability of MRI, a number of large-scale studies of typical structural brain development have demonstrated not only that the human brain continues to undergo substantial structural remodelling throughout childhood and adolescence and into adulthood, but have also documented region-specific differences in the timing and extent of these changes.

The earliest longitudinal brain imaging project began in 1989 under the direction of Markus Krusei at the Child Psychiatry Branch of the National Institute of Mental Health (CPB; Giedd et al., 1996). This longitudinal brain imaging project has acquired more than 6,500 structural MRI scans from thousands of individuals aged 3 years and older (see http://clinicalstudies.info.nih.gov/cgi/detail.cgi?A_1989-M-0006.html). The data for the following sections regarding quantification of brain structure sizes are from subgroups of typically developing individuals within this cohort, including Giedd et al., 1996 (cross-sectional, 104 participants, 4–18 years); Giedd et al., 1999 (243 scans, 145 participants, 4–21 years); Gogtay et al., 2004 (52 scans, 13 participants, 4–21 years); Lenroot et al., 2007 (829 scans, 387 participants, 3–27 years); Raznahan, Lerch, et al., 2011 (376 scans, 108 participants, 9–22 years) and Raznahan, Shaw, et al., 2011 (1274 scans, 647 participants, 3–30 years). Since this initial pioneering study began, many other studies have been initiated, and there is now a substantial literature documenting structural brain development in adolescence. To date, few of these have reported measures of pubertal development. I shall first summarise the main findings regarding adolescent structural brain development, and then focus on specific puberty-related findings.

1.7.2.1 Total Cerebral Volume

Total brain volume has been shown to double in the first year of life and to increase by a further 15% in the second year (Knickmeyer et al., 2008). It approaches adult volumes by mid-childhood, reaching approximately 95% of peak volume by age 6 (Lenroot et al., 2007). After this time, total brain volume remains relatively stable through the second decade into adulthood, with some studies suggesting a small reduction in volume (Brain Development Cooperative Group [BDCG], 2012; Koolschijn & Crone, 2013; Lenroot et al., 2007; Østby et al., 2009).

Consistent with the adult neuroimaging literature (e.g., Goldstein et al., 2001), mean total cerebral volume is approximately 10% larger in boys than girls throughout childhood and adolescence (Giedd, Raznahan, Mills, & Lenroot, 2012). This finding has often been attributed to differences in overall body size in adult populations, but the CPB study showed that the differences in brain size preceded the striking differences in body size, which predominantly occurred after puberty in the sample (Lenroot et al., 2007). This study demonstrated the large inter-individual variability that exists in brain volumes. Thus, healthy children at the same age may have nearly two-fold differences in total brain size, as well as large variation between volumes of various substructures. While the mean difference in total cerebral volume persisted between the sexes, there was significant variation within and between the sexes in terms of brain volume (Lenroot et al., 2007).

1.7.2.2 White matter development

Cortical tissue can be broadly divided into white matter (WM) and grey matter (GM) based on its appearance on MRI. WM is composed primarily of axons, many of which are myelinated, and associated vasculature and glia. Early cross-sectional MRI studies of WM development showed that volumes increased through childhood and adolescence (Jernigan & Tallal, 1990; Reiss, Abrams, Singer, Ross, & Denckla, 1996; Schaefer et al., 1990), complementing the anatomical studies describing on-going myelination during childhood described above. More recent longitudinal MRI studies have tracked the trajectories of WM volume development, revealing age-dependent patterns that were broadly similar across lobes (frontal, temporal and parietal; Lenroot et al., 2007). A key WM structure in the brain is the corpus callosum, which is made up of approximately 200 million mostly myelinated axons that connect homologous areas of the left and right cerebral hemispheres. The CPB study (Lenroot et al., 2007) and other studies (Pujol, Vendrell, Junqué, Martí-Vilalta, & Capdevila, 1993; Thompson et al., 2000) have shown increasing volume of the corpus callosum in childhood and adolescence (4-20 years). MRI data does not yet have the resolution to establish the underlying cellular mechanisms that lead to this increasing volume, but it is thought to

represent, at least in part, the changing axonal structure of neurons, with increasing axonal diameter of the fibres, and continued myelination (Paus et al., 2010).

With advances in MRI technology, additional methods to characterize WM structure have been developed. One important technique is diffusion tensor imaging (DTI), which quantifies diffusion of water through different regions of the brain (Mori & Zhang, 2006). If unconstrained, water molecules will randomly diffuse in all directions. Non-random diffusion can be used to infer constraints placed upon the motion of water by physical features such as cell membranes or interactions with large molecules (Le Bihan et al., 2001). Fractional anisotropy (FA) is a measure used to indicate the degree of non-randomness of the diffusion, providing both information on the microstructure of WM and the directionality of the axons contained within it. Mean diffusivity (MD), the overall speed of diffusion, tends to be decreased by these same factors.

As would be predicted by increasing myelination, overall FA has been shown to increase and MD to decrease during childhood and adolescence (Lebel & Beaulieu, 2011; Peters et al., 2012; Tamnes, Østby, et al., 2010). FA increases are generally driven more by reductions in radial diffusion (RD) (in the perpendicular plane to predominant diffusion direction) than by changes in axial diffusion (AD) (in the plane parallel to predominant diffusion direction) (Giorgio et al., 2008; Lebel, Walker, Leemans, Phillips, & Beaulieu, 2008), although some studies have reported a decrease in both modalities (Eluvathingal, Hasan, Kramer, Fletcher, & Ewing-Cobbs, 2007).

High FA indicates coherently bundled myelinated axons and axonal pruning, resulting in more efficient neuronal signalling (Suzuki, Matsuzawa, Kwee, & Nakada, 2003) and improved cognitive performance e.g. reading ability (Beaulieu et al., 2005) and IQ (Schmithorst, Wilke, Dardzinski, & Holland, 2005). This development varied between regions and specific tracts, with tracts connecting frontal and temporal regions e.g. cingulum, uncinate fasiculus, and superior longitudinal fasciculus showing more prolonged periods of maturation than other regions e.g. corpus callosum and fornix (Lebel et al., 2012). One study showed FA continuing to increase beyond 23 years of

age in frontal and temporal regions (Tamnes, Østby, et al., 2010). Each of these studies demonstrate substantial inter-individual variation in the age at which individual tracts stabilise, and some individuals continue to show changes in FA into the fourth decade of life (Lebel & Beaulieu, 2011).

1.7.2.3 Cortical grey matter development

Examining the developmental trajectory of total grey matter (GM) volume in the CPB study revealed inverted U-shaped trajectories with an increasing volume across childhood followed by a decrease in adolescence (Giedd et al., 1999; Lenroot et al., 2007). The rate of decreasing volume in adolescence reduces with age, suggesting relative stability of overall GM volume by early adulthood (Hedman, van Haren, Schnack, Kahn, & Hulshoff Pol, 2012; Lenroot et al., 2007; Østby et al., 2009). The timing at which GM volume peaks differs between lobes, with parietal lobes peaking before the frontal lobes and the temporal lobes peaking last (Giedd et al., 1999; Muftuler et al., 2011). Across the cortical surface, there is extensive heterogeneity in this developmental timing, with the earliest GM volume peak and subsequent decline occurring in the primary sensorimotor areas, and the latest in higher-order association areas that integrate those primary functions, such as the dorsolateral prefrontal cortex, inferior parietal cortex, and superior temporal gyrus (Gogtay et al., 2004; Muftuler et al., 2011). The underlying mechanisms associated with a reduction in GM volume are still debated (Carlo & Stevens, 2013; Paus, 2010), and to date there are no studies that have tested the relationship between developmental changes in underlying cellular or synaptic anatomy and structural MRI measures. Despite these limitations, it is thought that reductions in GM volume may reflect synaptic and glial reorganization and/or increases in WM resulting in GM encroachment and apparent GM volume reduction.

Improving MRI resolution and the development of new surface-based reconstruction tools e.g. CIVET, Freesurfer has enabled the study of distinct aspects of GM structure, such as cortical thickness and surface area, in addition to GM volume. Cortical thickness refers to the distance between the two surfaces of the GM cortex i.e. the GM-WM interface and the outer GM-pial interface. Surface area refers to the total or

regional area of the cortical surface. GM volume is a product of cortical thickness and surface area. Cortical thickness and surface area are driven by distinct genetic (Panizzon et al., 2009; Winkler et al., 2010), evolutionary (Rakic, 1995), and cellular (Chenn & Walsh, 2002) processes. Differences in surface area are pronounced across species (Hill et al., 2010; Rakic, 1995), whereas cortical thickness is highly conserved in comparison. Many areas of the brain that have expanded in surface area across evolution also show relatively greater surface-area expansion between infancy and adulthood (Hill et al., 2010).

The CPB dataset has been used to characterize changes in surface area and cortical thickness (Raznahan, Shaw, et al., 2011). In this study, both cortical thickness and surface area showed decreases throughout adolescence following peak measurements in childhood. In males, the changes in surface area explained two-thirds of the changes seen in cortical volume, while in females cortical thickness and surface area appeared to contribute equally (Raznahan, Shaw, et al., 2011). Reductions in cortical thickness during adolescence have also been demonstrated in other studies (Brown et al., 2012; Tamnes, Ostby, et al., 2010; van Soelen et al., 2012). Further work is needed to elucidate the developmental trajectories of cortical thickness during childhood, since the findings across these studies are not consistent in this younger age range.

1.7.3 Subcortical structural changes using MRI

The relatively small size and ambiguous MRI signal of the borders between subcortical structures mean that reliable automated techniques for extracting volumes on a large scale have only recently been developed, and to date there are few published large-scale studies documenting the developmental trajectories of subcortical structures.

1.7.3.1 Amygdala and hippocampus

Amygdala and hippocampus volumes increase during early to mid-childhood (1 month to 8 years; Uematsu et al., 2012). The developmental trajectory in late childhood and adolescence is less clear. An early cross-sectional analysis of amygdala and hippocampus volumes using data from the CPB cohort, validated by expert rater

manual tracing, showed increases in amygdala volumes in males only and increases in hippocampal volumes in females only (Giedd et al., 1996; N=104, 4–18 years). A later, larger cross-sectional study of amygdala and hippocampal development between 8 and 30 years of age (N=171) showed a nonlinear increase in volume with age of both the hippocampus and amygdala in both sexes (Østby et al., 2009), and a further cross-sectional study of 885 participants between 3 and 20 years of age showed nonlinear increases in hippocampus volume (Brown et al., 2012). However, a longitudinal study that incorporated the developmental trajectories of the amygdala and hippocampus described small decreases in both amygdala and hippocampus volume (n=85, 8–22 years; Tamnes et al., 2013). A decrease in amygdala volume (but not hippocampal volume) was also reported by Uematsu and colleagues (n=109, 1 month–25 years; Uematsu et al., 2012). The discrepancy between studies may reflect the large inter-individual variation in amygdala and hippocampus volume, where there is a twofold variation in absolute volume (Giedd et al., 1996; Østby et al., 2009; Tamnes et al., 2013), or different study designs and methods of measurement and analysis.

1.7.3.2 Thalamus

Analyses of the changes in thalamus volume across adolescence show a similar discrepancy between studies to those seen when examining the amygdala and hippocampus. One longitudinal study showed decreasing volume with age (8–22 years; Tamnes et al., 2013) and an early cross-sectional study also reported thalamic decreases with age (N=35, 7–16 years; Sowell, Trauner, Gamst, & Jernigan, 2002). In contrast, other cross-sectional studies have reported slight increases in thalamus volume over adolescence (BDCG, 2012; Brown et al., 2012; Koolschijn & Crone, 2013; Østby et al., 2009).

1.7.3.3 Basal ganglia

The basal ganglia are a collection of subcortical nuclei (caudate, putamen, globus pallidus, subthalamic nucleus, and substantia nigra). The caudate decreases in volume across adolescence (Lenroot et al., 2007; Tamnes et al., 2013). The CPB longitudinal study found that the caudate volume followed an inverted U-shaped trajectory, increasing in childhood and peaking before decreasing in adolescence (Lenroot et al.,

2007), while the longitudinal study by Tamnes et al. showed a steady decrease in volume throughout the study (Tamnes et al., 2013). This discrepancy may reflect the differing starting ages of the studies and emphasizes the relative paucity of information regarding the childhood trajectories of many subcortical regions. The large cross-sectional samples available support the longitudinal data showing a decrease in caudate volume during adolescence (BDCG, 2012; Østby et al., 2009).

The data for the putamen, globus pallidus, and nucleus accumbens are broadly in agreement, showing decreases in volume across adolescence, although the extent of these changes varies between studies and some do not show significant changes in all regions (BDCGroup, 2012; Koolschijn & Crone, 2013; Østby et al., 2009; Sowell et al., 2002; Tamnes et al., 2013).

1.7.4 Structural brain development associated with puberty

Cross-sectional studies have reported that puberty is associated with aspects of brain development in adolescence. A study focussing on the association between brain volumes and both pubertal stage and testosterone concentration found that males and females in later stages of puberty, and with higher circulating testosterone concentration, had larger amygdala volumes and smaller hippocampal volumes than their less well developed peers (Neufang et al., 2009). In contrast, a second study investigating puberty and pubertal hormone correlations with grey matter volume showed decreasing amygdala volume with increasing testosterone levels in girls (Bramen et al., 2011). Studies looking at cortical grey matter and pubertal measures have found region-specific correlations between grey matter density and both pubertal stage and oestradiol concentration in girls (Peper et al., 2009). These studies have limited power due to relatively small sample sizes. No longitudinal studies incorporating pubertal measures had been completed at the time of starting my PhD, constraining our ability to attribute causality to this association or to investigate the effect of sex steroids on brain developmental trajectories during adolescence.

Only two studies have considered whether pubertal factors also influence white matter microstructure during adolescence, in addition to effects of age. One study looked at RD in white matter tracts in males and females aged 8-28 years, focussing on tract regions of interest in which significant age effects occurred, to explore whether pubertal effects were also present (Asato, Terwilliger, Woo, & Luna, 2010). Several association and projection tracts demonstrated continued immaturity (through a relatively high RD) in early and mid-puberty, suggesting that pubertal changes may be more tightly coupled to white matter maturation than previously thought. The second study reported increased FA in boys in cortico-spinal, long-range association and cortico-subcortical white matter, and reduced MD in frontal and temporal white matter in boys compared with girls (Herting, Maxwell, Irvine, & Nagel, 2012). They also found that pubertal hormones such as testosterone explained variation in microstructure within some white matter regions (Herting et al., 2012).

1.8 Functional brain development in adolescence

In addition to the advances in our understanding of structural brain development in adolescence over the past two decades, there have also been extensive increases in our knowledge of the changing function of the brain over this period. Adolescent cognitive neuroscience, being a young and dynamic field, is fast-moving and new data are readily forthcoming. I will focus in this introduction on the relevant studies that were published when the work in this thesis was being conceived and designed.

1.8.1 Social behaviour and emotional development

As summarised in **Section 1.3.1**, social behaviours change dramatically during adolescence. Using fMRI, it is possible to explore whether these behavioural changes are associated with contemporaneous changes in neural activity. The collection of brain regions subserving social cognition is known as the 'social brain' (Frith, 2007). This includes the fusiform face area, the posterior superior temporal sulcus (pSTS), the amygdala, the temporo-parietal junction (TPJ), the medial prefrontal cortex (mPFC), the anterior cingulate cortex (ACC) and the anterior temporal cortex (ATC) (see **Figure**

1.7). fMRI studies show differences between adolescence and adulthood in patterns of activity within these regions during social tasks.

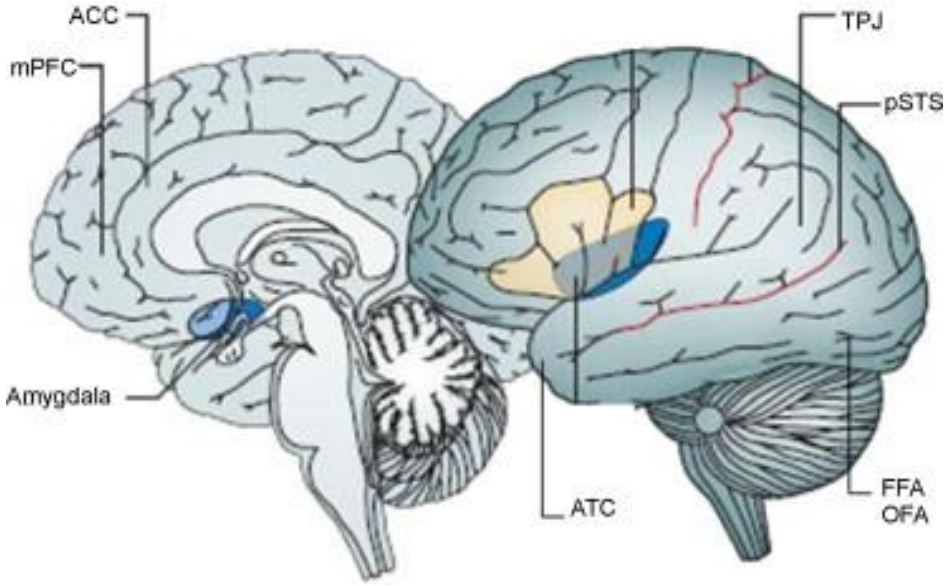


Figure 1.7: The social brain. *Regions shown are the medial prefrontal cortex (mPFC), anterior cingulate cortex (ACC), temporo-parietal junction (TPJ), posterior superior temporal sulcus (pSTS), fusiform face area), occipital fusiform area (OFA), anterior temporal cortex (ATC) and amygdala. From Burnett, Sebastian, Cohen Kadosh, & Blakemore, 2011, adapted by Blakemore, 2008.*

A key aspect of social interaction is the ability to read the visual cues of people, and particularly to interpret their facial emotions. Basic facial processing i.e. identifying that an object is a face, has consistently been shown to exhibit a prolonged developmental pattern, both in terms of processing abilities and of functional specialisation within the relevant brain regions, with adolescents showing an intermediate pattern of BOLD activation in the fusiform face area compared to children and adults (Golarai et al., 2007; Peelen, Glaser, Vuilleumier, & Eliez, 2009; Scherf, Behrmann, Humphreys, & Luna, 2007). The ability to interpret emotions from facial expressions recruits additional brain regions in addition to those required for facial recognition, including regions involved in emotion processing (e.g. amygdala), action and emotion regulation (e.g. ACC and orbitofrontal cortex (OFC)), and higher-level social processing (e.g. mPFC and ACC). Two studies comparing adolescent and

adult BOLD signal activation patterns when identifying fearful faces compared to neutral faces have reported increased activation of the amygdala in adolescents compared to adults (Guyer et al., 2008; Monk et al., 2003), suggesting on-going development of the neural processing of emotional faces.

As previously described in **Section 1.3.1**, mentalising is the ability to understand another person's intentions, emotions, desires and beliefs. It is a crucial capacity for a range of social behaviours and depends on components of the social brain network (Olsson & Ochsner, 2008). The network of brain regions recruited during mentalising tasks is a subset of the social brain regions, and includes the dorsal mPFC, pSTS/TPJ and ATC (see **Figure 1.7**). Developmental studies have shown a shift in relative activity within regions of the mentalising network between adolescence and adulthood (Blakemore, 2008). Specifically, a number of studies have consistently shown that signal in the mPFC during mentalising tasks decreases with age across adolescence (e.g. Blakemore, den Ouden, Choudhury, & Frith, 2007; Burnett, Bird, Moll, Frith, & Blakemore, 2009; Gunther Moor et al., 2011; Overgaauw, Güroğlu, Rieffe, & Crone, 2014; Pfeifer et al., 2009; Wang, Lee, Sigman, & Dapretto, 2006), despite using a variety of different mentalising tasks to investigate this (see **Figure 1.8** for results of meta-analysis). Many of these studies also report age-related increases in activity in other parts of the mentalising network, including the ATC, ACC and pSTS/TPJ during this period (see Burnett et al., 2011 for review).

A further aspect of social behavioural development that has been studied using neuroimaging techniques is social evaluation. As reported in **Section 1.3.1**, adolescents are highly affected by the perceived opinions of others. When adolescents are lying in an fMRI scanner, they show heightened recruitment of the mPFC when they think they are being watched compared with children or adults, in a pattern that mimics increasing embarrassment levels of the same participants (Somerville et al., 2013). In a study recreating a perceived sense of social rejection using the Cyberball task during fMRI data collection (Sebastian et al., 2010), female adolescents showed differential patterns of brain activation to adults. While adults showed increased levels of ventrolateral PFC activation when they were being excluded, the opposite pattern was

shown for adolescents (Sebastian et al., 2010). Different patterns of BOLD activation in the mPFC have been found in other studies comparing processing of social evaluation in adolescents with that of adults (Gunther Moor et al., 2011; Sebastian et al., 2011), and it is hypothesised that these changes may underlie differences in behaviour between adolescence and adulthood (Burnett, Sebastian, et al., 2011). It has further been hypothesised that these changes seen during adolescence may be triggered or driven by pubertal events (Blakemore et al., 2010), but none of the studies discussed above incorporated measures of pubertal development to accurately test this hypothesis.

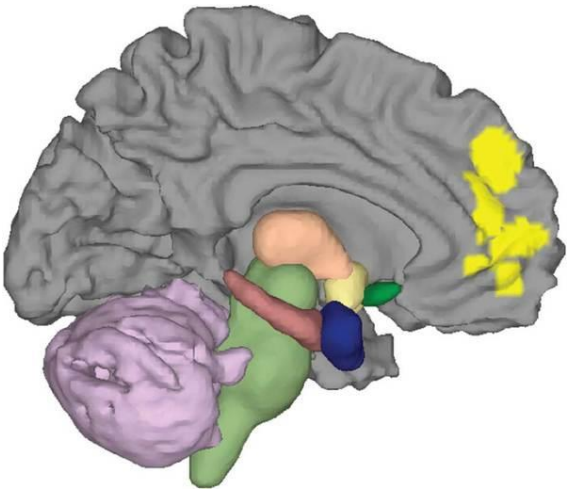


Figure 1.8: Meta-analysis showing voxels in mPFC exhibiting a negative relationship with age in fMRI studies of social cognition*. The yellow area delineates voxels that are within 10mm of the peak voxel in three or more of eight published developmental fMRI studies of social cognition. From Blakemore & Robbins, 2012.*

1.8.2 Decision-making and risk-taking

As outlined in **Section 1.3.2**, the real-life decisions made by adolescents, and their consequential behaviours and actions, are characteristically perceived to be different from and more risky than those chosen by adults in the same situations (Blakemore & Robbins, 2012). Decision-making cognition depends on the interaction of several component processes including response selection and inhibitory control and the value and representation of reward. These processes have been associated with on-going neural development during adolescence.

Go/no-go and stop-signal reaction time tasks can be used in an fMRI scanner to assess levels of inhibitory control. In both of these tasks, a participant is asked to repeat an

action or response unless they see or hear a particular cue. The process of having to inhibit the action when the cue is seen recruits frontal brain regions in adults including the ACC and the lateral PFC (Rubia et al., 2001). While performance on go-no-go and stop-signal tasks tends to improve during adolescence, studies investigating neural activation in the PFC in adolescents and adults have found varied results, with some studies showing increased PFC activity in adolescents relative to adults (Luna et al., 2001) and others showing gradually rising levels of PFC activity with age into adulthood (Rubia et al., 2000; Rubia et al., 2006). These differences are likely to relate to the specific task being used and the exact PFC region involved.

Inhibitory control, in addition to preventing a pre-potent response, can be studied using temporal discounting of reward paradigms. These involve scenarios where a participant is offered a small reward immediately, or a larger one after a specified time delay. Temporal discounting studies have been performed on animals, children and adults, and show that the probability of choosing the large reward is a hyperbolic function of this delay. There is considerable inter-individual variation in behaviour, with some individuals consistently showing more 'impulsive' behaviour and taking the immediate reward more often than others (Casey, Jones, & Somerville, 2011). With improving impulse control during childhood and adolescence, temporal discounting of reward decreases (Scheres et al., 2006), a behaviour that has been associated with an increase in the activity of the left ventromedial PFC (vmPFC), as well as a decrease in the ventral striatum (VS) (Christakou, Brammer, & Rubia, 2011). These regions are part of a network associated with reward-processing, emphasising the involvement of both reward-processing and inhibitory control in behavioural outcomes during a decision-making task.

Unlike inhibitory control, which has been shown to increase gradually during adolescence, and appears to be related to linear changes in neural activation, adolescents have been shown to exhibit different responses to reward from either children or adults (Ernst et al., 2005; Galvan et al., 2006). In particular, studies have focussed on activation within the VS, showing heightened activation during reward tasks in adolescents compared to adults across a number of studies (Cohen et al.,

2010; Ernst et al., 2005; Galvan et al., 2006; Geier, Terwilliger, Teslovich, Velanova, & Luna, 2010) although not all studies have demonstrated this (Bjork et al., 2004).

Evaluation of the changes in decision-making behaviour of adolescents (see **Section 1.3.2**) together with the evidence of differing patterns of maturation between brain regions, led to a proposed model of adolescent brain development (Casey et al., 2008; Steinberg, 2008). This model, known as the dual systems model, seeks to link the behavioural and neuroimaging data of adolescent risk-taking. As outlined in **Section 1.3.2**, the dual systems model proposes that increased risk-taking in adolescence is a result of a mismatch in developmental timing in the neural networks responsible for cognitive control, which develop gradually throughout adolescence and into adulthood, and reward-processing, which matures earlier in adolescence before stabilising in later adolescence (See **Figure 1.9**). The 'gap' in maturation during adolescence results in adolescents being biased towards more reward-seeking choices than children or adults (Casey et al., 2008; Steinberg, 2008).

A number of studies have been conducted exploring this dual systems model using a variety of different paradigms and populations (see **Table 1.1** from Mills, Goddings, Clasen, Giedd, & Blakemore, 2014). When considering the reward-processing network of regions, these studies have focussed on the nucleus accumbens (NA) or ventral striatum (VS), the ventromedial prefrontal cortex (vmPFC) and the orbitofrontal cortex (OFC) (see **Table 1.1**). Results for the NA and VS are mostly consistent, with studies showing greater activation during risk-taking tasks in adolescents compared to children and adults (Ernst et al., 2005; Galvan et al., 2006; Geier et al., 2010; Somerville, Hare, & Casey, 2011; Van Leijenhorst, Zanolie, et al., 2010); although see (Bjork et al., 2004; Van Leijenhorst, Gunther Moor, et al., 2010).

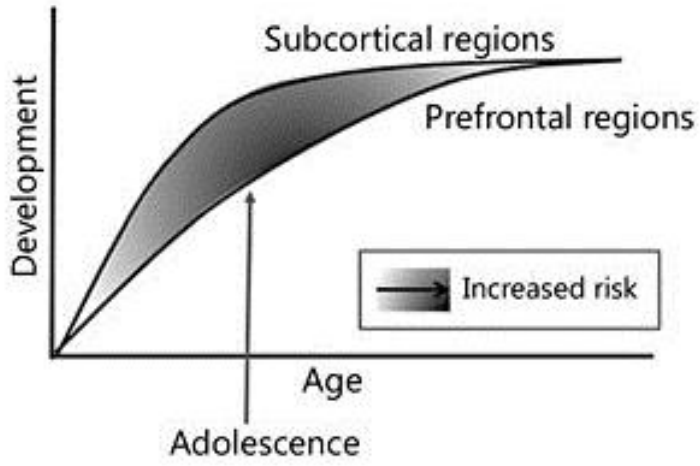


Figure 1.9: The dual systems model*. This schematic illustrates the proposed developmental mismatch in brain maturation, with subcortical regions (amygdala and VS) maturing during early adolescence, and the prefrontal regions maturing later, in adulthood. The authors hypothesised that the gap (shaded) in maturity increases the risk for affectively driven behaviours during adolescence. From Casey et al., 2011.*

Data for the vmPFC and OFC are more mixed. Only one of the studies in **Table 1.1** reported results for the vmPFC, and showed a peak in neural activation in adolescence when making risky decisions, with no differences between age groups when observing the outcome of the task (Van Leijenhorst, Gunther Moor, et al., 2010). Four publications reported results for the OFC, and showed a mixed picture of neural activation, with greatest activity seen in childhood (Galvan et al., 2006), adolescence (Van Leijenhorst, Gunther Moor, et al., 2010 during decision-making) and adulthood (Eshel, Nelson, Blair, Pine, & Ernst, 2007; Van Leijenhorst, Zanolie, et al., 2010 during reward outcome) across the studies. Again, these studies differ in the tasks used, and the process being analysed (decision-making vs. reward anticipation vs. processing of outcome), which may explain the differences found in activation patterns.

The data for prefrontal cognitive control regions are mixed, with conflicting results regarding the relative functional activation in different age groups (see **Table 1.1**). Cognitive control regions identified by these previous studies have included the dorsolateral and ventrolateral PFC, as well as the dorsal ACC (dACC) (Van Leijenhorst,

Gunther Moor, et al., 2010). Importantly, there is little consensus on which regions of the PFC are part of the cognitive control network in the dual systems model, and this varies depending on the study, the task and the element of decision-making that is being investigated. Neither of the studies that reported lateral PFC activation during the risk-taking task found any differences between children, adolescents and adults (Eshel et al., 2007; Van Leijenhorst, Gunther Moor, et al., 2010). The results for the dACC are inconclusive, with studies showing highest levels of BOLD signal in childhood (Van Leijenhorst, Gunther Moor, et al., 2010 during risky decision-making), adolescence (Geier et al., 2010 during reward anticipation) and adulthood (Eshel et al., 2007 during risky decision-making).

These conflicting findings may result from the number of differences between the studies in terms of the tasks and participant samples used, the aspect of the decision-making process that was evaluated and the analysis methods. This heterogeneity makes it difficult to draw clear conclusions from these data, but recent reviews of the literature have cautioned that the empirical evidence currently available suggest a more complex picture of neurodevelopment than is suggested by the dual systems model, particularly regarding the PFC development (Crone & Dahl, 2012; Pfeifer & Allen, 2012). As with the neural changes associated with social behaviour discussed earlier, the changes in brain maturation related to risky decision-making have been associated with puberty (Scherf, Behrmann, & Dahl, 2012; Steinberg, 2008). Yet, most of the studies that have investigated these changes have failed to incorporate a measure of pubertal maturation, meaning that evidence to support this theory is limited. In the next section, I shall summarise the limited data available to support the role of puberty in brain development in humans during adolescence.

Table 1.1: Results of studies investigating the dual systems model in adolescence.

Study	amygdala	NA	Lateral PFC	dACC	vmPFC	OFC	Age groups	Process	Task
Bjork et al., 2004		VS					12 adolescents (12-17 years); 12 adults (22-28 years)	Reward anticipation of gains versus non-gains	Monetary Incentive Delay
Ernst et al., 2005	amygdala	NA					16 adolescents (9-17 years); 14 adults (20-40 years)	Response to reward outcome feedback	Wheel of fortune
Galvan et al., 2006		NA*				OFC	13 children (7-11 years); 12 adolescents (13-17 years); 12 adults (23-29 years)	Reward anticipation/ response to outcome feedback	Pirate reward paradigm
Eshel et al., 2007			Lateral PFC	dACC		OFC/ vlPFC	**Same sample as Ernst et al., 2005**	Risky decision-making	Wheel of fortune
Hare et al., 2008	amygdala*						11 children (7-12 years); 24 adolescents (13-18 years); 24 adults (19-32 years)	Response to target/non-target emotional faces	Go/no-go with emotional faces
Van Leijenhorst, Zanolie, et al., 2010		VS*				OFC	17 young adolescents (10-12 years); 18 mid-adolescents (14-15 years); 15 adults (18-23 years)	Response to passive reward outcome feedback	Slot machine
Van Leijenhorst, Gunther Moor, et al., 2010		VS	Lateral PFC	dACC	vmPFC *	medial OFC *	12 pre-pubertal children (8-10 years); 15 pubertal adolescents (12-14 years); 15 post-pubertal adolescents (16-17 yrs); 15 adults (19-26 years)	Risky decision-making	Cake gambling
		VS*	Lateral PFC	dACC	vmPFC			Response to reward outcome feedback	Cake gambling
Geier et al., 2010		VS		ACC/ MFG			18 adolescents (13-17 years); 16 adults (18-30 years)	Reward anticipation	Monetary reward anti-saccade
Somerville et al., 2011		VS*					18 children (6-12 years); 19 adolescents (13-17 years); 25 adults (18-29 years)	Response to target/non-target emotional faces	Go/no-go with emotional faces

Key

BOLD signal magnitude	greater during childhood	greater during adolescence	greater during adulthood	no difference	* peak during adolescence

Table 1.1: Results of studies investigating the dual systems model in adolescence. *This table summarises the results of 10 studies that have investigated developmental changes (with an adult comparison group) in brain activity associated with reward-processing and risk-taking behaviour. vmPFC=ventromedial PFC; MFG=middle frontal gyrus; dACC=dorsal ACC; VS=ventral striatum; NA=nucleus accumbens. Outlined are the developmental changes reported in brain regions relevant to the dual systems model: regions associated with the reward system including the amygdala, VS/NA and the OFC; and regions associated with the cognitive control system including lateral PFC and the dACC. If the study used different nomenclature for ROIs, or reported a cluster that spanned more than one ROI, this nomenclature has been used. Developmental differences in BOLD signal magnitude for the process of interest are indicated by colour, where green indicates greater BOLD signal in childhood, pink indicates greater BOLD signal in adolescence, blue indicates greater BOLD signal in adulthood, and grey background indicating no difference between age groups.*

1.8.3 Functional brain development associated with puberty

At the time of starting the work for this thesis in 2011, only a few functional neuroimaging studies of the adolescent brain had included puberty measures. The field has expanded over the last few years, meaning that there is additional evidence of functional brain changes with pubertal development. For the sake of clarity, I shall summarise here the studies that were published at the time of the design of each of the studies in the thesis, and shall incorporate more recent findings where relevant in the discussions sections of the relevant chapters.

Two fMRI studies have assessed the changes in neural activation associated with face processing with puberty (Forbes, Phillips, Silk, Ryan, & Dahl, 2011; Moore et al., 2012). The first of these showed evidence for increased BOLD signal in the amygdala and ventrolateral PFC to threatening faces in a pre/early puberty group compared with a mid/late puberty group (aged 11-13; Forbes et al., 2011). In a different study, at 10 and 13 years, Moore and colleagues found that participants in later stages of pubertal development showed increased signal in face processing regions, including the

amygdala, the ventrolateral PFC and the dorsal mPFC, when looking at affective facial expressions (Moore et al., 2012). These studies report some discrepant findings, which may reflect the different methods of assessing pubertal development used, or the different tasks administered (Moore et al., 2012; Op de Macks et al., 2011). No previous fMRI study has investigated pubertal influences on mentalising and the social brain.

Two fMRI studies have previously investigated differences in reward processing and puberty (Forbes et al., 2010; Op de Macks et al., 2011). The first of these demonstrated differences in caudate and rostral mPFC BOLD signal between early and late puberty groups (aged 11-13), with adolescents at more advanced pubertal stages showing less caudate activation and more rostral mPFC activation when processing reward outcome in a gambling task (Forbes et al., 2010). Additionally, they reported a correlation between testosterone level and caudate BOLD signal, whereby testosterone was positively correlated with activation during reward anticipation in males only, and negatively correlated during reward outcome in both males and females (Forbes et al., 2010). A second fMRI study investigating reward and pubertal hormonal concentration showed a different, significant positive correlation between testosterone level and VS activation, with both males and females with higher testosterone levels showing greater activation during reward outcome (Op de Macks et al., 2011). These conflicting results emphasise the need for further, clarifying research, and highlight the potential complexity of the BOLD activation patterns during decision-making tasks.

1.9 Thesis overview

The aim of this thesis is to build a body of evidence that will allow a greater understanding of how puberty is related to brain development during adolescence. I shall achieve this through the investigation of three key research questions:

1: What is the relationship between pubertal status and structural brain development?

2: How does the functional activation of the brain during tasks incorporating key adolescent behaviours differ in relation to pubertal status?

3: To what extent can chronological age and pubertal status be disentangled when looking at brain development in human adolescents?

Following this introduction, **Chapter 2** provides an overview of the methods used to measure puberty, and highlights which of these were used in the empirical studies presented in this thesis. Chapters 3-7 describe five studies undertaken to investigate the research questions outlined above. Chapters 3 and 4 focus on changes in brain structure during adolescence, and how these may relate to pubertal development. **Chapter 3** describes a longitudinal study looking at how pubertal development relates to the structural development of the subcortical structures within the brain, using large dataset from the National Institute of Mental Health, USA, and employs mixed effects modelling analysis. **Chapter 4** investigates the relationship between pubertal status and white matter structural development in a cross-sectional sample of 12-16 year old boys, using diffusion tensor imaging. Pubertal status for Chapters 4-7 was assessed using a physical development scale and salivary sex steroid hormone levels.

Chapters 5-7 describe studies that explore the developmental changes in brain function during adolescence, and how these relate to pubertal maturation. Chapters 5 and 6 use data collected from a sample of 11-13 year old girls while performing a social emotion task. In **Chapter 5**, brain regions activated during the social emotion condition, but not the control basic emotion condition, are compared in relation to pubertal status. **Chapter 6** focuses on functional connectivity during the same task, i.e. how the co-activation of connected brain regions varies with pubertal maturation. **Chapter 7** uses a risky decision-making task, the Balloon Analog Risk-taking task (BART) to explore functional brain maturation in a cross-sectional sample of 12-14 year old boys using fMRI. The chapter analyses differences in BOLD activation with puberty during both the decision-making and outcome phases of the tasks, in relation to both pubertal stage and hormonal levels. Finally, in **Chapter 8**, the results of the empirical studies are summarised and the findings and implications of the thesis are discussed.

CHAPTER 2

Investigating puberty

There are two principal aims of this chapter. The first is to describe the participants included in the studies detailed in the subsequent experimental chapters (Chapters 3-7). This includes the recruitment information, inclusion and exclusion criteria and consent procedure.

The second aim of this chapter is to outline how puberty was measured in the different studies described in this PhD thesis and the rationale for the study designs. I have reviewed the most common methods of pubertal assessment, together with their advantages and disadvantages, to determine the best methods to use for each of my empirical studies. In order to investigate the role of puberty in the development of the adolescent brain, it is important to recognise that age and puberty are necessarily related to each other in adolescence, but may have differing effects on behavioural and brain development. In the design of all the studies of my thesis, I have attempted to acknowledge the issue of correlation between chronological age and pubertal development in adolescence, and have used different experimental and statistical methods to tackle this. This has been a key theme throughout my studies, and is relevant to all of the experimental chapters.

2.1 Study design

2.1.1 Dissociating puberty and chronological age

In each of the studies included in this thesis, the principal aim was to investigate the relationship between pubertal development and differing aspects of brain development during adolescence. Puberty is a fundamental component of adolescence, and it impacts on many of the other psychological and social changes that are seen in this period of life. As outlined in the introduction, puberty, once triggered, progresses in a predictable fashion. Therefore, age and puberty are necessarily correlated during development within an individual, since as the individual ages, they also become more pubertally mature. However, within a population, there is substantial inter-individual variation in the timing and tempo of puberty, and the design of the studies included in this thesis sought to take advantage of this natural variation to distinguish between the relationship of puberty and chronological age on brain development.

Two different methods were used in this thesis to distinguish age and pubertal effects. In **Chapter 3**, a large, longitudinal sample was used, where all participants had self-reported pubertal status information. The size of the sample and longitudinal nature of the data allowed the use of mixed effects modelling analyses to tease apart age and pubertal variables. In **Chapters 4-7** where cross-sectional samples were used, participants were selected from a narrow age range, within which typically developing individuals can be at any stage of puberty. This method aimed to maximise pubertal variation while minimising age differences.

2.1.2 Gender

As outlined in the Introduction, the biological changes of puberty, and the hormonal regulation of these changes, differ significantly between males and females (see **Section 1.4**). Furthermore, the age at which these changes occur also differs between the sexes. Therefore, including both males and females in studies of pubertal development can result in multiple confounding results. Published studies exploring

structural brain development in adolescence suggest that there may be sex differences in absolute size and in developmental trajectories, and that these may be region-specific (see **Section 1.7**). To date, there is insufficient data from adolescent functional MRI studies to have robust evidence as to whether gender differences in the timing and/or nature of the functional development exist.

Given these differences between males and females, the studies described in this thesis do not attempt to statistically disentangle the differing impacts of puberty between sexes. **Chapter 3** includes both males and females in the study, but each are analysed separately using different methods and no direct comparison is included. For each of Chapters 4-7, only one sex was included in the study sample, and the age range used was focussed on the expected age of maximal pubertal variation for that sex. **Chapters 4 and 7** include data collected only from males (an overlapping sample of participants), whilst **Chapters 5 and 6** include data from a sample of females. This strategy means that it is not possible to ascertain sex differences from the studies undertaken for this thesis, and it is hoped that future work may focus on this question.

2.2 Participants

For all the experimental chapters included in my thesis, participants were healthy volunteers recruited from the community. The sample used for the analysis in **Chapter 3** was taken from the NIMH CPB Section on Brain Imaging longitudinal dataset of structural MRI scans (Giedd et al., 1996). This large dataset consists of more than 6500 scans from more than 3000 participants of which approximately half are typically developing and half are from various diagnostic groups. Inclusion and exclusion criteria for the overall dataset can be found on the NIMH website (http://clinicalstudies.info.nih.gov/cgi/detail.cgi ?A_1989-M-0006.html). Specific details of the participants included in the analysis are outlined in **Section 3.2.1**.

The study participants for **Chapters 4-7** were recruited from London and the surrounding counties via advertisements posted around the University campus and

letters sent to local schools. All participants spoke English as their native language and had normal or corrected to normal vision. Potential participants were excluded based on a self- or parent-reported history of prematurity (<34 weeks gestation), previous neurosurgery, a known neurological, psychiatric or endocrine disorder, or any contraindications to MRI. Details of the ethical approval and recruitment incentives for each study are included in the individual chapters.

2.3 Measuring puberty

The biological process of puberty has been long recognised and during the first half of the 20th Century, a number of scientific papers documenting the physical changes associated with adolescence were published based on samples from the USA, UK and the Netherlands (Hogben, Waterhouse, & Hogben, 1948; Jones, 1939; Nicolson & Hanley, 1953; Reynolds & Wines, 1948). These empirical studies aimed to quantify the pubertal process in boys and girls so that puberty could be measured and its relationship to the physical, psychological and behavioural changes of adolescence could be studied. Following these initial studies, Tanner and colleagues published works in the 1960s detailing standardised pubertal categories, or 'Tanner stages' to document pubertal maturation for both males and females (Tanner, 1962).

2.3.1 Physician-assessment of Tanner stage

The classification system proposed by Tanner incorporated example photographs of each 'Tanner stage' (**Figure 2.1**) as well as a short description of the physical attributes associated with the stage (**Table 2.1**). This tool was developed by Tanner and his colleague Marshall to describe the variations in timing and pattern of development of secondary sexual characteristics in females (Marshall & Tanner, 1969) and males (Marshall & Tanner, 1970). The data they used were part of the Harpenden Growth study, a longitudinal investigation of adolescent growth conducted in a children's home in Hertfordshire, England. The study participants, 192 girls and 228 boys, were examined every three months during adolescence, collecting both anthropomorphic

measurements and photographic documentation of breast (girls), genitalia (boys) and pubic hair (both sexes) development (Marshall & Tanner, 1969, 1970).

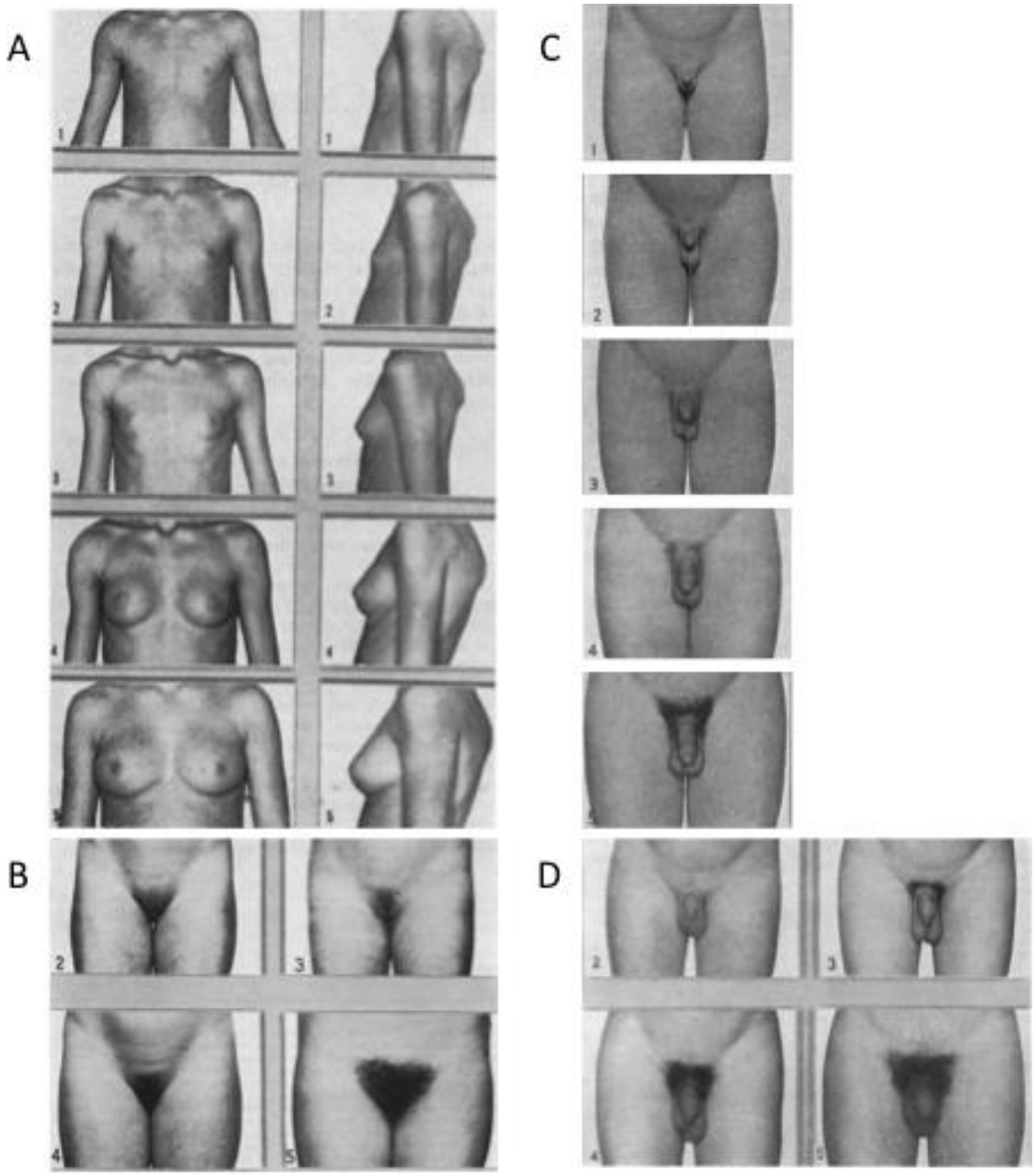


Figure 2.1: Photographs of Tanner stages of pubertal development. *Examples of the photographs taken during the Harpenden Growth Study. Panel A shows female breast development, panel B shows female pubic hair development, panel C shows male genital development and panel D shows male pubic hair development. Panels A and C show all 5 stages of development from Tanner stage 1 (pre-pubertal) to Tanner stage 5 (adult equivalent). Panels B and D show Tanner stages 2 (first appearance of pubic hair) to 5 (adult-like distribution of pubic hair). From Marshall & Tanner, 1969, 1970.*

TANNER STAGE DESCRIPTIONS FOR FEMALES		
Tanner stage	Breast development	Pubic hair development
Stage 1	Pre-adolescent. Elevation of papilla only	Pre-adolescent. The velus over the pubes is no further developed than that over the abdominal wall, i.e. no pubic hair.
Stage 2	Breast bud stage. Elevation of breast and papilla as a small mound, enlargement of areola diameter.	Sparse growth of long, slightly pigmented, downy hair, straight or only slightly curled, appearing chiefly along the labia.
Stage 3	Further enlargement of breast and areola, with no separation of their contours.	Considerably darker, coarser, and more curled. The hair spreads sparsely over the junction of the pubes.
Stage 4	Projection of areola and papilla to form secondary mound above the level of the breast.	Hair is now adult in type, but the area covered by it is still considerably smaller than in most adults. There is no spread to the medial surface of the thighs.
Stage 5	Mature stage. Projection of papilla only, due to recession of the areola to the general contour of the breast.	Adult in quantity and type, distributed as an inverse triangle of the classically feminine pattern. Spread to the medial surface of the thighs but not up the linea alba or elsewhere above the base of the inverse triangle.
TANNER STAGE DESCRIPTIONS FOR MALES		
Tanner stage	Genitalia development	Pubic hair development
Stage 1	Pre-adolescent. Testes, scrotum, and penis are of about the same size and proportion as in early childhood.	Pre-adolescent. The velus over the pubes is no further developed than that over the abdominal wall, i.e. no pubic hair.
Stage 2	The scrotum and testes have enlarged and there is a change in the texture of the scrotal skin. There is also some reddening of the scrotal skin.	Sparse growth of long, slightly pigmented, downy hair, straight or only slightly curled, appearing chiefly at the base of the penis.
Stage 3	Growth of the penis has occurred, at first mainly in length but with some increase in breadth. There has been further growth of testes and scrotum.	Considerably darker, coarser, and more curled. The hair spreads sparsely over the junction of the pubes.
Stage 4	Penis further enlarged in length and breadth with development of glans. Testes and scrotum further enlarged. There is also further darkening of the scrotal skin.	Hair is now adult in type, but the area covered by it is still considerably smaller than in most adults. There is no spread to the medial surface of the thighs.
Stage 5	Genitalia adult in size and shape. No further enlargement takes place after Stage 5 is reached.	Adult in quantity and type, distributed as an inverse triangle of the classically feminine pattern. Spread to the medial surface of the thighs but not up the linea alba or elsewhere above the base of the inverse triangle.

Table 2.1: Written descriptions of Tanner stages*. From Marshall & Tanner, 1969, 1970.*

These photographs were retrospectively examined and compared to each other, and each time-point was assigned to a developmental stage using Tanner's proposed scale. Using this method, Marshall and Tanner produced estimates of both the chronological timing and duration of pubertal maturation in both girls and boys (see **Figure 2.2**). In girls, onset of puberty occurred between 8.5 and 13 years in 95% of subjects, with the duration of puberty from onset to completion varying from 1.5 to more than 6 years (Marshall & Tanner, 1969). In boys, onset of puberty occurred between 9.5 and 14 years in 95% of subjects, with the duration of puberty from onset to completion varying from 2 to 4.5 years (Marshall & Tanner, 1970).

Since the publication of Tanner and Marshall's seminal work, the 'gold standard' for assessing pubertal development in both girls and boys has continued to be direct physician-assessment of pubertal stage using the 5 Tanner stages they proposed. However, this method is not necessarily practical in a number of research studies, where there may be a paucity of skilled personnel to assess pubertal development, lack of an appropriate room to conduct an assessment, and concerns or objections from potential study participants and their parents. Where a measure of pubertal maturation is being sought solely for research purposes, and there is no associated clinical need, care must be taken to balance the accuracy of the pubertal assessment with the potential ethical considerations of physician assessment, and its potential ability to cause emotional distress or harm to the participants in the study.

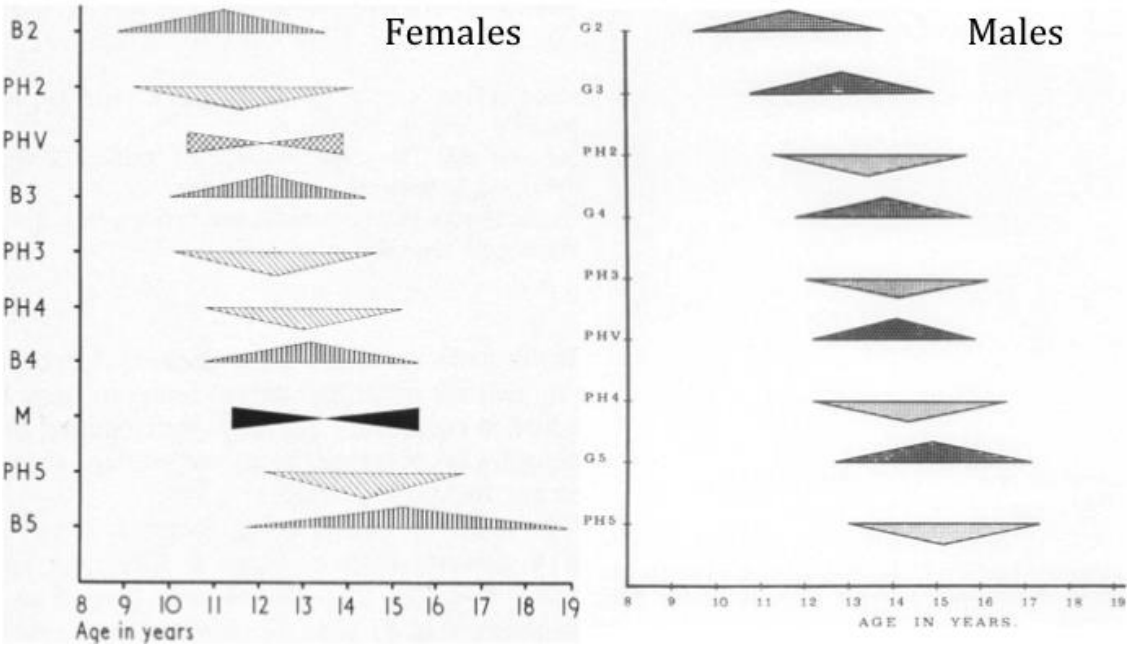


Figure 2.2: Chronological timing and duration of puberty in females and males. *These graphs show the range of ages that participants in the Harpenden Growth Study were at each of the Tanner stages of puberty. The centre of each symbol represents the mean and the symbol width displays 2 S.D. either side of the mean. The y-axis shows the different Tanner stages, where B=stage of breast development, PH=pubic hair development, G=genital development, M=menarche, and PHV=peak height velocity.*

2.3.2 Self-assessment of puberty

To balance the requirement for indicators of pubertal maturation with the desire to limit the need for potentially embarrassing and distressing pubertal assessments, self-reported indicators of pubertal development have been proposed. The most commonly used of these are self-report of Tanner stage using standardised line diagrams (Morris & Udry, 1980; Taylor et al., 2001) or anonymised photographs (Dorn, Susman, Nottelmann, Inoff-Germain, & Chrousos, 1990; Duke, Litt, & Gross, 1980; Neinstein, 1982) and the Pubertal Development Scale (PDS; Petersen, Crockett, Richards, & Boxer, 1988).

2.3.2.1 Self-assessment of Tanner Stage

A number of studies have chosen to obtain an estimate of pubertal development by asking adolescents to rate their own physical development, using either photographs

(Dorn et al., 1990; Duke et al., 1980) or line drawings (Morris & Udry, 1980; Schlossberger, Turner, & Irwin, 1992; Taylor et al., 2001). Self-assessment methods are advantageous because they are less embarrassing/unpleasant for the participants, can be a quicker process that does not necessarily require a dedicated space, and do not necessarily require formal training. However, studies comparing self-report of Tanner stage to physician-assessment have had variable results (for reviews, see Coleman & Coleman, 2002; Dorn & Biro, 2011).

I undertook a search of published studies or abstracts that compared self-reported Tanner stage to physician-assessed Tanner stage. This was conducted using Pubmed, JSTOR, SCOPUS and PsychINFO in August 2014. The Pubmed search strategy was as follows: (puberty [MeSH term] OR pubert* OR "sexual maturation") AND (self-assessment [MeSH term] OR self-assess* OR self-report*) AND (validity OR evaluation OR "evaluation studies"[Publication type] OR "evaluation studies as topic" [MeSH term]). Search terms for the other databases were similar. Additionally, I reviewed all the reference lists of the studies highlighted by the search strategy to check for other relevant papers. The search included cross-sectional and longitudinal studies in any language. Studies were included that incorporated either community or clinical sample populations. A total of 30 studies were found, which are listed in **Table 2.2a** (female data) and **Table 2.2b** (male data). These data span 32 years of publication, and use a variety of self-report methods. Details of nationality are included where defined in the original studies, as well as specific information regarding medical diagnoses of participants included in the studies. The statistical analyses presented vary between studies, and measures of correlation between physician and self-report (using Pearson, Spearman, Kendall or concordance correlation analyses) as well as measures of agreement between physician and self-report (using kappas, weighted kappas and percentage agreement as available). The studies listed do not represent an exhaustive systematic review but instead are a summary of accessible and available literature.

Direct comparison of the studies is challenging since the statistical analytical methods used vary significantly, and few of the studies include the full raw data from which to make any additional comparisons. Furthermore, the studies used different methods of

self-report including: photographs (Azevedo, Brasil, Macedo, Pedrosa, & Arrais, 2009; Boas, Falsetti, Murphy, & Orenstein, 1995; Desmangles, Lappe, Lipaczewski, & Haynatzki, 2006; Dorn et al., 1990; Duke et al., 1980; Hardoff & Tamir, 1993; Neinstein, 1982; Wacharasindhu, Pri-Ngam, & Kongchonrak, 2002); line drawn pictures with or without written descriptions (translated into a variety of languages) (Bonat, Pathomvanich, Keil, Field, & Yanovski, 2002; Brooks-Gunn, Warren, Rosso, & Gargiulo, 1987; Chan et al., 2008; Finkelstein et al., 1999; Hergenroeder, Hill, Wong, Sangi-Haghpeykar, & Taylor, 1999; Hick & Katzman, 1999; Lee, Valeria, Kochman, & Lenders, 2006; Morris & Udry, 1980; Norris & Richter, 2008; Sarni, de Toni, & Gastaldi, 1993; Schall, Semeao, Stallings, & Zemel, 2002; Schlossberger et al., 1992; Schmitz et al., 2004; Sun, Tao, Su, & China Puberty Research Collaboration, 2012; Taylor et al., 2001); picture-based interviews, involving detailed verbal explanation of line drawings (Shirtcliff, Dahl, & Pollak, 2009); and alveolar descriptions (used in Wu, Schreiber, Klementowicz, Biro, & Wright, 2001 instead of breast development stages). In some studies, it was not possible to ascertain which self-assessment method was used (Leone & Comtois, 2007; Matsudo & Matsudo, 1994; Rollof & Elfving, 2011).

The most frequently quoted statistic across the papers was a measure of correlation between physician and self-assessment of puberty. Correlations between physician and self-report vary significantly between studies, with coefficients ranging from 0.37-0.90 for female breast staging; 0.28-0.91 for female pubic hair staging; 0.10-0.84 for male genitalia; and 0.35-0.88 for male pubic hair (see **Table 2.2**). There are many possible reasons for the wide variety in reported correlations. The studies used different methods of self-report in populations of different nationalities and cultural backgrounds, and chose samples with different characteristics (community vs. clinical samples; healthy vs. co-morbid conditions). Furthermore, the age ranges of the participants across the studies showed significant variability. Despite these differences, the majority of studies report moderate to strong correlation coefficients of >0.6, supporting the use of self-report of Tanner stage methods, but emphasising the need to take into account the potential limitations. Importantly, correlation statistics provide an indication of how well the two types of assessment varied with each other

in each sample population, but do not indicate whether both assessments assigned individuals to the same Tanner stage.

In addition to, or instead of correlation statistics, many of the studies quoted levels of agreement between physician and self-reported pubertal stage, either using percentage agreement or a Cohen's kappa statistic (κ), which takes into account the possibility that agreement occurred by chance. Measures of agreement are more informative than correlations when dealing with categorical data, since they assess whether the two different assessment tools assigned the same category to the same individuals, and can therefore be used to assess the validity of the tools. The measures of agreement again varied significantly across studies. κ agreement ranges from 0.16 to 0.81 for female breast staging; 0.33 to 0.91 for female pubic hair staging; 0.18-0.85 for male genitalia staging; and 0.39-0.85 for pubic hair staging (see **Table 2.2;** where κ statistics are averaged across genders or pubertal measures, this is indicated in the table. Where κ are not quoted, they have been calculated if sufficient information is included in the paper, and percentage agreement is quoted if not). There are variable findings regarding the proportions of each sex who over- and underestimated their pubertal status. Based on previously published magnitude guidelines (Fleiss, Levin, Paik, & Fleiss, 2003; Landis & Koch, 1977), many of these κ values suggest only fair to moderate agreement between the Tanner stage allocated to an individual by a physician and self-assessment. The authors of many of these studies therefore advise caution in using self-assessment of Tanner stage as a direct proxy for physician-staging, particularly in small cross-sectional studies.

A subset of studies additionally calculated weighted κ values for their data (Boas et al., 1995, Taylor et al., 2001; Wacharasindhu et al. (2002) only provide weighted κ values; Hardoff & Tamir (1993) analyse data where ratings are one stage apart). Weighted κ values provide additional information regarding agreement between scores, as they weight scores based on how close they are to agreement. All of the studies quote high levels of agreement between ratings using weighted κ analyses, indicating that whilst there may not be perfect agreement between ratings, the ability of self-reported

methods to assess puberty within one stage of physician-assessment is very good, giving the possibility of reliably using self-reported pubertal stage data to group adolescents by maturity, particularly where sample sizes are small.

Other factors that must also be considered are the ethnicity of participants, their body composition and any concurrent illnesses that may impact on their self-perception. Since Tanner stages were developed in the UK, and the photographs and drawings used to help categorise participants are based on Caucasian young people, there may be additional inaccuracy when assessing young people from different ethnic backgrounds. Obesity can make an accurate pubertal assessment difficult, especially for breast development, since adipose tissue can be difficult to distinguish from breast tissue, and may lead to an underestimate of male genitalia size and development. This was highlighted in a study assessing pubertal stage in obese and non-obese participants (Bonat et al., 2002), which reported that correlations differed between the two groups (Kendell rank correlations: girls breast development obese 0.37/non-obese 0.54; girls pubic hair development obese 0.64/non-obese 0.66; boys pubic hair obese 0.45/non-obese 0.35). These studies therefore highlight the potential limitations of a relying on self-assessments, and care should be taken to ensure that self-assessment data are considered to be approximate estimates of pubertal development and are interpreted as such.

							Tanner stage breast		Tanner stage pubic hair	
Authors	Date	N	Age	Diagnoses in sample	Nationality	Method	Correlation	Agreement	Correlation	Agreement
Morris & Udry	1980	47	12-16		US	D	r=0.63	0.29	r=0.81	
Duke et al.	1980	43	9-18		US	P		0.81		0.91
Neinstein	1982	22	11-18		US	P	r=0.88	77%	r=0.86	73%
Brooks-Gunn et al.	1987	151	11-13		US	D	r=0.72		r=0.82	
Dorn et al.	1990	44	9-15		US	P	r=0.88	43%	r=0.91	48%
Schlossberger et al.	1992	37	11-14		US	D		0.59		0.64
Sarni et al.	1993	50	9-17		Italy	D		16%*		16%*
Matsudo & Matsudo	1994	174	6-26		Brazil	ns		61%		71%
Hergenroeder et al.	1999	107	8-17		US	D		0.34		0.37
Wu et al.[1]	2001	621 (1396)	11-14		US	Other		0.32-0.51		0.36-0.55
Taylor et al.	2001	41	12-16		UK	D		0,43		0.6
Wacharasindhu et al	2002	100	7-15		Thailand	P		0.76w		0.79w
Norris &Richter	2005	90	10-18		South Africa	D	τ=0.90	0.81	τ=0.86	0.71
Desmangles et al.	2006	130	6-16		Not stated	P	τ=0.76	0.49	τ=0.88	0.68
Chan et al.	2008	182	8-18		Hong Kong	D	τ=0.64	0.72	τ=0.76	0.83
Shirtcliff et al.	2009	78	9-14		US	PBI	r=0.83	0.36*	0.88	0.43*
Azevedo et al.	2009	150	8-18		Brazil	P		0.27-0.29^		0.42-0.46^
Rollof & Elfving	2012	44	10-16		Sweden	ns		0.32		0.50
Hardoff & Tamir	1993	54	13-14	learning disabilities	Israel	P				0.55/0.43^
Finkelstein et al.[1]	1999	17 (136)	11-19	delayed puberty	US	D	C=0.62-0.88		C=0.28-0.76	
Hick & Katzman	1999	40	8-18	anorexia nervosa	Canada	D		30%		50%
Bonat et al.	2002	135	6-12	obesity	US	D	τ=0.37/0.54	49%	τ=0.66/0.64	59%
Schall et al.	2002	34	8-18	Crohn's disease	US	D		0.74		0.81
Schmitz et al.	2004	93	8-18	diabetes	US	D	r=0.79	0.16	r=0.81	0.33
Lee et al.	2006	39	8-18	overweight	US	D	p=0.74	0.21	p=0.86	0.5
Leone et al.	2007	23	12-17	elite athletes	Canada	ns		0.85		0.75
Sun et al.	2012	9132	7-18	obesity	China	D	τ=0.77/0.74	0.62/0.53	τ=0.78/0.79	0.59/0.57

Table 2.2a: Published studies evaluating the validity of self-reported Tanner stage (female). For full legend, see page 91

							Tanner stage genitalia		Tanner stage pubic hair	
Authors	Date	N	Age	Diagnoses in sample	Nationality	Method	Correlation	Agreement	Correlation	Agreement
Morris & Udry	1980	48	12-16		US	D	r=0.59		r=0.63	
Duke et al.	1980	23	9-18		US	P		0.88*		0.88*
Neinstein	1982	22	11-18		US	P	r=0.72	45%	r=0.69	50%
Williams et al.	1988	49	10-14		US	P & D	τ =0.65		τ=0.82	
Dorn et al.	1990	46	9-15		US	P	r=0.84	33%	r=0.77	50%
Schlossberger et al.	1992	46	11-14		US	D		0.18		0.66
Sarni et al.	1993	50	9-17		Italy	D		14%*		14%*
Taylor et al.	2001	62	12-16		UK	D		0.51		0.75
Wacharasindhu et al.	2002	94	7-15		Thailand	P		0.59w		0.73w
Norris &Richter	2005	92	10-18		South Africa	D	τ =0.80	0.6	τ =0.85	0.63
Desmangles et al.	2006	110	6-16		Not stated	P			τ =0.82	0.49
Chan et al.	2008	172	8-18		Hong Kong	D	τ =0.52	0.58	τ =0.75	0.8
Shirtcliff et al.	2009	82	9-14		US	PBI	r=0.6	0.36*	r=0.71	0.43*
Azevedo et al.	2009	169	8-18		Brazil	P		0.27-0.29^		0.42-0.46^
Rollof & Elfving	2012	44	10-16		Sweden	D				75%
Rabbani et al.	2013	190	8-16		Iran	D		0.63		0.74
Hardoff & Tamir	1993	89	13-14	learning disabilities	Israel	P				0.55/0.43^
Boas et al.	1995	61	11-18	healthy/cystic fibrosis	US	P		0.35/0.49		0.73/0.80
Finkelstein et al.[1]	1999	32 (256)	11-19	delayed puberty	US	D	C=0.10-0.50		C=0.02-0.61	
Bonat et al.	2002	109	6-12	obesity	US	D			τ =0.35/0.45	48%
Schall et al.	2002	66	8-18	Crohn's disease	US	D		0.85		0.85
Schmitz et al.	2004	85	8-18	diabetes type I and II	US	D			r=0.88	0.39
Lee et al.	2006	38	8-18	overweight	US	D	ρ=0.68	0.25	ρ=0.85	0.39
Leone et al.	2007	24	12-17	elite athletes	Canada	ns		0.67		0.79
Sun et al.	2012	6924	7-18	healthy/obesity	China	D	τ =0.78/0.64	0.50/0.35	τ =0.83/0.74	0.64/0.45

Table 2.2b: Published studies evaluating the validity of self-reported Tanner stage (male). For full legend, see page 91

Table 2.2 (Pages 89 and 90): Published studies evaluating the validity of self-reported Tanner stage. *Table 2.2a shows data for females, Table 2.2b shows data for males. Where a sample specifically recruited patients with diagnoses, these are included.*

Different methods of Tanner stage self-assessment were used: P=Tanner stage photographs; D=Tanner stage drawings with descriptions (language appropriate to nationality); PBI=Picture-based interview; Other=Wu et al. used alveolar and public hair staging; ns=not stated in the paper.

Different statistical analyses were performed across studies. Results indicating both correlation and agreement between physician and self-report are included in the table where available. r=Pearson correlation coefficient; ρ=Spearman rank coefficient; τ=Kendall rank coefficient; C=concordance correlation coefficient. Measure of agreement are Cohen's κ unless marked as percentages (%); w=weighted κ.

**combined scores across features for sex; ^combined score for equivalent feature across sex*

[1]Whese studies included longitudinal data, N=number of participants in the study, with the total number of data-points in brackets. Results were presented with separate indices of agreement for each wave. The range is shown here.

2.3.2.2 Petersen Pubertal Development Scale

The Petersen Pubertal Development Scale (PDS) was developed by Petersen and colleagues, and was first described in a 1988 publication (Petersen et al., 1988). The scale incorporates a series of 5 questions regarding physical changes in adolescence. For both sexes, questions are asked about development including growth spurt, body hair development and skin changes. Additional questions for girls include breast development and menarche, while boys are asked about facial hair growth and voice change (Petersen et al., 1988). For each question, participants are asked to rate themselves on a four-point scale from '1 - no development', '2 - development has barely begun', '3 - development definitely underway' or '4 - development already complete'. The original designers of the PDS scale advocated averaging the scores from the five different variables to provide an overall PDS score of between 1 and 4. The scale was designed to be a simpler, more understandable tool than the Tanner stage self-assessment for use in research situations.

Reported correlations between the PDS and the gold standard of physician-assessed Tanner stage are variable. Brooks-Gunn and colleagues compared PDS scores to physician-assessed Tanner stage scores in 151 Caucasian girls aged 11-13 years (Brooks-Gunn et al., 1987). This study showed correlations of between 0.61 and 0.67, which are considered accurate enough for a rough estimate, but would limit confidence in the data in terms of findings from any such studies. As with Tanner self-assessment, the PDS was developed using a Caucasian sample. A study of the reliability of the PDS in a multi-ethnic community of 10-18 year olds in Soweto, South Africa (Norris & Richter, 2008) reported very low κ coefficients between the PDS and an expert rating of sexual maturity (girls κ =0.34; boys κ undetermined). This study also highlighted the relatively poor convergence between the different items measured on the PDS questionnaire i.e. between facial hair, skin changes and growth. These studies emphasise that the PDS should be used with caution in research, and has a number of potential limitations.

2.3.3 Timing of menarche

Self-reported menarcheal status is frequently used as an indicator for pubertal development in girls for research purposes. Compared to the assessment of other physical changes, it is relatively easy to ascertain by simply asking a participant if they have experienced menarche, and if so, at what age they experienced this. Assessing menarcheal status and age of menarche by report from the participant (or a parent or teacher) is the only acceptable method to obtain this information. Asking a group of adolescents about their current status gives a relatively accurate dichotomy into Yes and No groups (Parent et al., 2003), and this can be analysed using a probit method to provide an approximate mean menarcheal age for the whole group. It is important to note that even a simple question such as this is subject to reporting bias, and responses may be influenced by socioeconomic and cultural factors (Artaria & Henneberg, 2000).

Studies assessing the reliability of retrospective self-report of age of menarche have had variable results. One longitudinal US cohort (Must et al., 2002) produced reassuring data showing excellent recall of age of menarche after 30 years ($r=0.79$; $p<0.001$) but a second study (Koo & Rohan, 1997) assessing a recall interval of 1-2 years since menarche showed reducing accuracy in adolescents as the time since menarche was experienced increased (66.1% accuracy if recall length 266-360 days, 44.8% accuracy if recall length 477-698 days; Koo & Rohan, 1997). A recent longitudinal study collected data of menarcheal age at 12 timepoints over 3 years (every 3 months) from 253 girls initially aged 11-17 years (Dorn, Sontag-Padilla, Pabst, Tissot, & Susman, 2013). Overall reliability of self-reported menarcheal age was found to be moderately good (intraclass correlation coefficient [ICC] 0.64), but there was substantial variability seen in the study. The reliability of report was significantly affected by the method of data collection, with face-to-face interviews giving more reliable data than telephone interviews (ICC 0.77 vs. 0.64; $p<0.05$). The average variation in age of reported menarche across all the interviews was 2.3 years, with some participants varying in their reported age of menarche by up to 5 years (Dorn et al., 2013), suggesting substantial inconsistency in self-reported menarcheal age during adolescence.

2.3.4 Measuring sex steroid hormones

An alternative method of calculating pubertal maturation, as opposed to assessing the observable physical changes, is to measure levels of pubertal hormones. Sex steroid hormones are expected to rise in the circulation in participants through puberty (see **Section 1.4**), and measuring these can therefore provide information about a participant's maturation. Many of the hormones that circulate within the bloodstream can also be detected within saliva. Since the 1970s, it has been possible to assess sex steroid hormone levels in saliva as an alternative to serum assays. Since saliva can be collected non-invasively, it has many practical advantages over serum, particularly in children. It can be collected in a community setting e.g. at home, and at repeated times without subjecting an individual to venepuncture, and it is a relatively stress-free

process, limiting the impact that the sample collection might have on the hormones that are being assessed.

Hormones are transferred from blood to saliva via passive diffusion from capillaries to the salivary glands. The rate at which this process occurs depends on the molecular structure of the hormones found within the bloodstream (Vining, McGinley, & Symons, 1983). Lipophilic molecules including steroids are transferred more rapidly than hydrophilic molecules, but diffusion is limited if the hormone is conjugated within the plasma, as is the case with DHEA-S (Vining et al., 1983). This affects both the relative concentration of hormone found in saliva, and also whether this concentration is flow-dependent. Conjugated steroids like DHEA-S will be found in small concentrations relative to serum (less than 1% of that found in the plasma; Vining et al., 1983), and the level of hormone in saliva is dependent on the flow rate of the saliva production. In contrast, unconjugated steroid hormones like testosterone, oestradiol and DHEA are not dependent on the flow rate, and salivary levels accurately represent the unbound, biologically active fraction of the hormone found in the general circulation (Granger, Schwartz, Booth, Curran, & Zakaria, 1999; Vining et al., 1983).

In blood, each of testosterone, oestradiol and DHEA can be found both in their bound, inactive form, and their unbound, active form. Circulating levels of testosterone and oestradiol in children and adolescents are much lower than in adults, and the portion transferred into saliva is small (1-15% of serum unbound levels; Vining et al., 1983). This means that an assay must be ultrasensitive and measurements highly reproducible to be valid for research purposes. DHEA is only weakly bound to albumin or sex hormone binding globulin (SHBG), and the unbound fraction of DHEA is known to be the biologically active form. Early concerns about the reliability and sensitivity of salivary assays, especially for children and adolescents who have lower hormone levels than adults, have largely been resolved with the development of reliable, efficient and ultrasensitive immunoassays using reproducible protocols for use on salivary samples (Granger, Schwartz, Booth, & Arentz, 1999, 1999; Shirtcliff et al., 2000). The correlation between serum and saliva levels of unbound DHEA, oestradiol and testosterone have

been shown to be high (Granger, Schwartz, Booth, & Arentz, 1999; Shirtcliff, Granger, Schwartz, & Curran, 2001; Vining et al., 1983; Worthman, Stallings, & Hofman, 1990).

Accuracy of salivary hormone assays is dependent on a number of technical factors relating to sample collection and storage. Salivary hormone levels can be affected by different collection methods, with both cotton swabs and chewing gum being associated with inaccurate levels (Granger, Shirtcliff, Booth, Kivlighan, & Schwartz, 2004). Saliva contaminated with blood (even from microinjury) can also result in falsely high results of hormone levels, especially testosterone and oestradiol (Granger et al., 2004; Kivlighan, Granger, & Schwartz, 2005). Storage conditions for saliva samples can also result in unreliable results, with testosterone levels shown to significantly increase when stored in a normal (4^{o}C) refrigerator over the course of just a few days, and decrease when stored in freezers at -20^{o}C or -40^{o}C. The nearly exponential increase in testosterone levels seen in samples stored at 4^{o}C suggests that bacterial growth may be interfering with antibody binding, or that there is ongoing hydrolysis within the samples (Granger et al., 2004). In contrast, when stored at -20^{o}C or -40^{o}C, levels of testosterone were found to be artificially low. This is consistent with the degradation reported for other analytes over time (Granger et al., 2004). Fortunately, levels have been shown to be stable when stored in a -80^{o}C freezer, and could be preserved for two years without degradation (Granger et al., 2004). Careful consideration was given to each of these factors when designing the studies described later in the thesis.

2.4 Pubertal indicators used in this thesis

As described in the introduction (**Section 1.4**), puberty is a complex process and can be measured in a variety of ways. Chapters 4-7 incorporated at least two measures of pubertal development for all participants including an indicator of phenotypic pubertal stage and a measure of sex steroid hormonal level using salivary assays. Phenotypic puberty stage is an integrative measure of the body's exposure to pubertal hormones over time (assuming roughly similar sensitivity of different individuals to the hormones). Hormone levels at the time of the study provide a measure of current

circulating amounts. While these two measures are related, they assess different aspects of pubertal development and sex steroid hormone exposure. Phenotypic puberty stage was estimated using either self-reported assessment of puberty (Chapters 4 and 7; males) or physician-assessed Tanner stage (Chapters 5 and 6; females) with a self-reported indication of menarcheal status. Chapter 3, which used a large longitudinal dataset previously collected at the NIMH (see **Section 3.2.1** for more details), used only a phenotypic measure of self-reported pubertal stage.

2.4.1 Physician-assessed Tanner stage

The phenotypic pubertal data used for the analyses presented in Chapters 5 and 6 (both studies on female participants) was obtained by physician-assessment of pubertal stage. I performed visual assessments of breast and pubic hair stage using established Tanner stages (Marshall & Tanner, 1969) having previously been trained in pubertal stage assessments while in outpatient clinics assessing healthy young people as well as young people with endocrine or other medical conditions. The pubertal assessments took place in a private room, and the young person was accompanied by a chaperone (usually their parent, or a female researcher if preferred). Written consent for this assessment, as part of the research study, was obtained from each participant's parent, and written assent was given by the participant. If a participant chose not to be examined (N=2), they were asked to rate their own developmental stage using Tanner stage diagrams (Taylor et al., 2001), as per **Section 2.3.1**. Participants also provided self-reported menarcheal status.

2.4.2 Self-assessment of Tanner stage

The phenotypic pubertal data used for the analyses in Chapters 3, 4 and 7 were obtained using self-report questionnaires of pubertal stage completed by the participants on the day of their MRI scan. Physician-assessed data were not collected due to the recruitment and assessment challenges that such assessment presented. Participants were shown gender specific line drawn pictures of pubertal stages with short written descriptions (Taylor et al., 2001) and were asked to rate which picture most resembled their current stage of development (see **Section 1.4.2.1**). Such self-

report measures have been shown to be a valid method for pubertal development assessment, with adolescents being reasonably accurate observers, especially where allocation is into two categories of Early and Late puberty and measures of gonadal and pubic hair stages are combined (Shirtcliff et al., 2009, see **Section 2.3.2**).

2.4.3 Grouping of participants into Early and Late puberty groups

For each of the studies described in Chapters 4-7, participants were grouped into two groups (Early and Late), based on their phenotypic pubertal stage. The reasons for this were two-fold. Firstly, as outlined above, where phenotypic puberty stage was collected using self-report measures, this has been shown to be most reliable when used to group adolescents into two categories. Secondly, the nature of MRI studies, in terms of the time and financial resources required for recruitment and data collection, prohibited a study design that would have adequate numbers of participants representing every puberty stage. Participants were characterised as early-mid puberty if they were Tanner Stage 1, 2 or 3 in both measures (pubic hair and gonadal development for boys, or pubic hair and breast development for girls) and if they were pre-menarcheal (girls only). Participants were characterised as late-post puberty if either breast/gonadal or pubic hair stage was 4 or 5, or they were post-menarcheal (girls only) (Dorn, 2006). From here on, these groups are referred to as 'Early puberty' and 'Late puberty'.

Since the data analysed in Chapter 3 represent a larger dataset that is longitudinal in nature, these data were not grouped in the same way as the other chapters. For each participant at each time point, a single combined Tanner stage score was assigned based on the overall stage that the participant felt best described themselves from looking at the separate breast/genital and pubic hair scores. Tanner stage was then treated as a continuous variable within the mixed effects model analyses.

2.4.4 Measurement of salivary hormone levels

Chapters 4-7 include analyses involving sex steroid hormone levels. The hormones measured in these studies were testosterone, oestradiol and DHEA, the principal

hormones that drive the physical and behavioural changes of puberty. Salivary hormonal assays rather than serological assays were to minimise invasive testing. Upon waking on the morning of their scan, before 9am, each participant collected up to 3.5 ml passive drool (unstimulated) samples of saliva after rinsing their mouths with water, and before brushing their teeth, eating or drinking anything (except water). This was in accordance with the recommendations of Salimetrics Europe Ltd (http://www.salimetrics.com/) who undertook the assay analyses. On arrival at the testing centre, the parents and participants were asked to verify that they had followed these instructions. The samples were transported on the day of collection by participants to the testing centre on ice in an insulated polystyrene box. A small number of samples across the studies were not brought in by participants on the day of scanning. In these cases the samples were collected following the same procedure within 2 weeks of the MRI scan. Samples were stored at -80^{o}C and later analysed as a batch containing all the samples for the same study by Salimetrics.

Duplicate assays for testosterone, oestradiol and DHEA were performed for each participant, with intra-assay variation of <7% for all results. Therefore, the mean values were used for all analyses. The oestradiol range of sensitivity was from 1-32pg/mL. The average intra-assay coefficient of variation was 1.8%. The testosterone range of sensitivity was from 1-600pg/mL. The average intra-assay coefficient of variation was 1.4%. The DHEA range of sensitivity was from 5-1000pg/ml. The average intra-assay coefficient of variation was 1.6%.

2.5 Next Chapter

The next chapter is the first of five experimental chapters in this thesis. It describes an empirical study designed to investigate the role of puberty in the structural development of the subcortical structures of the brain using a longitudinal dataset.

CHAPTER 3

The influence of puberty on subcortical brain development

As outlined in Chapter 1, the human brain undergoes significant change between childhood and adulthood, but little is known about how puberty influences its development. The principal aim of this thesis is to establish whether there is empirical evidence supporting an association between puberty and the development of the adolescent brain. This chapter describes a study that examined structural brain development during adolescence. This study used a longitudinal sample of 711 magnetic resonance imaging scans from 275 individuals aged 7-20 years to examine how subcortical brain regions change in relation to puberty. The regions of interest included the amygdala, hippocampus and corpus striatum including nucleus accumbens, caudate, putamen and globus pallidus.

Pubertal development was significantly related to structural volume in all six regions in both sexes. Pubertal development and age had both independent and interactive influences on volume for the amygdala, hippocampus and putamen in both sexes, and the caudate in females. There was an interactive puberty-by-age effect on volume for the NA and GP in both sexes, and the caudate in males. These findings suggest a significant role for puberty in structural brain development.

3.1 Introduction

As outlined in **Section 1.7**, the past 20 years have seen a major expansion in research on the structural development of the human adolescent brain, based largely on the results of cross-sectional and longitudinal MRI studies. Studies of brain growth trajectories over adolescence to date have predominantly considered growth in relation to chronological age, with few exceptions (Paus et al., 2010; Raznahan et al., 2010). Examining brain development in relation to pubertal maturation may provide additional information regarding the mechanisms associated with adolescent brain development. This study aimed to investigate how the developmental trajectories of subcortical regions that are linked to stereotypical behaviours are associated with pubertal development (Forbes & Dahl, 2010; Steinberg, 2008): the amygdala and hippocampus, which play an important role in emotion and mood regulation (Davidson et al., 2002); and the corpus striatum including the nucleus accumbens (NA), caudate, putamen and globus pallidus (GP), which are involved in decision-making and reward-seeking behaviours (Gottfried, 2011).

It has been hypothesized that the brain restructuring and development seen in adolescence may be specifically related to the hormonal influences that control the onset of and progression through puberty (Giedd et al., 1999; Lenroot et al., 2007; Peper, Hulshoff Pol, Crone, & van Honk, 2011; Sowell et al., 2002). Sex steroids such as testosterone (an androgen) and oestradiol (an oestrogen) have been shown to be capable of inducing both synaptogenesis and synaptic pruning in rats and nonhuman primates (Ahmed et al., 2008; Hajszan, MacLusky, & Leranth, 2008; Sato, Schulz, Sisk, & Wood, 2008; see **Section 1.7.1**). These differential effects across brain areas may provide an explanation for the diverging growth trajectories of particular brain structures between males and females documented across studies, and the resultant increasing sexual dimorphism in adolescence reported in some regions (BDCG, 2012; Lenroot et al., 2007; Neufang et al., 2009; Sowell et al., 2002).

Cross-sectional studies have reported that puberty is associated with aspects of brain development in adolescence. A study examining the association between brain volumes and both pubertal stage and testosterone concentration found that males and females in later stages of puberty, and with higher circulating testosterone concentration, had larger amygdala volumes and smaller hippocampal volumes than their less well developed peers (Neufang et al., 2009). In contrast, a second study investigating puberty and pubertal hormone correlations with grey matter volume replicated the positive association between amygdala volume and pubertal stage in boys, but showed decreasing amygdala volume with increasing testosterone levels in girls (Bramen et al., 2011). These studies have limited explanatory power due to their relatively small sample sizes and cross-sectional methods. At the time of planning this study, no longitudinal studies incorporating pubertal measures had been published. Longitudinal analysis allows comparison of brain volumes both between-subjects and also within each subject over time, and therefore can provide a measure not just of brain volume at a particular time-point, but also of developmental trajectories for each of these subcortical brain regions by following what happens to each participant. This is particularly advantageous when looking at brain volumes, which vary substantially between individuals (BDCG, 2012; Giedd et al., 1999; Tamnes et al., 2013).

3.1.1 The current study

Using a large sample of scans from 275 individuals scanned longitudinally (using an unstructured multiple cohort design), the current study examined how subcortical brain regions change in relation to puberty as measured by Tanner stage (Tanner & Whitehouse, 1976), and compared developmental trajectories in females and males. The study used a large dataset containing information on pubertal stage and chronological age to examine growth trajectories over adolescence in each of these subcortical regions. Based on previous cross-sectional findings, it was hypothesised that the volume of the amygdala and hippocampus would increase between 7 and 20 years (Giedd et al., 1999; Østby et al., 2009), while the volumes of the corpus striatum (NA, caudate, putamen and GP) would decrease (BDCG, 2012; Østby et al., 2009; Sowell et al., 2002). Furthermore, it was hypothesized that volume change for all

structures of interest would be related to pubertal development as measured by Tanner stage, and that puberty and age would have independent effects on volume in these regions. It was therefore predicted that models incorporating both Tanner stage and chronological age as explanatory variables for volume change would provide a significant fit for the developmental data of the structures examined.

3.2 Methods

3.2.1 Participants

The sample used for the analysis was taken from the NIMH CPB Section on Brain Imaging longitudinal dataset of structural MRI scans (Giedd et al., 1996). Details of inclusion and exclusion criteria for the overall sample can be found in **Section 2.2** of the Methods chapter. For each participant, the outcomes of interest that were measured included ethnicity, socioeconomic status (SES; using Hollingshead scales), IQ (estimated using age-appropriate Wechsler Intelligence Scales) and handedness (using Physical and Neurological Examination of Soft Signs inventory; Denckla, 1985), each of which were collected at the time of the first scan, and pubertal status, ascertained at the time of each scan using Tanner stage diagrams (Taylor et al., 2001).

The subgroup used for the current analysis consisted of 275 unrelated individuals (117 females), and incorporated the scans of all individuals who met the following criteria:

1. Healthy individuals at the time of scanning. Participants were screened for neurological or psychiatric illness using a telephone screening interview and completion of a parent-report screening questionnaire (CBCL) at each time-point, and only healthy participants were included in the analysis as established by standardized scoring (Achenbach, 1991). All participants included had an IQ of greater than 80.
2. Two or more MRI scans between the ages of 7.0 and 20.0 years. This age range was estimated using two large US population-based studies on pubertal timing (Herman-Giddens et al., 1997; Sun et al., 2002), and incorporating ages from two 2 S.D. below the mean age of being in Tanner stage 2 (6.3-7.8 years for females and 6.9-9.7 years for males depending on the measure (breast/pubic hair/gonadal development) used),

to 2 SD above the mean age of being in Tanner stage 5 (20.5-21.0 years for females, 19.6-20.1 years for males depending on the measure used). This range was refined based on the particular characteristics of the study dataset to 7.0 to 20.0 years, as this incorporated the ages at which there was variation in Tanner stage between individuals.

3. Complete data for age and self-reported Tanner stage for each MRI scan. One female individual who reported regression of puberty during adolescence (as indicated by reducing Tanner score) was excluded from the analysis, since pubertal regression is essentially biologically implausible and likely represents error. Pubertal arrest, which may be perceived as regression, is associated with significant systemic illness or malnutrition.

4. Only one individual per family. The original dataset incorporates a high number (>400 individuals) of monozygous and dizygous twin pairs, as well as siblings. The heritability of brain structure and development is still not well understood, but there are likely to be both genetic and environmental effects on structural brain volume and developmental trajectory, as well as pubertal timing, which are not independent between family members, and therefore might bias the analysis. Where data were available for more than one family member, only one individual was included in the analysis; the family member included was determined by the participant with a higher number of high quality scans, more complete demographic data, or, if these were equal, by random selection.

Of the 275 participants whose data were included in the study, 87.3% were right-handed (7.3% left-handed, 5.4% mixed). The majority (89.5%) were Caucasian (5.1% African-American; 2.2% Hispanic; 0.7% Asian; 2.5% Other). Details of SES, IQ, puberty status and number of scans can be seen in **Table 3.1**. Participants were recruited from the community through local advertisement and reimbursed for their participation in the study. The study research protocol was approved by the Institutional Review Board of the NIMH and written informed consent and assent to participate in the study were obtained from parents/adult participants and children respectively.

FEMALES	Total sample	Tanner stage 1	2	3	4	5
N subjects/scans*	117/296	36/41	37/42	57/72	79/105	30/36
	Mean (SD)	Mean (SD)				
Age (years)	12.8 (3.3)	8.6 (1.3)	9.8 (1.1)	11.5 (1.5)	14.6 (1.5)	18.1 (1.4)
Height (m)	1.52 (0.14)	1.30 (0.09)	1.40 (0.09)	1.47 (0.09)	1.63 (0.06)	1.65 (0.06)
Weight (kg)	46.7 (14.8)	28.1 (5.5)	35.4 (7.9)	42.2 (9.2)	57.2 (10.3)	61.7 (10.2)
IQ	113 (12.0)	117 (12.9)	112 (10.3)	111 (12.5)	113 (11.5)	112 (12.7)
SES	41 (17.2)	41 (18.2)	44 (15.8)	41 (16.4)	41 (17.2)	39 (19.7)
MALES	Total sample	Tanner stage 1	2	3	4	5
N subjects/scans	158/415	41/45	51/56	82/104	110/136	59/74
	Mean (SD)	Mean (SD)				
Age (years)	13.8 (3.4)	9.2 (1.7)	10.0 (1.3)	12.3 (1.4)	15.7 (1.3)	18.0 (1.5)
Height (m)	1.60 (0.18)	1.35 (0.12)	1.40 (0.08)	1.55 (0.10)	1.73 (0.08)	1.78 (0.07)
Weight (kg)	55.6 (19.3)	32.3 (9.3)	35.8 (8.0)	47.6 (10.3)	66.7 (12.8)	76.2 (14.2)
IQ	115 (11.1)	120 (10.5)	115 (11.3)	116 (10.5)	113 (11.2)	117 (11.1)
SES	40 (19.1)	30 (15.8)	44 (18.6)	41 (19.7)	43 (19.7)	36 (17.0)

Table 3.1: Participant demographics. *The table shows the mean and S.D. for age, height, weight, IQ and SES for the whole sample and for the participants in each of the 5 Tanner stages. *Included in the table are the Tanner stage reported by each participant at the time of their MRI scan, as well as the total number of MRI scans performed on participants at each Tanner stage. Some participants had more than one scan while in the same developmental Tanner stage, hence there are more scans than participants.*

3.2.2 Image acquisition

All MRI scans were T1-weighted images with contiguous 1.5 mm axial slices and 2.0 mm coronal slices, obtained on a 1.5-T General Electric Signa scanner (Milwaukee, WI) using a 3D spoiled gradient recalled echo sequence with the following parameters: echo time: 5 ms; repetition time: 24 ms; flip angle: 45°; acquisition matrix: 256 x 192; field of view: 24 cm. The same scanner, hardware and software were used throughout the scanning period. All scans were assessed by a clinical neuroradiologist for gross abnormalities. All scans performed as part of the NIMH project have been rated for motion-related quality by trained technicians. Only scans given a high-quality rating were included in the analysis.

3.2.3 Image processing

Subcortical volume estimation was performed with the Freesurfer 5.1 image analysis suite using the programme's automated segmentation procedure (http://surfer.nmr.mgh.harvard.edu/). This procedure has been described in detail previously (Fischl et al., 2002), and is summarized here. An optimal linear transform is computed that maximizes the likelihood of the input image, given an atlas constructed from manually labelled images. A nonlinear transform is then initialized with the linear one, and the image is allowed to further deform to better match the atlas. Finally, a Bayesian segmentation procedure is carried out, and the maximum *a posteriori* estimate of the labelling is computed. The segmentation uses three pieces of information to disambiguate labels: (1) the prior probability of a given tissue class occurring at a specific atlas location; (2) the likelihood of the image given that tissue class; and (3) the probability of the local spatial configuration of labels given the tissue class. The automated segmentations have been found to be statistically indistinguishable from manual labelling (Fischl et al., 2002).

Automated subcortical segmentation using Freesurfer has previously been compared to manual tracing techniques for the amygdala and hippocampus in adults (Morey et al., 2009; Tae, Kim, Lee, Nam, & Kim, 2008). The correlation between Freesurfer and manual tracing methods for hippocampal volume was very high in both studies (Morey et al., 2009 κ=0.82; Tae et al., 2008 κ=0.82). For amygdala volume, which was only studied by Morey and colleagues, the correlation with manual tracing was more moderate (κ=0.56) but still highly significant (Morey et al., 2009). As a further test of automated segmentation reliability, Morey and colleagues calculated the within-subject scan-rescan reliability of Freesurfer (Morey et al., 2010). Participants (N=23, mean age 23.4 years, SD 3.3 years) each had a total of 4 structural MRI scans, of which two were performed 1 hour apart on day 1, and two more were performed 1 hour apart on day 7-9). ICCs were calculated for each of the subcortical structures for each participant across their four scans. The ICCs were very high for each of the subcortical structural volumes (>0.9) except for the NA (right 0.68; left 0.86) and the amygdala (right 0.82; left 0.87), which still showed acceptably high levels of correlation (Morey

et al., 2010). This suggests that there is high scan-rescan reliability of subcortical automated segmentation using Freesurfer, although these validations have only taken place in adult samples, and have not been repeated in children and adolescents.

The analysis focussed on the following subcortical brain regions, based on *a priori* hypotheses regarding changes in volume over adolescence and pubertal effects: amygdala, hippocampus, NA, caudate, putamen and GP. Each of these regions is defined by the automated Freesurfer segmentation procedure based on location, likelihood of tissue class and spatial configuration. An example of raw T1-weighted scans and Freesurfer's automated segmentation can be seen in **Figure 3.1**.

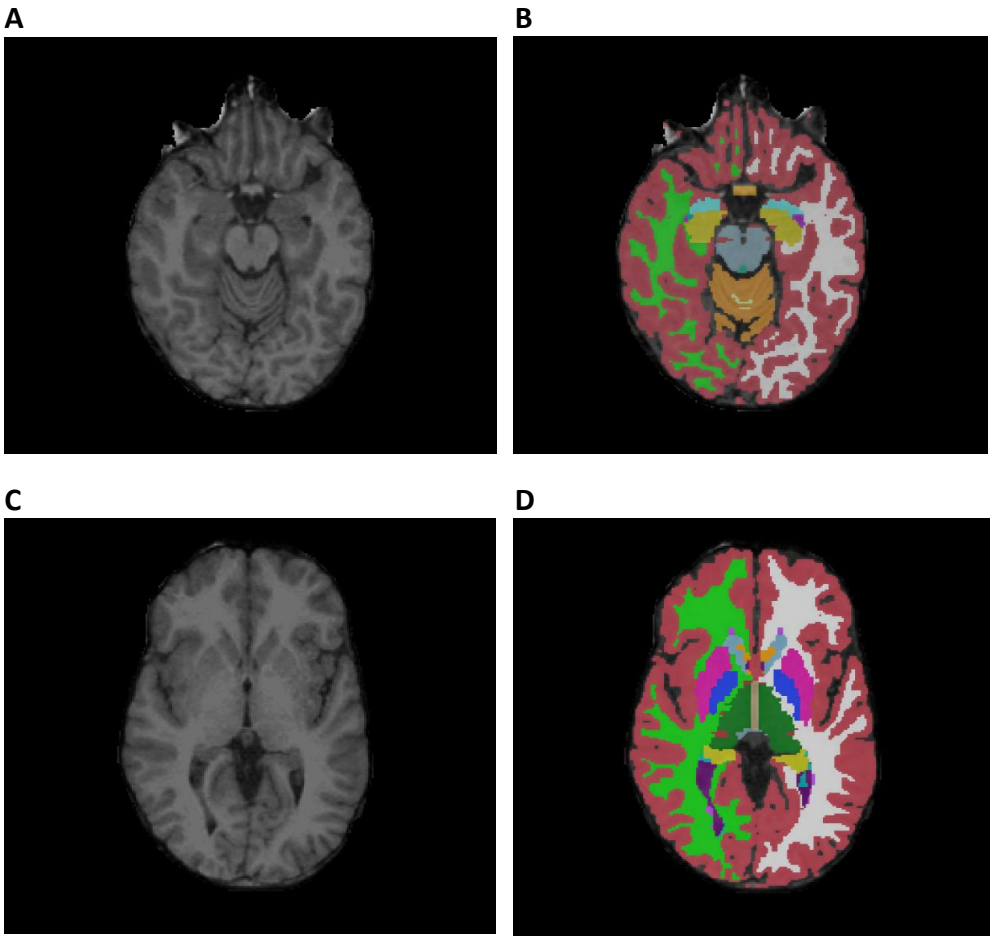


Figure 3.1: Examples of the Freesurfer 5.1 automated segmentation procedure. *Figures (A) and (C) show the raw T1-weighted axial scans; figures (B) and (D) show the superimposed automated segmentation of the subcortical structures and surrounding cortical regions. (A) and (B) include the hippocampus (mustard yellow) and amygdala (sky blue), while (C) and (D) include the caudate (medium blue), nucleus accumbens (orange), putamen (pink) and globus pallidus (dark blue).*

3.2.4 Statistical Analysis

The analysis was conducted using the average volume across hemispheres, to produce one value for each ROI. Previous studies have demonstrated no evidence of developmental difference between hemisphere in these regions (BDCG, 2012; Østby et al., 2009), and the dataset used for this analysis shows high correlations between hemispheres for all volumes ($r=0.5$-0.9, $p<0.001$). Raw volumes were analysed for each region and percentage change was calculated for each volume over time. Mixed effects modelling was used (R version 3.1-102; nlme package; Pinheiro, Bates, DebRoy, & Sarkar, 2011) to analyse the data, thereby allowing an estimation of the fixed effects of measured variables on volume change, while incorporating the longitudinal nature of the data by including within-person variation as nested random effects. Age was centred on 7 years, which represented the minimum age included in the sample. Tanner stage was treated as a continuous variable for this analysis, allowing the model to incorporate changing Tanner stage and changing brain volume for each individual.

For each structure, volume was first modelled against Tanner stage (Tanner-only model), and linear, quadratic and cubic developmental trajectories were evaluated. Males and females were modelled separately to allow for different trajectories of growth through adolescence (Lenroot et al., 2007). Tanner stage was treated as a continuous variable to allow the model to account for movement of individuals between stages and to maintain the ordinal nature of the data. The equations for volume growth of a structure in relation to Tanner stage are:

$$\text{Linear model: Volume} = \text{Intercept} + \beta_1(\text{Tanner})$$

$$\text{Quadratic model: Volume} = \text{Intercept} + \beta_1(\text{Tanner}) + \beta_2(\text{Tanner}^2)$$

$$\text{Cubic model: Volume} = \text{Intercept} + \beta_1(\text{Tanner}) + \beta_2(\text{Tanner}^2) + \beta_3(\text{Tanner}^3)$$

where β_1, β_2 and β_3 represent the constant terms defining the effects of each fixed term. To determine whether a cubic, quadratic or linear growth model with Tanner stage best-fit the sample, an F-test was performed on models where the marginal *p*-value of the highest order variable was significant ($p<0.05$).

Given that pubertal maturation is a developmental process, Tanner stage is necessarily highly correlated with chronological age, and this was found to be the case in this study sample (r=0.89 for males and r=0.88 for females). It was therefore necessary to consider the effects of age on the developmental trajectories of the subcortical volumes, to see whether this could explain all of the puberty-related effects, and to establish whether models including age with puberty were a better fit than puberty models alone. Therefore, age was incorporated into the model to ascertain whether there were (i) dissociable main effects of puberty and age, and/or (ii) puberty-by-age interactive effects. The model including the main effects of both puberty and age and the puberty-by-age interaction is referred to as the *combined model* (Volume = Intercept + β_1(Tanner) + β_2(Age) + β_3(Tanner*Age)), while the model including only the puberty-by-age interaction is referred to as the *interactive-only model* (Volume = β_1(Tanner*Age). Lastly, an age-only model was estimated (using linear, quadratic and cubic growth options as above).

Comparison of puberty-only, combined, interaction-only and age-only models was undertaken using likelihood ratio (LR) tests where possible. Where LR tests could not be performed, i.e. if the models were not nested, Akaike Information Criterion (AIC) values were used to compare models. Models were considered to be a significantly improved fit if the difference between AIC values was 5.9 or greater, equating to an Akaike weight of the poorer model of less than 0.05. If the difference between AIC values was <5.9, both models were considered equally valid based on relative likelihood (Wagenmakers & Farrell, 2004).

3.3 Results

Demographic details for all participants are included in **Table 3.1** and **Figure 3.2**. The raw data for each sex and each structure are displayed in **Figure 3.3**.

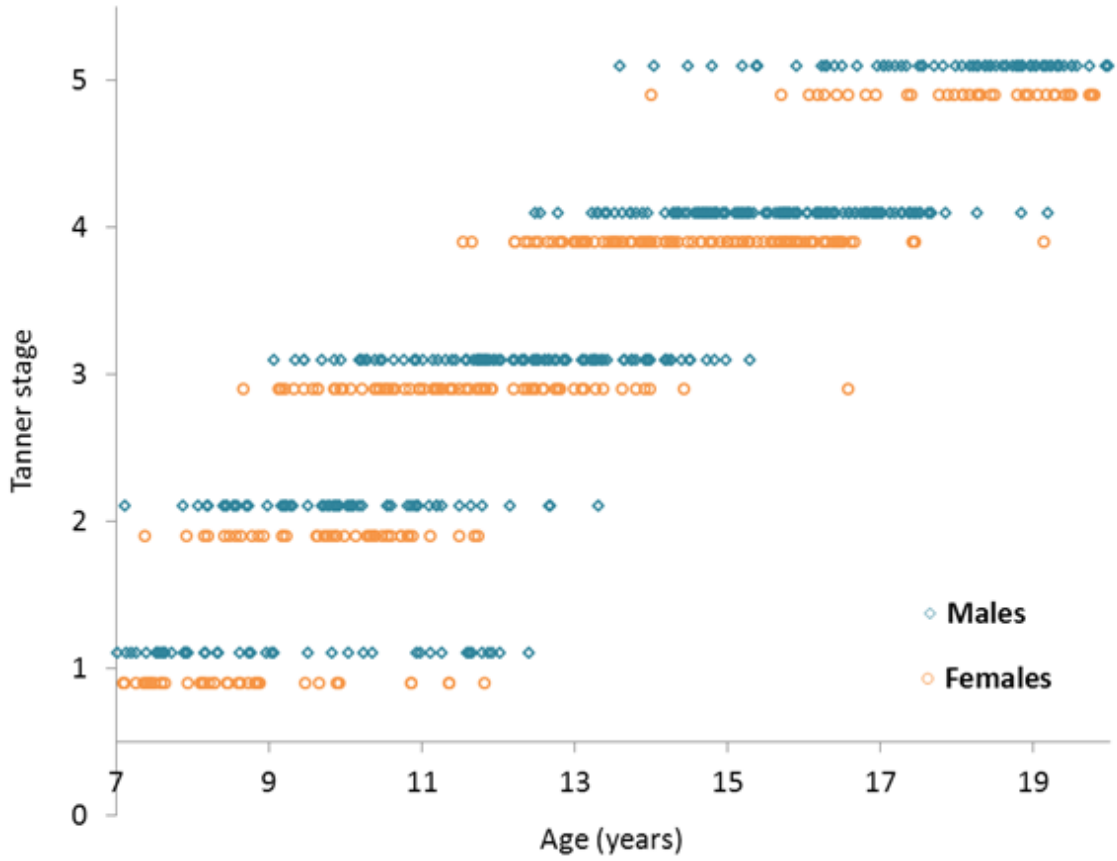


Figure 3.2: Age and Tanner stage of each participant at each study time-point. *Males are shown in turquoise, and females are shown in orange.*

3.3.1 Models using Tanner stage as an explanatory variable for subcortical volume change

Pubertal development, as measured by Tanner stage, was significantly related to the structural development of all six subcortical regions in both males and females (see **Table 3.2** and **Figures 3.4 and 3.5**). In both sexes, amygdala and hippocampus volume increased across puberty, while the other structural volumes (NA, caudate, putamen and GP) decreased (See **Figures 3.4 and 3.5**). In females, the growth trajectories were either linear (NA, GP) or quadratic (amygdala, hippocampus, caudate and putamen), whilst in males the trajectories were linear (hippocampus, GP), quadratic (putamen) or cubic (amygdala, NA, caudate) (see **Table 3.2 and Figure 3.4**). The proportional volume change over puberty varied between structures from a 7.5% increase in male amygdala volume, to a 9.9% reduction in female GP volume, with some regions showing more modest volume changes e.g. caudate 2.3% reduction in males (see **Figure 3.5**).

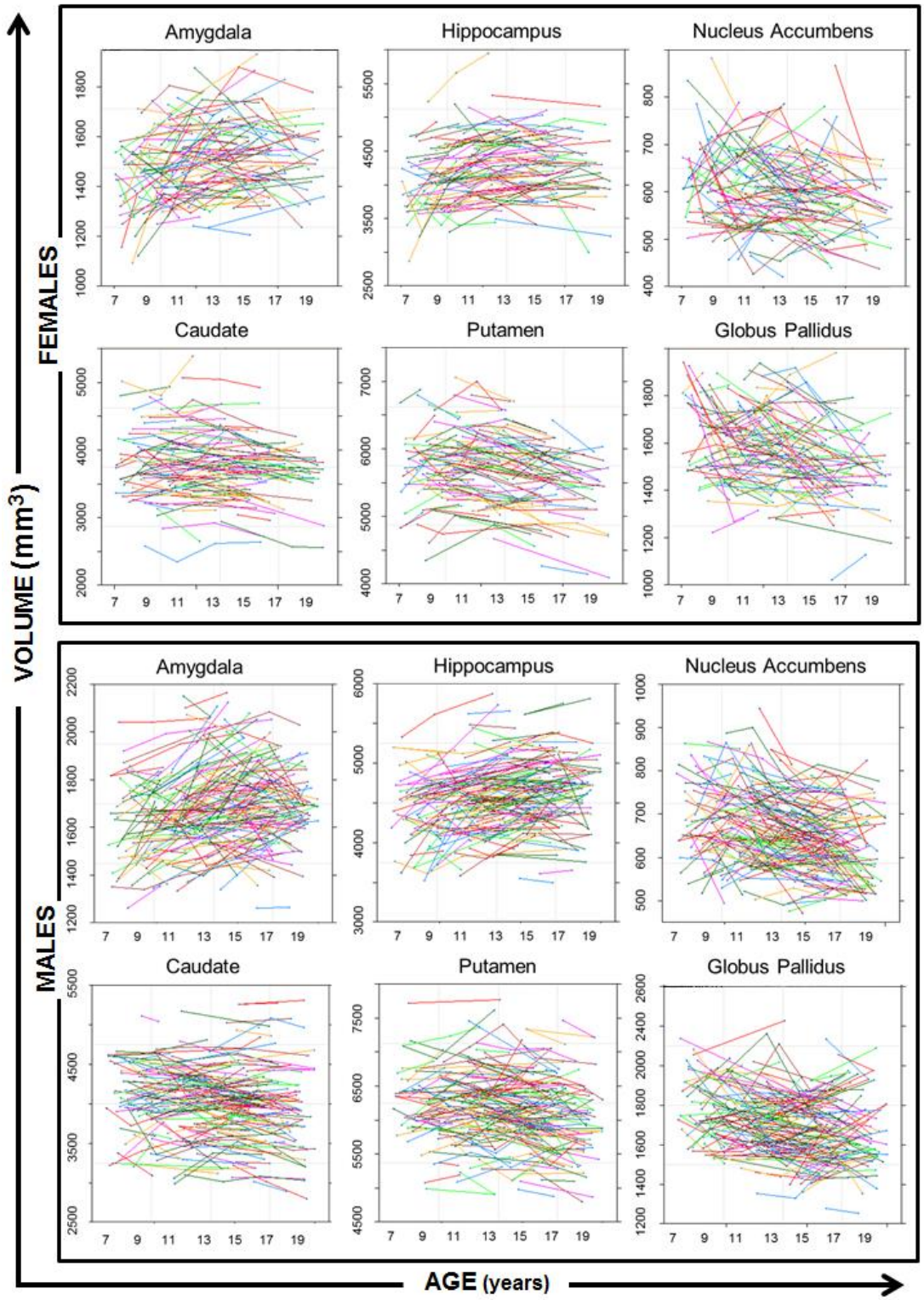


Figure 3.3: Volume against age for females (above) and males (below) for each of the six subcortical regions of interest. *Scans corresponding to the same participant are joined by a connecting line (different colours used to aid visual interpretation). Each scan point is indicated by a dot on the graph (Females: 296 scans of 117 participants; Males 415 scans of 158 participants).*

	Best-fitting model	Volume change across puberty		Significance of highest order variable	p-value
		Absolute (mm^3)	% change		
FEMALES					
Amygdala	**Quadratic**	80	5.3%	$F_{(1, 177)} = 6.38$	0.012
Hippocampus	**Quadratic**	191	5.1%	$F_{(1, 177)} = 6.26$	0.013
Nucleus accumbens	**Linear**	-50	-8.6%	$F_{(1, 178)} = 18.73$	<0.0001
Caudate	**Quadratic**	-131	-3.6%	$F_{(1, 177)} = 10.78$	0.001
Putamen	**Quadratic**	-282	-5.5%	$F_{(1, 177)} = 16.15$	0.0001
Globus Pallidus	**Linear**	-149	-9.9%	$F_{(1, 178)} = 45.02$	<0.0001
MALES					
Amygdala	**Cubic**	130	7.5%	$F_{(1, 254)} = 7.40$	0.007
Hippocampus	**Linear**	296	6.3%	$F_{(1, 256)} = 69.01$	< 0.0001
Nucleus accumbens	**Cubic**	-55	-8.7%	$F_{(1, 254)} = 4.44$	0.036
Caudate	**Cubic**	-92	-2.3%	$F_{(1, 254)} = 4.21$	0.041
Putamen	**Quadratic**	-159	-2.6%	$F_{(1, 255)} = 7.91$	0.005
Globus Pallidus	**Linear**	-79	-4.7%	$F_{(1, 256)} = 19.22$	<0.0001

Table 3.2: Tanner-only best-fit model for each of the six subcortical regions in females and males. *For each region, volume change across puberty is given in absolute (mm^3) and relative (% change for the structure compared to initial volume) terms. The table includes the F test for the model and the p-value of the highest order variable.*

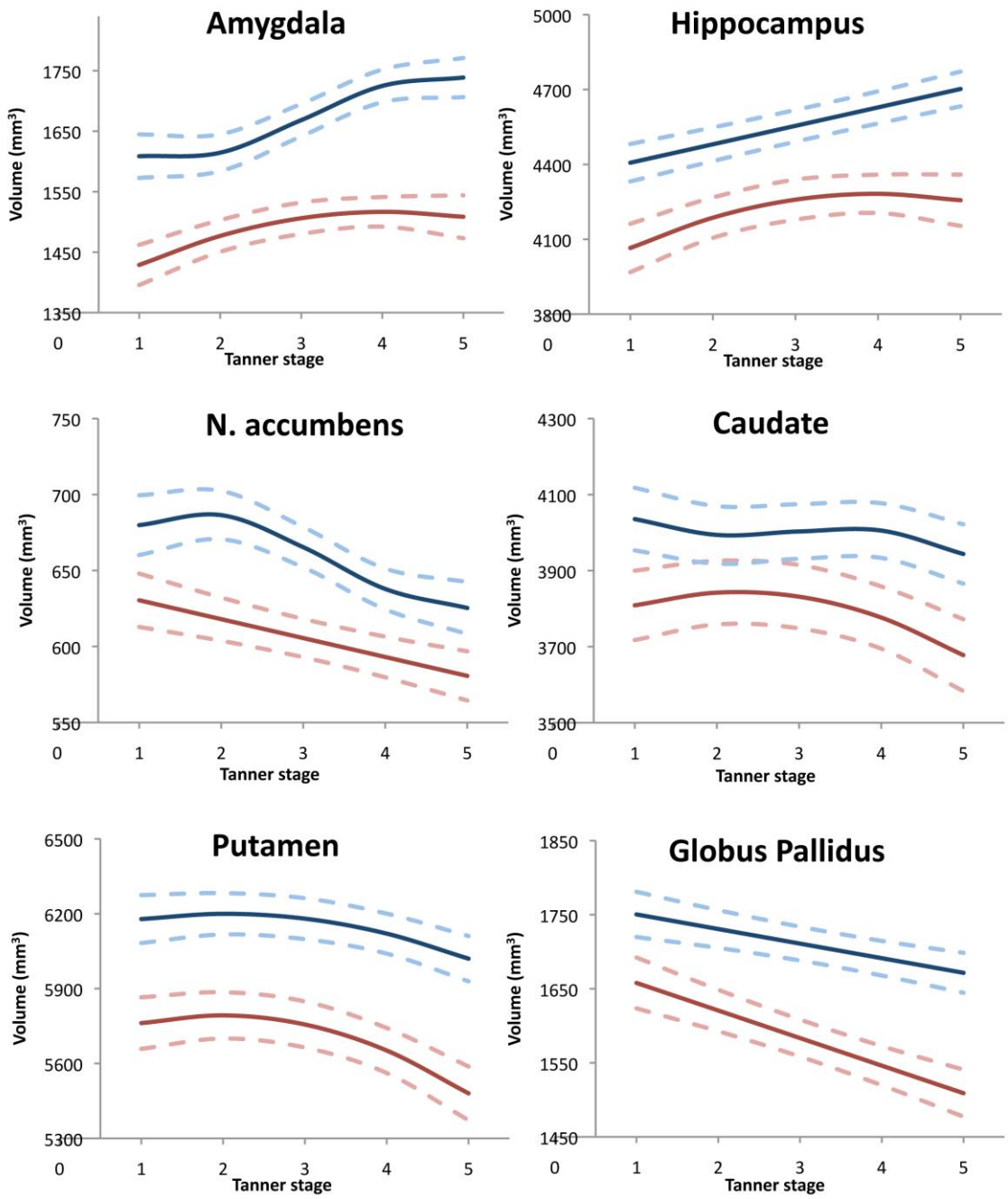


Figure 3.4: Growth trajectories for each subcortical region modelled against Tanner stage in females and males. *For both sexes, the amygdala and hippocampus increase in volume over puberty, while the NA, caudate, putamen and GP decrease in volume. The figure shows the models for each of the regions separately. Pink lines represent females and blue lines represent males. The solid line represents the best model fit, with 95% confidence intervals shown by the dashed lines.*

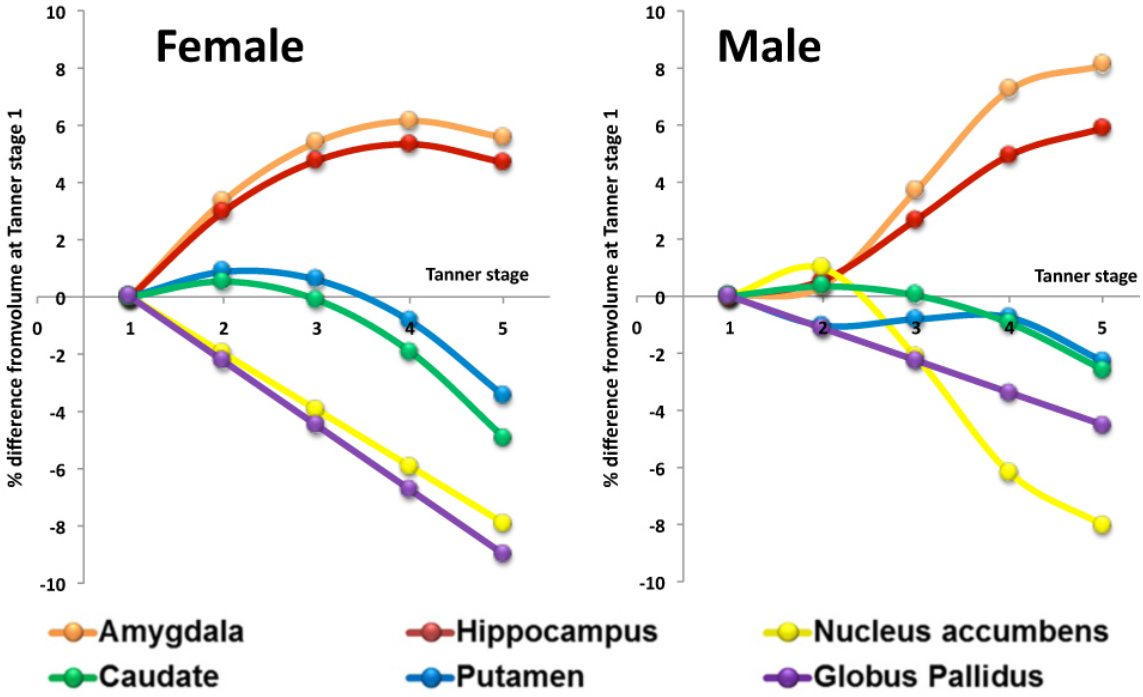


Figure 3.5: Percentage volume change for each subcortical region (y-axis) modelled against Tanner stage (x-axis) in females and males. *For each structure, the % volume was calculated for each pubertal stage as a proportion of volume at Tanner stage 1.*

3.3.2 Models using Tanner stage and chronological age as explanatory variables for subcortical volume change

For the amygdala, hippocampus and putamen in both sexes, as well as the caudate in females, a combined model (including Tanner stage and age as main effects, and a Tanner stage-by-age interaction) provided a significantly better fit than the Tanner-only model (see **Table 3.3**). For each of these structures, individuals in later stages of puberty (i.e. higher Tanner stage score) than their age-matched peers had volumes that are further along the developmental trajectory than peers in earlier stages of puberty. For females, individuals who were more pubertally mature than their age-matched peers had a larger volume in these structures in late childhood and early adolescence, an earlier peak volume and then a smaller volume until the end of puberty than their less mature, age-matched peers (see **Figure 3.6**). In males, for the amygdala and hippocampus, individuals in later stages of puberty (i.e. higher Tanner stage score than age-matched peers) had larger structural volumes throughout the studied age range (7-20 years) than age-matched peers in earlier stages of puberty

(see **Figure 3.6**). In contrast, for the putamen in males, individuals in later stages of puberty had smaller structural volumes throughout the studied age range than age-matched peers in earlier stages of puberty **(**see **Figure 3.6)**.

Structure	Best-fitting Tanner and age model[a]	Likelihood ratio test compared to Tanner only model	p-value	Difference between AIC
FEMALES				
Amygdala	**Combined**	**7.67**	**0.006**	
Hippocampus	**Combined**	**23.96**	**<0.0001**	
NA	Interactive	3.21*	*	3.21
Caudate	**Combined**	**14.16**	**0.0002**	
Putamen	**Combined[b]**	**28.13**	**<0.0001**	
GP	Interactive	3.43*	*	3.43
MALES				
Amygdala	**Combined**	**9.48***	*****	**9.48**
Hippocampus	**Combined**	**23.06**	**<0.0001**	
NA	Interactive	4.63	0.099	
Caudate	Interactive	5.29	0.071	
Putamen	**Combined**	**5.16**	**0.023**	
GP	Interactive	1.51*	*	1.51

Table 3.3: *B**est-fit models using a combination of Tanner stage and chronological age variables.** The table includes likelihood ratios and differences in AIC. **Structures in BOLD** show structures where the mixed Tanner stage and age model is a significantly better fit than the Tanner stage only model. NA=nucleus accumbens: GP=globus pallidus.*

(a) *Combined model refers to a model incorporating independent effects of Tanner stage and chronological age as well as an interactive Tanner stage-by-age effect. Interactive model refers to a model using only an interactive Tanner stage-by-age effect. (**b**) In females, the best-fit model for the putamen was a combined model including independent effects of Tanner stage, chronological age and quadratic as well as an interactive Tanner stage-by-age effect.*

** For these structures, a likelihood ratio test is not valid as the models are not nested and have the same number of degrees of freedom. Therefore, significance of the models has been judged using AIC differences. If AIC difference ≥5.9, the model is a significantly better fit (equivalent to Akaike weight of <0.05).*

For the NA and GP in both sexes, and for the caudate in males, an interactive-only model provided a significant fit to the data, but the model was no improvement over the Tanner stage only model (see **Table 3.3**). Based on this interactive model, individuals in a later stage of puberty than their age-matched peers had a smaller NA or GP (both sexes) or caudate (males only) volume throughout the age range investigated (7-20 years) **(Figure 3.6)**.

3.3.3 Models using chronological age as an explanatory variable for subcortical volume change

For some structures in males, the hippocampus and the GP, age-only models gave a significantly better fit of the data than models incorporating Tanner stage (hippocampus LR test compared to interactive model=4.69, p=0.030; GP LR test compared to interactive model=9.29, p=0.002). There was no statistically significant age-only model for volume change in the caudate in males over the age range. For the remaining structures in males (amygdala, NA and putamen) and all the six investigated structures in females, an age-only model did not improve the model fit over the models incorporating puberty measures.

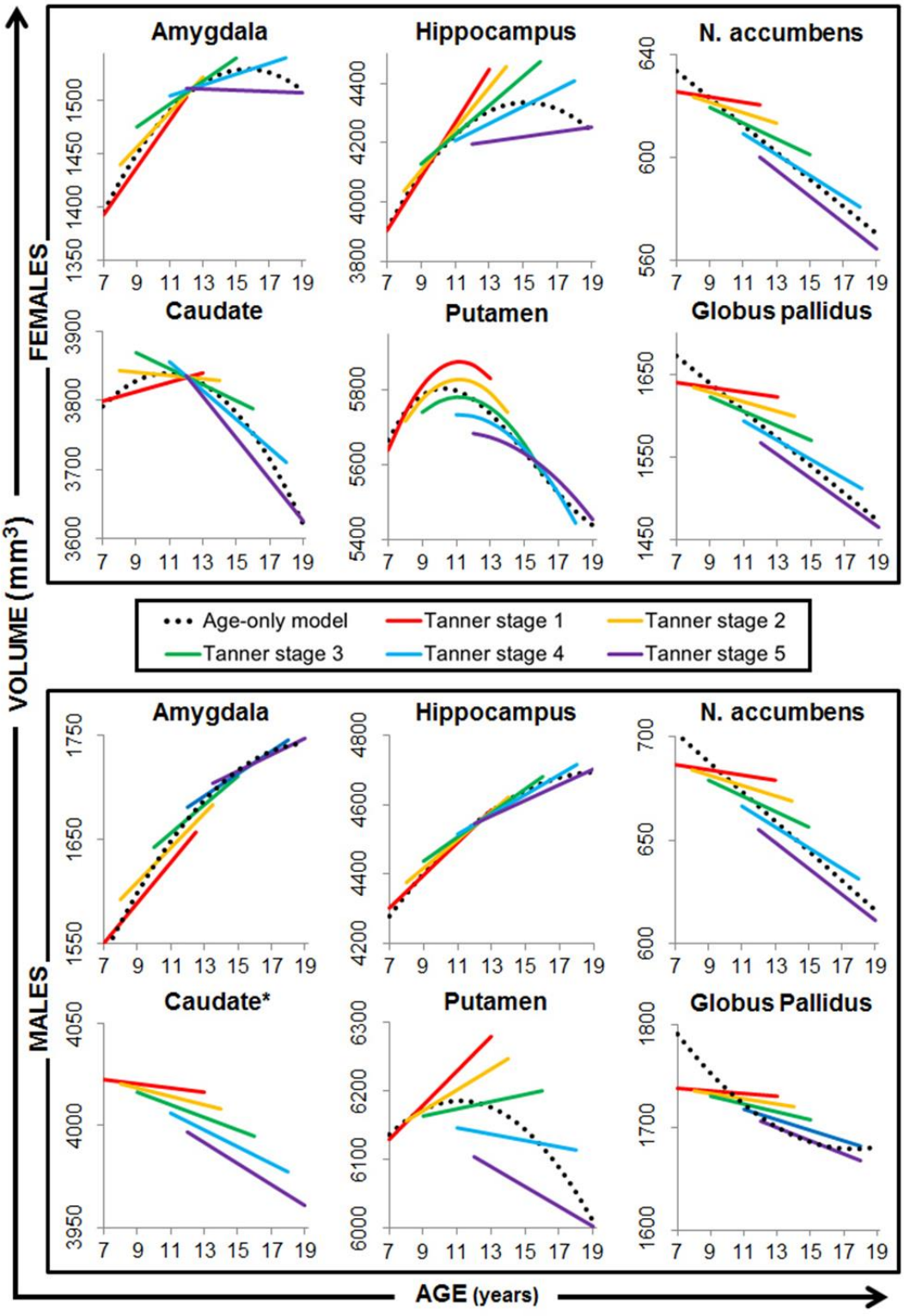


Figure 3.6: Changing subcortical volumes with age and puberty stage in (a) females and (b) males using the best-fit combined or interactive model for each structure. *Age is presented on the x-axis, while puberty stage is indicated by the coloured lines. Age ranges for each Tanner stage line were decided using the ages and pubertal variation in the study sample (see* ***Figure 3.2*** *for range). *For the caudate in males, there is no significant model that explains the developmental trajectory.*

3.4 Discussion

The current study examined the relationship between puberty and growth trajectories of subcortical regions during adolescence using a large longitudinal dataset. Each of the regions studied showed significant longitudinal associations with puberty **(Figure 3.4)**. For many of the structures (Females: amygdala, hippocampus, caudate, putamen; Males: amygdala, putamen), models including both age and Tanner stage modelled development better than Tanner-only models. This suggests that both variables are important factors when modelling volume change over adolescent development. THE hippocampus and GP in males were best modelled using only age as an explanatory variable, while the caudate in males was best modelled using only Tanner stage.

Despite the close proximity of the subcortical structures explored, there were clear differences in their structural development during adolescence. In both males and females, the amygdala and hippocampus continued to increase in volume during puberty, while the other structures examined decreased in volume, with structures changing between 2.3% and 9.9% across adolescence **(Figure 3.5)**. These results may reflect the different mechanisms that influence macroscopic volume changes between structures. Alternatively, the regions may undergo similar growth patterns, but do so at different chronological time-points, resulting in different growth patterns within the restricted age and developmental range.

3.4.1 Physiological association between Tanner stage development and structural brain development

Observation of pubertal development during adolescence provides an integrative marker of an individual's systemic sex steroid hormone exposure over time. The principal hormones involved are testosterone and DHEA, both androgens, and oestradiol, an oestrogen. Rising hormone levels, triggered by re-activation of the HPG axis, lead to the series of physical changes classically associated with puberty (see **Section 1.4**). Androgens signal the development of adult-type body hair and skin changes in both females and males, in addition to gonadal development in males,

while oestradiol primarily affects females, and causes breast and gonadal development. Tanner staging categorises pubertal maturation by describing five stages of development from pre-pubertal to full maturation for each of pubic hair development, genital development and breast development (see **Section 2.3**). An individual's puberty stage is related both to how long they have been exposed to the sex steroid hormones, and to their current level of hormones (Dorn & Biro, 2011; Shirtcliff et al., 2009). These results, showing that growth trajectories of subcortical structures were related to pubertal development, suggest that these same pubertal hormones may influence structural brain growth.

Systemic pubertal hormones cross the blood-brain barrier in small concentrations (Marynick, Havens, Ebert, & Loriaux, 1976), and systemic concentrations of testosterone have been shown to be related to amygdala volume in both males and females (Neufang et al., 2009). Both androgens and oestrogens induce synaptogenesis and synaptic pruning in rat and non-human primates (Ahmed et al., 2008; Hajszan et al., 2008; Sato et al., 2008) and it is likely that this process also occurs in humans, modulating brain growth across puberty (Matsumoto, 1991). Sex hormone receptors for both oestrogens and androgens are found throughout the brain in varying concentrations, with high levels in subcortical regions, particularly the hippocampus and amygdala (Abdelgadir et al., 1999; Clark et al., 1988; Sholl & Kim, 1989). The difference in regional receptor concentrations in the brain potentially helps to explain why different patterns of growth are seen across structures, and the resultant sexual dimorphism reported to emerge during adolescence in some regions (BDCG, 2012; Lenroot et al., 2007; Neufang et al., 2009; Sowell et al., 2002).

3.4.2 Amygdala development

This study found that both males and females demonstrated pubertal effects related to amygdala growth, with increases in volume over puberty in both sexes. Although the overall volume change was similar between the sexes, the growth trajectories were quite different (**Figure 3.4**), with females showing a large increase in volume in early puberty before peaking and decreasing, and males showing an increasing volume

until the end of puberty. This is consistent with the different patterns of testosterone concentration in puberty, with males showing larger increases in concentration and a longer period of increase, and females showing a much smaller rise and earlier plateau of testosterone concentration (Ankarberg & Norjavaara, 1999). The smaller increase and earlier peak in testosterone concentration in females compared with males might explain the differences in trajectories between the two sexes, where the changes in neural structure that are seen are being modulated by systemic testosterone concentrations (Zuloaga, Puts, Jordan, & Breedlove, 2008). This connection might reflect a direct effect of testosterone on amygdala volume, via the testosterone receptors found in high concentrations in the amygdala, or indirect effects e.g. via aromatisation and effects on oestrogen receptors (Schwarz & McCarthy, 2008), or through interaction with growth hormone (Meinhardt & Ho, 2006) and its receptors present in the brain.

Previous cross-sectional studies investigating changes in amygdala volume with puberty have reported volume increases with increasing pubertal stage (and testosterone level) in males (Bramen et al., 2011; Neufang et al., 2009), but have had conflicting results in females, where both increases (Neufang et al., 2009) and decreases (Bramen et al., 2011) in amygdala volume have been reported. The different findings between these two studies may result from the relatively small sample sizes or the differing age ranges (Neufang et al., 2009: N=46, 23 male, 8-15 years; Bramen et al., 2011: N=80, 32 male, 10.7-14.0 years). As the results from the current sample show, there is substantial variation in brain volumes between participants (see **Figure 3.3** for raw values), and small samples may not be able to reflect the variation in the population, and within each group (Early vs. Late puberty or Tanner stages 1-5). These results indicate that both age and puberty impact on the structural development of the amygdala in males and females, and differences in the findings of these two previous studies may reflect that they were sampling participants of different ages.

3.4.3 Hippocampus development

In the hippocampus, the best model to explain growth in females was the combined model incorporating puberty and age, whilst in males the age-only model provided a better fit. This is consistent with an oestrogen-modulated growth pattern. During puberty, oestrogen concentrations in females increase by 4-9 times (Ikegami et al., 2001), and oestrogens are dominant in many pubertal changes. In contrast among males, relatively minor rises in oestrogens are seen during puberty due to aromatization of testosterone. The hippocampus in non-human primates has high levels of oestrogen receptors (Sholl & Kim, 1989), and the increasing concentration of oestrogen that occurs predominantly in females coincides with the growth trajectories seen in the hippocampus in this study.

3.4.4 Nucleus accumbens development

Both males and females showed a decrease in NA volume with puberty in this analysis (see **Figure 3.4**), and both sexes show on-going development throughout adolescence. Both androgens and oestrogens modulate the function of the NA, changing levels of dopamine release (Thompson & Moss, 1994), and these results are consistent with the existence of macroscopic structural effects of both hormones on NA volume. For the NA, there was no statistical difference between the amount of volume change explained by the model based on puberty status alone, the model based on chronological age alone, or the model incorporating the interactive effects of both puberty and age. This may relate to the high correlation between age and pubertal stage. Note that such co-linearity reduces the precision of the estimates of coefficients but does not affect estimates of model fit. Importantly, all the models appear to show on-going structural changes across adolescence in the NA.

3.4.5 Development of the caudate, putamen and globus pallidus

The caudate, putamen, and GP lie in close proximity to one another, and have related functions. Growth trajectories of these three regions across puberty are similar, with decreases in volume seen with greater pubertal development. The relative changes in volume for the caudate and putamen are the smallest of all the structures over the

course of puberty (**Figure 3.5**). The caudate in males was the only structure analysed that showed no significant relationship with age, emphasizing the importance of considering alternative variables that influence development over this age range. Previous cross-sectional studies of the caudate have shown similar findings, with weak or no age-related changes (BDCG, 2012; Østby et al., 2009; Sowell et al., 2002); the results of this study using a longitudinal dataset support this. Less is known about the sex hormone receptor concentrations in the caudate, putamen and GP. Sex hormones have been shown to impact upon receptor densities in these regions (Sumner & Fink, 1998), giving one potential indirect mechanism for the changes in structure seen, but further work exploring how sex hormones influence macroscopic structural changes is needed to help ascertain this.

3.4.6 Strengths and limitations

One of the major strengths of this study is the longitudinal nature of the dataset, which enabled the modelling of growth trajectories based on the trajectories of real individuals instead of using cross-sectional points. There is wide variability in structural volumes in the brain between individuals, and repeated measurements of the same individual will therefore produce more accurate trajectories than assuming that cross-sectional data can be extrapolated to define trajectories, making the analysis more powerful to detect small but significant changes in brain volume. The structural development of the brain is likely to be affected by a number of variables and the use of a large longitudinal dataset maximised the study's ability to characterise the relationships of different variables with changing structural volume. The longitudinal nature of the data, the replication of previous cross-sectional findings and the presence of plausible biological mechanisms means that it is reasonable to hypothesize that puberty may have causal effects on brain development. However, this study does not provide scope to address whether the effects of puberty on structural brain growth are direct or indirect. Previous research has not tackled this subject, and further work exploring how pubertal hormones influence brain structural development in both animal and human studies, and combining hormonal and pubertal stage variables, may help to establish how this relationship is modulated.

The data used for these analyses are subject to a number of limitations. Some of these are common across studies included in this thesis, and will be discussed further in **Section 8.4**. Despite the large sample size, and longitudinal nature of the current dataset, there are limited numbers of participants at the extreme ages for each Tanner stage. This would be expected because the NIMH dataset is representative of a normal population undergoing typical development, with a normal distribution of ages for each puberty stage, and therefore relatively small numbers of individuals at the extreme ends of normal puberty timing. These smaller numbers may reduce the accuracy of the model at these extreme ages and further research targeting narrower age ranges, and focusing on these extremes of normal pubertal development, and using different methods to measure pubertal maturation, would help to validate and further expand on the findings of this study.

Polynomial models, as used in this study, have been shown to be susceptible to the age range and age-centring used (Fjell et al., 2010) when performing age-based analyses of development. For this reason, clear *a priori* methods for the analysis were developed, based on the primary aim of exploring the relationship between puberty and structural development of subcortical regions. The age range was clearly defined, based on the largest available recent US-population based studies of pubertal timing, to incorporate the reasonable ages associated with pubertal development. The use of the full range of pubertal variation in the analysis, the primary variable of interest, should minimise any impact of age-centring on the reliability of the model fit. A further limitation is the use of automated segmentation software to extract structural volumes for the six regions of interest. This method was chosen in view of the large number of scans (711) included in the study, and is widely accepted to be appropriate for very large scale-studies where manual tracing techniques are prohibitively time-consuming and resource intensive. Correlations between amygdala and hippocampus volumes for Freesurfer vs. manual tracing are high (Fischl et al., 2002; Morey et al., 2009; Tae et al., 2008).

3.5 Conclusion

This study has shown that the structural development of subcortical brain regions is related to pubertal development during adolescence. This relationship likely reflects the effects of systemic sex hormones on structural brain development. Examining brain development in relation to pubertal development may provide additional information regarding the control mechanisms behind adolescent brain development, and in particular may shed light on how many of the behaviours classically associated with puberty come to arise. It may also help explain the development of a marked sexual dimorphism in psychiatric disorders around the time of puberty (Zahn-Waxler, Shirtcliff, & Marceau, 2008), as there is some published evidence of associations between volumes of subcortical structures and psychiatric diagnoses (Karchemskiy et al., 2011; Rigucci, Serafini, Pompili, Kotzalidis, & Tatarelli, 2010).

3.6 Next Chapter

This chapter focussed on structural brain development, quantified using volumetric measurements, and has shown how changing subcortical volumes during adolescence relate differentially relate to puberty and age. In Chapter 4, I will focus on a different aspect of structural brain development by using diffusion tensor imaging (DTI) to quantify aspects of white matter microstructure. These indices will be compared between individuals who are at different stages of puberty using a cross-sectional design, with the aim of describing possible relationships between puberty and white matter development.

CHAPTER 4

The effects of puberty on white matter development

Neuroimaging studies demonstrate considerable changes in white matter volume and microstructure during adolescence. As with studies looking at structural brain volume (see Chapter 3), most studies investigating white matter microstructure have focused on age-related effects, whilst puberty-related changes are not well understood. The study described in this chapter was designed to address this gap in the literature. The effects of pubertal status on white matter mean diffusivity (MD) and fractional anisotropy (FA) were investigated using diffusion tensor imaging (DTI) and tract-based spatial statistics (TBSS) in 61 males aged 12.7-16.0 years. Participants were grouped into Early puberty (≤Tanner Stage 3 in pubic hair and gonadal development; N=22) and Late puberty (≥Tanner Stage 4 in pubic hair or gonadal development; N=39) groups as described in ***Section 2.4.3****. Salivary levels of pubertal hormones (testosterone, DHEA and oestradiol) were also measured.*

*Pubertal stage was significantly related to MD in diverse white matter regions. No relationship was observed between pubertal status and FA. Regression modelling of MD in the significant regions demonstrated that an interaction model incorporating puberty, age and puberty*age best explained these findings. In addition, testosterone was correlated with MD in these pubertally significant regions. No relationship was observed between oestradiol or DHEA and MD. In conclusion, pubertal status was significantly related to MD in this study sample, but not FA, and this relationship could not be explained by changes in chronological age alone.*

4.1 Introduction

Cross-sectional and longitudinal structural MRI studies have demonstrated that white matter volume increases across the brain during adolescence (see **Section 1.7.2.2**). These increases in white matter have been proposed to reflect increased axonal calibre within fibre bundles (Paus, 2010) and/or myelination, which in humans continues well into the second and even the third decade of life (Miller et al., 2012). Diffusion tensor imaging (DTI) is an imaging technique that provides *in vivo* quantitative information about white matter microstructure as opposed to classical structural MRI which assesses volumetric changes in white matter (Basser & Pierpaoli, 1996; D Le Bihan et al., 2001).

DTI fits a tensor to each voxel in the region of interest that estimates diffusion in three dimensions, measured on three axes (Le Bihan & Johansen-Berg, 2012). A variety of quantitative indices can be derived from this estimated diffusion tensor of which the two most frequently considered DTI measures are mean diffusivity (MD) and fractional anisotropy (FA) (Basser, Mattiello, & LeBihan, 1994). Studies exploring age effects on diffusion indices have consistently identified decreases in MD and increases in FA during adolescence (see **Section 1.6.2.2**). MD is the mean of the diffusion distance along all three axes, and therefore represents the overall magnitude of water diffusion in any direction. MD is sensitive to the number of cells and their processes in a region. Therefore, MD will be high in CSF where there is little restriction on diffusion, and lower within cortical tissue. In a tight bundle of axons, in which diffusion is restricted due to the large myelin lipids, water diffusion is restricted and MD is low. MD reduces further as myelination increases or cellular architecture becomes more complex, since these changes will reduce water diffusion.

FA provides a measure of overall directionality of movement, and ranges from 0 when the diffusion is isotropic (and the tensor is a perfect sphere and water diffuses equally in all three dimensions) to 1 when diffusion occurs along a single axis. FA increases as the extent of axonal myelination increases, or as axons become more coherently

organised in a uniform direction (Beaulieu, 2002). Two additional measures of the tensor that can be estimated are axial diffusivity (AD), the diffusion distance in the main direction of movement (along the main axis of the tensor) and radial diffusivity (RD), the diffusion distance across the main axis of the tensor (i.e. the mean of the other two distances). These measurements are all related, since they are calculated from the same tensors, but can be used to infer different information about the underlying white matter microstructure. FA increases are generally driven more by reductions in radial diffusion (RD) (in the perpendicular plane to predominant diffusion direction) than by changes in axial diffusion (AD) (in the plane parallel to predominant diffusion direction) (Giorgio et al., 2008; Lebel et al., 2008), although some studies have reported a decrease in both modalities (Eluvathingal et al., 2007).

Only two studies, both cross-sectional in design, have investigated whether pubertal factors also influence white matter microstructure in adolescence, in addition to effects of age. The first study looked at RD in white matter tracts in a cross-sectional sample of males and females aged 8-28 years (N=114, 63 females), and explored whether pubertal effects were present in tract regions of interest that showed significant age effects (Asato et al., 2010). Several association and projection tracts across the brain demonstrated continued immaturity (that is, a relatively high RD) in early and mid-puberty, suggesting that pubertal changes and white matter maturation may be more tightly coupled than previously thought. A second cross-sectional study (N=77, 39 female; 10-16 years) reported increased FA in boys in cortico-spinal, long-range association and cortico-subcortical white matter tracts, and reduced MD in frontal and temporal white matter compared with girls, and found that pubertal hormones such as testosterone explained variation in microstructure within some white matter regions (Herting et al., 2012). Other supporting evidence for pubertal effects on white matter in humans comes from a study looking at white matter volumetric and density changes. It has been shown that white matter density in frontal, parietal and occipital lobes increases with pubertal maturation in boys only, but decreases in cortico-spinal tracts (Perrin et al., 2009).

4.1.1 The current study

The current study aimed to investigate pubertal effects on white matter microstructure in boys, and to examine to what extent these effects these can be dissociated from correlations with chronological age. Since age and pubertal developmental are tightly coupled with a high degree of shared variance, removing significant effects of age when looking at DTI changes associated with puberty may mask potentially valuable results. This co-linearity was minimised by using a narrow age range at a developmental stage during which a full range of pubertal stages is seen, therefore maximising the study's ability to detect pubertal changes in white matter microstructure. Since the timing of puberty and the hormones associated with physiological changes differ between sexes, this study focused on exploring this question in males only.

The principal hypotheses of this study were that boys in later stages of puberty would show (i) higher FA and (ii) lower MD than boys in earlier stages of puberty. I predicted, based on findings of age-related changes in white matter microstructure, which show widespread development in adolescence (Bava et al., 2010; Qiu, Tan, Zhou, & Khong, 2008; Tamnes, Østby, et al., 2010), that these changes in white matter microstructure would be widespread and not region-specific. Given the correlation between pubertal development and age, I sought to disentangle these two explanatory variables by comparing regression models considering both factors and their interaction term, and hypothesised that puberty would explain additional variance over and above that explained by age alone.

4.2 Methods

4.2.1 Participants

The sample consisted of 61 boys aged 12.7 to 16.0 years. Details of recruitment, as well as inclusion and exclusion criteria, are outlined in **Chapter 2**. Informed consent for participation was obtained from each participant's parent or legal guardian, and participants provided informed assent for the study. Subjects received travel expenses,

and were reimbursed up to a maximum of £10/hour for their participation. The study was approved by the local University Ethics Committee and the National Research Ethics Committee.

To ensure that puberty groups were matched on cognitive ability, participants completed the Vocabulary and Matrices subscales of the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999), which was used as an estimation of IQ. IQ data were unavailable for two participants. Height and weight were recorded and Body Mass Index (BMI) was then calculated.

4.2.2 Puberty Measures

Pubertal development was assessed through a self-report pictorial questionnaire (Taylor et al., 2001) according to established Tanner criteria (Tanner & Whitehouse, 1976; see **Section 2.3.2.1** for details). Participants were divided into two groups: early-mid puberty (≤Tanner Stage 3 in both pubic hair and gonadal development) (N=22) and late-post puberty (≥Tanner Stage 4 in either pubic hair or gonadal development) (39), which are referred to as 'Early puberty' and 'Late puberty' from here on (see **Section 2.4.3**).

In addition, salivary hormone assays were collected to measure testosterone, DHEA and oestradiol (each measured in pg/ml) (see **Section 2.4.4** for full details). Saliva samples were not available (either not provided or unable to be processed due to insufficient volume) for 5 individuals for testosterone and DHEA (N=56 available samples), and 8 individuals for oestradiol (N=53 available samples).

4.2.3 Image acquisition

MRI data were acquired using a 1.5T Siemens Sonata MRI scanner with a 32-channel head coil. Head movement and scanner noise were minimised using soft cushions to support the participant's head within the head coil. Diffusion weighted images were acquired using a standard Siemens DTI echo planar imaging (EPI) sequence; gradient encoded pulses were applied in 64 directions, with AP-phase encoding, in addition to

the collection of a b0 (non-diffusion weighted) image. The following parameters were used: TR=7500ms, TE=104ms, FOV=224mm^2. 46 contiguous axial slices were acquired, with an isotropic voxel size of 2.3mm^3, and a b-value of 1000 s/mm^2.

4.2.4 Image processing

DTI data were pre-processed using tools from the FDT (Functional MRI of the Brain (FMRIB) Diffusion Toolbox) part of FSL (FMRIB Software Library) (Smith et al., 2004; Woolrich et al., 2009), initially undergoing correction of eddy currents, head motion and magnetic field inhomogeneities via a 12 parameter affine registration to the first non-diffusion weighted (b0) volume. The parameters used in this correction were also applied to the diffusion gradient vectors such that both the image data and the vector data were appropriately aligned. In addition, motion-related parameters in each of the 6 possible aspects (x, y, z, pitch, roll, yaw) were acquired and used to calculate a summary motion parameter indicating the extent of subject head motion between each volume acquisition (Jenkinson, 1999). Subjects with a high mean relative motion of >2mm, a previously recommended threshold (Yendiki, Koldewyn, Kakunoori, Kanwisher, & Fischl, 2014), were excluded prior to statistical analysis (N=1). Brain extraction (skull-stripping) was performed using FSL's Brain Extraction Tool (BET) (Smith 2002). A diffusion tensor model was fitted to the data in a voxel-wise fashion to generate whole-brain maps of the three orthogonal eigenvectors and eigenvalues, and these were used to calculate the summary measures of MD and FA. Maps of axial diffusivity (the principal eigenvalue) and radial diffusivity (the average of the remaining two eigenvalues) were stored for *post hoc* analyses.

4.2.5 Statistical analysis using TBSS

A voxel-wise statistical analysis of the DTI data was carried out using TBSS (Tract-Based Spatial Statistics; Smith et al., 2006), part of FSL. All participants' FA data were aligned into a common space using the nonlinear registration tool FNIRT (Andersson, Jenkinson, & Smith, 2010), which uses a b-spline representation of the registration warp field (Rueckert et al., 1999). This mean FA image was created and thinned to create a mean FA skeleton, which represents the centres of all tracts common to the

group. Each participant's aligned FA data were then projected onto this skeleton to create a skeletonised FA image, and the same transform was applied to the MD data to create a skeletonised MD image. Skeleton voxels with a mean FA of <0.2 were excluded to reduce partial volume effects. The skeletonised FA and MD data for all participants were entered into voxel-wise between-subject statistical analysis.

4.2.5.1 *A priori* TBSS voxel-wise analysis of pubertal group

The *a priori* hypothesis that there would be a significant effect of pubertal group on white matter microstructure was tested on the FA and MD maps using a Student's t-test. Assessment of significance was by non-parametric permutation tests using the randomise tool in FSL (Nichols & Holmes, 2002; Winkler, Ridgway, Webster, Smith, & Nichols, 2014) with 5000 permutations and the threshold-free cluster enhancement (TFCE; (Smith & Nichols, 2009) correction for multiple comparisons. The threshold for significance after TFCE correction (α) at a whole brain level was 0.05. For regions of the skeletonised maps where a main effect of puberty was identified, anatomical location was identified using the Johns Hopkins University White Matter atlas, available through FSL. The number of voxels lying within each named tract that demonstrated a significant effect of pubertal group was also calculated.

4.2.6 *Post hoc* regression analyses

4.2.6.1 Puberty and age

For clusters in which pubertal group was significantly related to either FA or MD, the cluster-averaged FA or MD values were extracted, allowing application of three *post hoc* linear regression models to the data. Specifically, the following were modelled: 1) a confirmatory analysis of the main effect of puberty; 2) an analysis of the main effect of age without considering pubertal group; and 3) an analysis of the main and interaction effects of age and puberty, taking into account their shared variance. Age was mean-centred prior to regression modelling. All regression models and group-based t-tests were computed using IBM SPSS Statistics for Windows, Version 20.0.

In light of recent concerns that apparent group differences in DTI indices may sometimes be driven by group differences in head motion, a motion parameter variable was also added to the regression models to confirm that head motion did not contribute significantly to group differences (Yendiki et al., 2014). The best-fit regression model was identified through calculation of AIC values (Burnham & Anderson, 2002). The model with the lowest AIC is the best-fitting model and is used as the reference model (with an AIC difference of 0; the numerical difference between the AIC of each model can be used to compare models. If the AIC difference was >5.9 (equating to an Akaike weight of the poorer model of <0.05), then the model with the smaller AIC was considered a significantly better fit to the data. This selection criterion is the same as that used in **Chapter 3** for the MRI structural data analysis.

4.2.6.2 Contributions of axial and radial diffusivity to MD and FA parameters

AD and RD values are used in the calculation of both FA and MD, and are therefore necessarily correlated with them, they were not investigated as *a priori* hypotheses. Instead, their influence on any regions that showed a significant relationship between either MD or FA and pubertal maturation was investigated, to help characterise the changes being measured. For any significant clusters in the FA and MD *a priori* TBSS analysis of pubertal group on white matter microstructure, correlations between the four DTI parameters (FA, MD, AD and RD) were performed to see if there was a differential contribution of each aspect of the diffusion tensor to the result.

4.2.6.3 Puberty and salivary hormone level

Further *post hoc* regression analyses were conducted to investigate, in clusters where a main effect of pubertal group was identified, whether MD or FA were significantly related to pubertal salivary hormone levels. Correlations were performed between MD or FA and salivary levels of testosterone, DHEA and oestradiol. For hormones where this indicated a significant relationship between the hormone and the DTI parameter, I investigated whether inclusion of that salivary hormone was then included in the regression model to see if this resulted in an improved model fit.

4.2.6.4 Investigation of outliers

Clusters demonstrating a significant effect of pubertal group were assessed to look for any evidence of outliers. MD or FA values identified following the analysis that were >3 standard deviations (SD) from the mean were considered possible outliers. Both the *post hoc* regression analyses and the initial TBSS analysis were repeated after excluding any such participants to assess their impact on the results.

4.3 Results

4.3.1 Participant demographics

Age, IQ and head motion in the scanner for each group are shown in **Table 4.1**, in addition to pubertal salivary hormone levels.

	Early Puberty (N=22) Mean (S.D.)	**Late Puberty (N=39) Mean (S.D.)**	**Test statistic**	**P value**
Age, years	**13.7 (0.72)**	**14.4 (0.89)**	**t= -3.29**	**p=0.002**
IQ (N=59)	108 (14.2)	114 (10.6)	t = -1.59	p=0.12
Testosterone, pg/ml (N=56)	**63.5 (20.8)**	**120 (42.4)**	**t = -6.65**	**p<0.001**
DHEA[#], pg/ml (N=56)	112 (94.4) [#]	131 (98.8) [#]	U= 317[#]	p=0.5[#]
Oestradiol, pg/ml (N=53)	1.51 (0.69)	1.74 (0.12)	t = -1.12	p=0.3
BMI (N=48)	21.0 (4.1)	19.9 (2.7)	t = 1.12	p=0.3
Head movement (mm)^	**0.90 (0.21)**	**0.75 (0.19)**	**t = 2.92**	**p=0.005**

Table 4.1: Participant demographics. *Two-tailed t-tests were performed to compare group differences unless otherwise stated. [#]DHEA was positively skewed and not suitable for parametric summary statistics. Therefore, median and interquartile range are displayed, and group differences are compared using a Mann-Whitney U-test.*

Bold *p values indicate significance at p<0.05.*

^Head movement was determined by mean volume-to-volume displacement.

The median Tanner stage in the Early puberty group was 2, and in the Late puberty group was 4. All 5 stages of pubertal development were represented. The Early and Late puberty groups differed significantly in age, Tanner stage, head movement and their salivary testosterone levels (all *p value*s <0.007, the Bonferroni corrected level for α=0.05 and 7 tests), but did not significantly differ in terms of their IQ, BMI, DHEA or oestradiol levels (all p values >0.1).

4.3.2 A priori TBSS voxel-wise analysis of pubertal group

4.3.2.1 Fractional anisotropy and puberty group

Use of TBSS to conduct a whole brain analysis of Early puberty vs. Late puberty showed that there were no significant between-group effects on FA. Although FA tended to be higher in the Late puberty group compared with the Early puberty group, there were no regions that survived the whole brain correction at p<0.05 for either increasing or decreasing FA with advancing pubertal stage. This negative finding persisted when the threshold was lowered, and there were no significant between-group effects above a threshold of p=0.2 for increased FA with increasing pubertal stage, and p=0.9 for decreased FA with increasing pubertal stage. Therefore no further analyses were conducted on FA.

4.3.2.2 Mean diffusivity and puberty group

When considering MD, there was evidence of a main effect of puberty that was significant at a whole brain level (p<0.05 corrected). Specifically, there was a decrease in MD from Early to Late puberty in a large single cluster comprising a number of anatomical tracts (see **Figure 4.1**; mean cluster MD for Early puberty group: 0.778mm^2/s, late puberty group: 0.753mm^2/s). White matter regions within the significant cluster demonstrating a pubertal effect are summarized in **Table 4.2** and include voxels within association tracts such as the superior and inferior longitudinal fasciculus, cortico-subcortical (limbic) tracts such as the uncinate fasciculus that connects subcortical regions such as the hippocampus and amygdala with OFC, and projection tracts such as the corticospinal tracts.

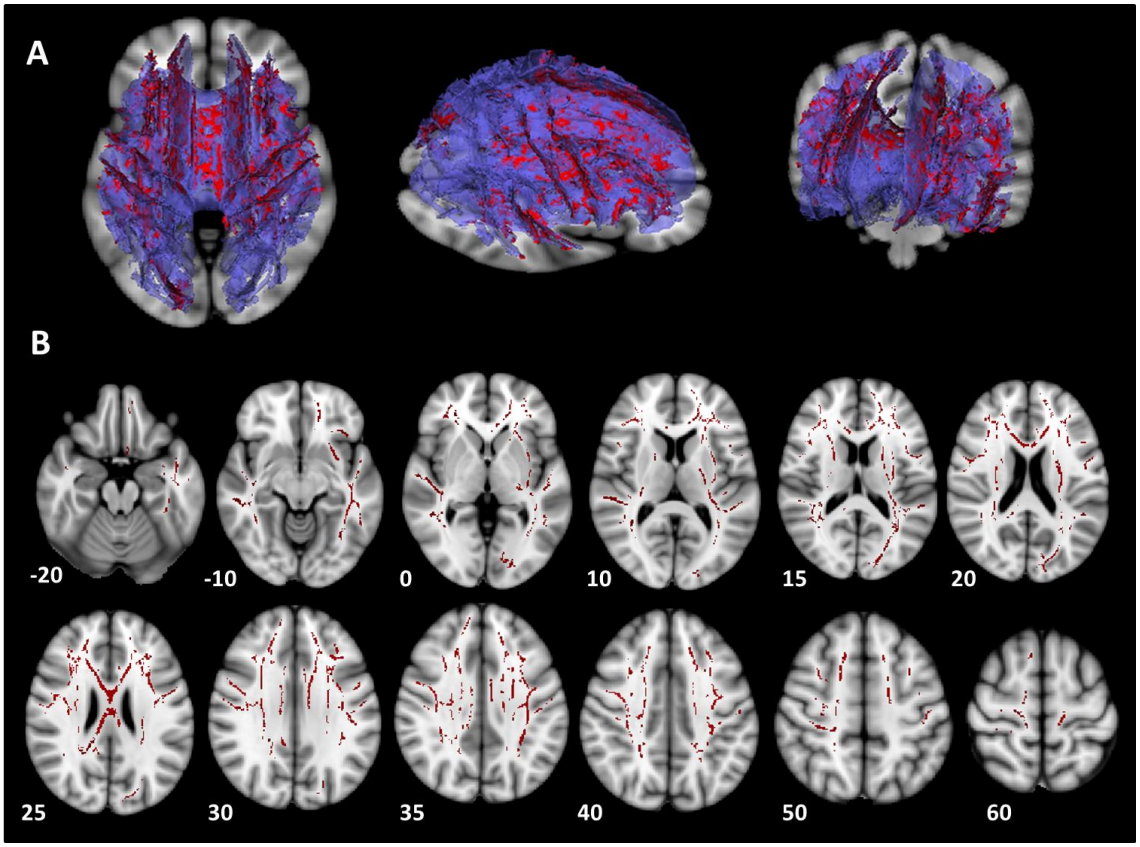


Figure 4.1: White matter regions demonstrating a significant effect of pubertal status on mean diffusivity.

A) 3D images in axial, sagittal and coronal dimensions, showing areas demonstrating a significant effect of puberty on MD (red), superimposed on 3D reconstruction of the mean white matter tract skeleton (purple). These images are superimposed onto a 2D brain slice in MNI space for orientation purposes at z=-2, z=-8 and y=-35. Images created using Slicer (www.slicer.org; Fedorov et al., 2012).

B) 2D axial slices showing the mean skeleton regions demonstrating a significant effect of puberty (red), shown in MNI space on a standard template (MNI z coordinates are indicated for each axial slice).

Tract Name		No of voxels demonstrating significant effect of pubertal status within this tract
Superior longitudinal fasciculus (temporal part)	L	63
	R	202
Superior longitudinal fasciculus	L	3047
	R	2306
Inferior longitudinal fasciculus	L	1637
	R	905
Corticospinal tract	L	718
	R	1015
Uncinate fasciculus	L	435
Inferior fronto-occipital fasciculus	L	1458
	R	1099
Anterior thalamic radiation	L	1084
	R	669
Cingulum (cinguEgyrus)	L	567
	R	438
Forceps minor		1660
Forceps major		810
Cingulum (hippocampus)	L	18
	R	21

Table 4.2: The anatomical tracts overlapping with the significant cluster that demonstrated a group difference between Early and Late puberty groups. *This significant cluster was identified using TBSS. The anatomical tracts included in this cluster are detailed in the table, with the number of voxels listed for each tract.*

4.3.3 *Post hoc* regression analyses

4.3.3.1 Modelling puberty and age effects on mean diffusivity

Despite the relatively narrow age range in the study sample, there remained a significant age difference between the Early and Late puberty groups (**Table 4.1**). Therefore, *post hoc* analyses were performed using regression models to assess whether the whole brain voxel-wise pubertal effects described above could simply be due to age differences. Three models were compared: a puberty-only model, an age-only model and a combined model (puberty + age + age*puberty). The model of best-

fit was found to be the combined model, which incorporated information from both measures to best explain MD across the two groups (**Table 4.3)**.

	Model 1 Puberty only			Model 2 Age only			**Model 3 Combined**		
Variable	B	SE B	β	B	SE B	β	B	SE B	β
Puberty	-0.025	0.006	-0.48***				-0.260	0.007	-0.48***
Age				-0.110	0.004	-0.36**	0.007	0.007	0.24
Puberty x Age							-0.018	0.008	-0.50*
R^2	0.227			0.132			0.332		
AIC difference	3.76			11.2			**0 (reference)**		

Table 4.3: Post hoc regression models. *The best-fitting model was the combined model (Model 3), so was set to be the reference model (**in bold**). The relative fit of other models was compared to this best model by comparing differences between the AIC for each model. The next best-fitting model was that with puberty alone, and the lowest ranking model was that with age alone. Unstandardised (B) and standardised (β) coefficients, as well as the standard error of the unstandardized coefficient (SE B) are shown for each variable. * p<0.05; ** p<0.005; ***p<0.001*

After the combined model, the puberty-only model was the next best-fit, followed by the age-only model, which was the poorest fit (**Table 4.3**). In order to illustrate the best-fitting combined model, this model is plotted graphically alongside the raw and group mean MD data in **Figure 4.2**.

The addition of the motion parameter to each of the three models confirmed that head motion did not improve the fit of any of the regression models, as the AIC increased (indicating a less well-fitting model) when motion was included in each of the models (AIC difference between combined model and combined model incorporating motion: 2.45; AIC difference between puberty model and puberty model incorporating motion 2.29; AIC difference between age model and age model incorporating motion: 0.5). This indicates that MD differences were not driven by motion artefact.

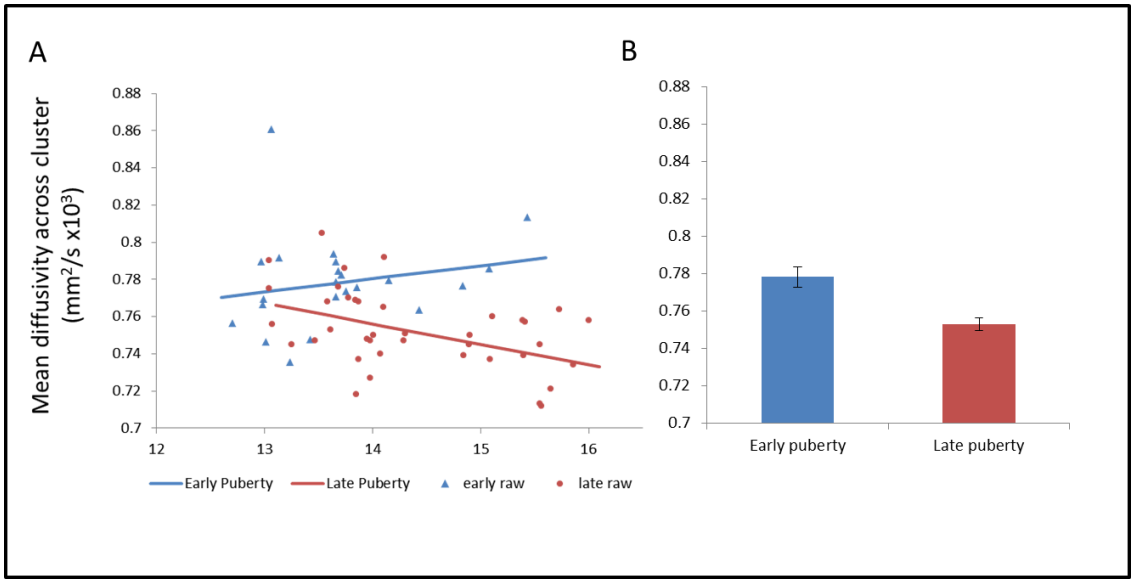


Figure 4.2: Graphical representation of the combined model of MD (within the significant cluster) with age and pubertal stage.

A) Scatterplot showing individual participants' raw data and linear regression model fit lines. Boys in the Early puberty group (blue) do not show the expected decrease in mean diffusivity as age increases. In contrast, boys in the Late puberty group (red) undergo a reduction in mean diffusivity as age increases.

B) Bar chart showing the mean MD values in the significant cluster for each puberty group, with error bars representing standard error.

4.3.3.2 Investigation of axial and radial diffusivity

Average values of AD and RD were extracted from the single significant cluster that showed pubertal differences in MD and were correlated against the average MD value. Within this cluster both AD and RD were positivity correlated with MD (AD: $r=0.83$, $p<0.001$; RD: $r=0.96$, $p<0.001$) and with each other ($r=0.64$, $p<0.001$). All three measures of diffusivity were negatively correlated with FA (MD: $r=-0.78$, $p<0.001$; AD: $r=-0.30$, $p=0.021$; RD: $r=0.92$, $p<0.001$).

When FA, AD and RD were included in the age and puberty regression models in place of MD, the same pattern of results was replicated: an combined model always provided the best model with the next best being Model 1 (puberty alone) and finally Model 2 (age alone). For AD and RD all three models were significant with p values

<0.005, while for FA only Models 1 and 3 (puberty alone and the combined model) were significant (p<0.05). Given how strongly correlated the four DTI measures were, and the fact that their inclusion did not change the interpretation of the regression model, FA, AD and RD were not analysed for the remaining models.

4.3.3.3 Hormonal variation and puberty group differences

To assess whether the pubertal effects on white matter tracts were related to salivary hormone levels, correlations were performed between MD and each of the three hormone levels. There was a significant correlation between salivary testosterone and MD (r=-0.30, N=56, p=0.023) with a decrease in MD as testosterone levels increased. There was no relationship with MD for either DHEA or oestradiol (**Figure 4.3**). Addition of testosterone to the puberty only and combined regression models of MD development did not improve the fit of either model.

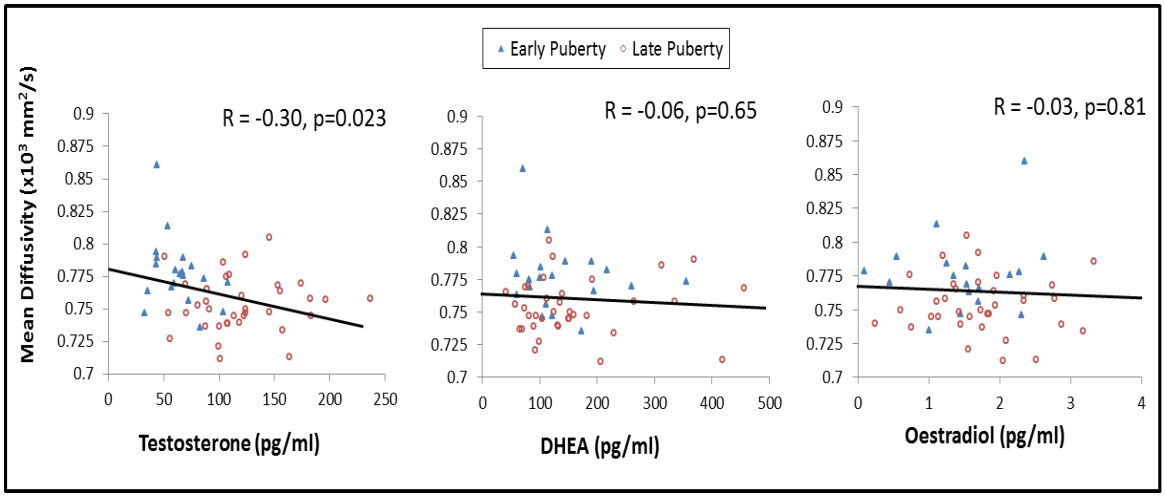


Figure 4.3: Scatter plots showing the relationship between testosterone, DHEA and oestradiol levels and MD in white matter regions that showed a significant effect of puberty group. *Markers indicate raw data; black lines indicate linear regression lines. Significant correlation was seen only between MD and testosterone.*

4.3.3.4 Investigation of possible outliers

Closer examination of the extracted MD values showed that one individual in early puberty had considerably higher MD than the rest of the early puberty group (>3 SD above the mean extracted MD value). As detailed in the Methods section, to ensure

that this participant's high MD was not driving the regression models for age and puberty, we repeated the regression after removing them from the analysis (N=60). The model fit comparisons after excluding the outlier were the same as reported before, with the combined model (standardised coefficients: puberty β -0.51; age β 0.48; puberty*age β -0.75) representing a better fit to the data than the puberty alone model (standardised coefficients: puberty β -0.46: AIC difference from combined model 9.33), which in turn was still a better fit than the age alone model (standardised coefficients: age β -0.33: AIC difference from combined model 16.4). Again, none of the models excluding the outlier were improved by the addition of a motion term.

In addition, we also repeated the entire TBSS analysis without this participant. There was a very high degree of overlap between the regions identified which demonstrated a main effect of puberty, although there was a reduction in the whole brain corrected significance level of the findings from α=0.05 to a trend level of α=0.1. After re-running the TBSS analysis, the regression models were repeated for age and puberty on the extracted MD data, similarly finding qualitatively identical results. Again the combined model demonstrated the best-fit (standardised coefficients: puberty β -0.65; age β 0.39; puberty*age β -0.62), the next best-fitting model was the puberty only model (standardised coefficients: puberty β -0.61: AIC difference from combined model 8.71) and the poorest fitting model was the age only model (standardised coefficients: age β -0.37: AIC difference from combined model 28.9). Once again, the addition of the motion variable reduced the fit of each of the models. Repeat correlation after repeating TBSS without outlier demonstrated a significant correlation between MD and salivary testosterone ($r=-0.36$, $p=0.007$) but no correlation between MD and either DHEA or oestradiol (both $p>0.3$).

These analyses support the earlier conclusion that these data demonstrate the existence of pubertal effects on white matter microstructure that are not simply ascribable to age, and that they are not driven by outliers.

4.4 Discussion

The current study identified a main effect of pubertal status on MD in a diverse set of white matter regions, where MD was reduced in male adolescents with more advanced pubertal development compared to their less well-developed peers. No significant relationship between pubertal status and FA was found. In keeping with the *a priori* hypotheses, the effect of pubertal status on MD was not region-specific, and the significant cluster from the analysis incorporated voxels from many different anatomical tracts. These pubertal effects were distinct from age-related differences in the same regions, and the best-fitting model to explain the differences in MD incorporated both age and puberty measures. In addition, there was a relationship between MD in the regions significantly associated with pubertal status and salivary testosterone, such that higher testosterone levels were associated with decreased MD.

4.4.1 Comparison with previous DTI findings in adolescence

In the current study, there were no significant effects of puberty on FA. Although increases in FA during adolescence have been found in studies looking at white matter changes with increasing age, these studies have typically been conducted over a much wider age range than the current study, often showing increases in FA from late childhood into the third decade of life (Ladouceur, Peper, Crone, & Dahl, 2012). Over the narrow age range within this study sample (3.3 years), pubertal development was associated with a significant reduction in MD but no change in FA. Although the DTI studies in adolescence described above have shown that in general increases in FA tend to occur concurrently with decreases in MD, these changes are not necessarily reciprocal in nature (Lebel & Beaulieu, 2011; Tamnes, Østby, et al., 2010). Detailed comparisons of changes in FA and MD during the course of adolescence have shown that MD tends to change predominantly in frontal, temporal and medial parietal lobes, whereas FA changes most strongly in lateral parietal, lateral occipital and frontal regions (Tamnes, Østby, et al., 2010). These anatomical differences between FA and MD developmental trajectories suggest that each parameter differently captures aspects of development of white matter microstructure. In addition, FA and MD were

differentially related to cortical thickness and white matter volume measures, again indicating differences between the microstructural changes best characterised by each measure (Tamnes, Østby, et al., 2010).

4.4.2 Previous DTI studies of puberty and white matter change

The present study focused specifically on the pubertal effects on white matter maturation. Two previous DTI studies have reported pubertal-associated changes, but these have been in the context of wider investigations of age effect on white matter microstructure. While these previous studies employed different analytical approaches, both initially accounted for effects of chronological age, thereby removing any shared variance between age and puberty from the remaining analysis concerning pubertal effects on brain structure. The first studied a cross-sectional sample of young people between 8-28 years (males and females), reporting radial diffusivity (RD), a measure of diffusion in the direction perpendicular to the predominant direction of diffusion (Asato et al., 2010). This study identified a diverse set of 19 regions in which there was a significant effect of age on RD, finding a reduction in RD with increasing age, and then explored whether this age effect could be accounted for by pubertal maturation.

Similar to the findings reported here, Asato and colleagues (2010) found continued decreases in the diffusion measure into post-puberty, though MD reflects overall magnitude of diffusion independent of direction, whereas RD considers diffusion only in the direction perpendicular to the predominant diffusion direction (Asato et al., 2010). This reduction in RD occurred across several major tracts including the superior longitudinal fasciculus, the uncinate fasciculus and the anterior thalamic radiation. The same study also found evidence for age-by-sex-by-pubertal status, suggesting that RD in these regions varied with both age and pubertal stage in different ways for boys versus girls. In addition, all but one of the 19 clusters showed an immature pattern in both the early and mid-pubertal stages, i.e. RD remained high, indicating that these regions within both association and projection tracts only became ‘adult-like’, with a lower RD, within the post-pubertal stage (Asato et al., 2010).

The second DTI study investigating puberty included a sample of males and females aged 10-16 years (Herting et al., 2012). Herting and colleagues found a significant relationship between FA and puberty in only a single white matter region (the right insular gyrus), after co-varying for age (and thereby removing any shared variance between age and puberty, which are tightly coupled). They found no relationship between MD and pubertal score after co-varying for age (Herting et al., 2012). When investigating the relationship between sex steroids and DTI measures, again after co-varying for age, the authors reported a significant relationship between testosterone and FA (in regions including the superior temporal gyrus, corpus callosum and superior frontal gyrus) and MD (in the superior frontal gyrus; Herting et al., 2012). In line with the findings of Herting et al. (2012), the current study also found evidence for a relationship between testosterone and white matter microstructure changes during adolescence, although the analyses found that pubertal development was related to changes in MD rather than FA. These discrepancies could relate to a number of methodological differences between the studies, including different age ranges (3 years in the current study compared with 6 years in Herting et al., 2012), method of assignment of pubertal group/score (self-report pictorial questionnaire vs. PDS), and hormone sampling method (blood vs. salivary assays).

4.4.3 Evidence for pubertal influences on white matter microstructure

There is evidence for pubertal effects on brain development from both human and animal studies. A review summarising the effects of pubertal hormones on connectivity in the human brain concluded that sex steroids such as testosterone and oestradiol play a key role in both the initial organisation of structural connections *in utero*, and in activating areas linked by such connections during adolescence (Peper, Hulshoff Pol, et al., 2011). For example, there is evidence from electroencephalographic (EEG) data and functional connectivity MRI data for a role for testosterone in decreasing subcortical-cortical connectivity, and increasing connectivity between sub-cortical areas (Miskovic & Schmidt, 2009; Schutter & van Honk, 2004; van Wingen, Mattern, Verkes, Buitelaar, & Fernández, 2010; Volman, Toni, Verhagen, & Roelofs, 2011). Whilst it is difficult to extrapolate these functional studies

(particularly for EEG given its high temporal but low spatial resolution) to these DTI results demonstrating white matter changes in specific regions, these data at least indicate that sex steroids such as testosterone can impact on brain function.

In the sample of adolescent boys used in this study, there were correlations between DTI measures and salivary testosterone, but neither of the other sex hormones, during adolescence. This may reflect a greater importance of testosterone than other hormones in white matter development during puberty in males. Testosterone is the primary androgen released from the gonads in males, and shows the greatest rise across puberty, being 45 times higher in adult men than in pre-pubertal boys (Biro, Lucky, Huster, & Morrison, 1995). Sex steroids such as testosterone play a critical role specifically in white matter development during adolescence, acting as trophic factors impacting on development of axons themselves and also their supporting cells (McEwen et al., 1982; Melcangi, Magnaghi, Galbiati, & Martini, 2001; Peper, Hulshoff Pol, et al., 2011). It is known from both animal and post-mortem human studies that myelination continues during adolescence and is more extensive in males than females (Kim & Juraska, 1997; Yakovlev & Lecours, 1967), possibly underlying the larger increases in white matter volume seen in human males than females during adolescence (Lenroot et al., 2007).

There is also evidence that sex steroids are important in determining diverse structural characteristics within the brain including white matter microstructure, affecting synapse number, neurite outgrowth, dendritic branching and myelination (Cooke & Woolley, 2005; Garcia-Segura & Melcangi, 2006; Jordan & Williams, 2001; Melcangi et al., 2001; Romeo, 2003). Sex hormones exert this control through mechanisms including acting as transcription factors for gene expression and cellular proliferation (Melcangi et al., 2001), and through activation of signalling cascades (Nguyen, Yao, & Pike, 2005). Sex steroids can also directly impact glial cells and thereby influence myelination (Garcia-Segura & Melcangi, 2006), and a role for testosterone and oestradiol as neuro-protective agents in demyelination disorders such as multiple sclerosis and in spinal cord nerve injury is being advocated (Leonelli et al., 2006; Melcangi & Mensah-Nyagan, 2006). Furthermore, gonadectomy led to demyelination

of the corpus callosum in intact, gonadectomised and hormone-replaced gonadectomised rats, and this effect was more deleterious in males than in females (Patel et al., 2013).

The importance of the androgen receptor for modulation of brain structure and function is supported by animal work confirming its presence in axons, dendrites and glial cells within specific cortical regions (Sarkey, Azcoitia, Garcia-Segura, Garcia-Ovejero, & DonCarlos, 2008). In addition, studies in human adolescents have found an association between testosterone levels and increasing white matter that is modulated by a functional polymorphism in the androgen receptor gene, the short arm polymorphism of which leads to more efficient androgen signalling than the long arm polymorphism (Raznahan et al., 2010). Increased testosterone levels had a more robust effect on white matter volume in boys with the short arm androgen receptor polymorphism (Paus et al., 2010; Raznahan et al., 2010), and this was associated with an increased likelihood of depression in adolescence (Perrin et al., 2008).

In summary, there is evidence from both animal and human studies for pubertal effects on brain structure, including white matter microstructure, and for mechanisms by which pubertal hormones such as testosterone might mediate such changes. The finding of this study, that pubertal development is associated with changes in white matter characteristics, is consistent with this. These changes were partly correlated with testosterone, although addition of testosterone to the regression models did not improve their fit. Pubertal group and testosterone are highly correlated in the current sample, and are therefore likely to be capturing similar aspects of pubertal maturation. Addition of testosterone to the regression models is therefore likely to be redundant, and reduces the efficiency of the model.

4.4.4 Interpretation of DTI parameter results as indices of white matter microstructure

The *post hoc* analysis of the four DTI parameters replicated the results of a recent study of 17 adults by de Santis et al. (2014) in which MD and FA in 42 white matter

regions of interest were not correlated with each other (de Santis, Drakesmith, Bells, Assaf, & Jones, 2014). In addition, in this previous study and the current one, MD and FA were both positively correlated with AD and showed opposite directions of correlation with RD (positive for MD and negative for FA).

de Santis and others found that FA but not MD showed a significant correlation with the regions' myelin water fraction (de Santis et al., 2014; Deoni, Rutt, Arun, Pierpaoli, & Jones, 2008; MacKay et al., 1994). Rather than representing myelination, MD decreases have instead been associated with proliferation and/or growth of astrocytes in grey matter (Blumenfeld-Katzir, Pasternak, Dagan, & Assaf, 2011; Sagi et al., 2012). The results from this study show that puberty is associated with MD more significantly than with FA. This suggests that changes in white matter microstructure associated with pubertal development may relate more to reduction in the overall magnitude of diffusion within the white matter than to changes in the directionality of diffusion. This decrease in MD is likely to indicate more cells and cell components in the white matter regions shown in **Figure 4.1**. The findings of this study are not specific to MD, so it is possible that the decrease in MD is caused by an increase in myelin, but since MD explains more variance in age and puberty than the three other DTI parameters (FA, AD and RD) it is more likely that alternative or additional cellular processes are driving the differences seen between Early and Late puberty. The exact nature of these changes cannot be elucidated with human *in vivo* studies.

4.4.5 Methodological considerations and future directions

These findings add to the current available literature to help understand the differential contributions of chronological age and puberty on adolescent brain development. As with all studies, there are a number of limitations that must be taken into consideration when interpreting the results. Some of these limitations are relevant for a number of chapters in the thesis; these will be described in **Chapter 8**. The current study investigated white matter microstructure in males only: males and females experience differences in the timing of pubertal processes during adolescence, and therefore direct comparisons between the sexes are confounded by such variations in the timing of puberty and the associated hormonal influences (see

Section 2.1.2). Future studies should additionally consider pubertal relationships with white matter development in females. There has been recent focus on the effects that head movement within the MRI scanner can have as a potential confound in studies comparing DTI data between groups (Yendiki et al., 2014). In the current study, head movement was greater in the Early puberty group compared with the Late puberty group, and therefore possible effects of movement are an important consideration, although overall movement was relatively small. Unlike many previous DTI studies of adolescence and puberty, motion parameters were incorporated into the analysis, confirming that head motion had a minimal impact on the presented results and did not contribute to models predicting differences between the Early and Late puberty groups.

4.5 Conclusion

This study provides evidence that pubertal effects on white matter microstructure are evident in boys. These findings were not simply due to age effects, but were more closely related to an interaction effect of puberty and age, and to pubertal status alone, than to age. In addition, white matter changes appeared to relate to salivary testosterone levels in boys, indicating that these differences in white matter may be underpinned partially by hormonal processes. This study provides evidence that pubertal processes impact on adolescent white matter development in addition to effects of chronological age.

4.6 Next chapter

The first two empirical studies (Chapters 3 and 4) of this thesis have focussed on structural brain development, and how this varies with pubertal development. The next chapter is the first of three to consider functional brain development in adolescence and how this may be affected by pubertal changes.

CHAPTER 5

The relationship between puberty and social emotion processing

Having established a relationship between puberty and the developing structure of the brain during adolescence in Chapters 3 and 4, the next three studies (Chapters 5-7) focus on the evolution of particular functions of the brain during adolescence. As outlined in the introduction (Chapter 1, section 1.6), one key area of change during adolescence is in the development of a network of brain regions referred to as the 'social brain network'. This network of brain regions allows us to mentally represent the thoughts, feelings and emotions of other people. The study described in this chapter used fMRI to explore how pubertal indicators (salivary concentrations of testosterone, oestradiol and DHEA; pubertal stage; menarcheal status) relate to brain activity during a social emotion task that recruits this network of brain regions.

42 females aged 11.1 to 13.7 years underwent fMRI scanning while reading scenarios pertaining either to social emotions, which require the representation of another person's mental states, or to basic emotions, which do not. Pubertal stage and menarcheal status were used to assign girls to Early or Late puberty groups. Across the entire sample, the contrast between social versus basic emotion resulted in activity within the social brain network, including dmPFC, pSTS and the ATC in both hemispheres. Increased hormone levels (independent of age) were associated with higher left ATC activity during social emotion processing. More advanced age (independent of hormone levels) was associated with lower dmPFC activity during social emotion processing. These results suggest functionally dissociable effects of pubertal hormones and age on the adolescent social brain.

5.1 Introduction

Despite the hypothesised links between pubertal development and functional brain development (see Chapter 1), only a few functional neuroimaging studies of the adolescent brain have included puberty measures. One fMRI study demonstrated differences in caudate and rostral medial prefrontal BOLD signal between early and late puberty groups (aged 11-13) when processing reward outcome in a gambling task, and a correlation between testosterone level and caudate BOLD signal (Forbes et al., 2010). A second fMRI study investigating reward and pubertal hormonal concentration showed a different, significant correlation between testosterone level and striatum activation (Op de Macks et al., 2011). Two fMRI studies have been published assessing changes in face processing with puberty. One showed evidence for increased BOLD signal in the amygdala and ventrolateral prefrontal cortex to threatening faces in a pre/early puberty group compared with a mid/late puberty group (aged 11-13; Forbes et al., 2011). In a different study, at 10 and 13 years, Moore and colleagues found that participants in later stages of pubertal development showed increased signal in face processing regions when looking at affective facial expressions (Moore et al., 2012). These studies report some discrepant findings, which may reflect the different methods of assessing pubertal development used, or the different tasks administered (Moore et al., 2012; Op de Macks et al., 2011). However, no previous study has investigated pubertal influences on the 'mentalising network' of the social brain.

One of the hallmarks of adolescent development is the dramatic change that occurs in social behaviours. Adolescents show heightened self-consciousness, develop increasingly complex and important peer relationships, experience sexual feelings and form romantic relationships, and demonstrate better understanding of other people compared to younger children (Spear, 2009; Steinberg & Morris, 2001). The emergence of these behaviours coincides with the physical changes of puberty, which prompts the hypothesis that social behavioural changes of adolescence result from increasing pubertal hormone levels, perhaps via a direct influence on brain structure and function (Forbes & Dahl, 2010). Mentalising, the ability to recognise and interpret

the feelings, intentions, beliefs and desires of others (Frith & Frith, 2003), is important for all of these social behaviours. For example, to experience self-consciousness, an individual must be aware of the perspectives and opinions of other people.

The network of brain regions recruited during mentalising tasks comprises the dorsomedial prefrontal cortex (dmPFC), posterior superior temporal sulcus (pSTS) at the temporo-parietal junction (TPJ) and the anterior temporal cortex (ATC; see **Figure 1.7**). Developmental studies have shown a shift in relative activity within regions of the mentalising network between adolescence and adulthood (Blakemore, 2008; see **Section 1.8.1**). Specifically, a number of studies have shown that signal in the dmPFC during mentalising tasks decreases with age across adolescence, while signal in temporal regions increases during the same period (e.g. Blakemore et al., 2007; Burnett et al., 2009; Pfeifer et al., 2009; Wang et al., 2006; see **Figure 1.8**).

5.1.1 The current study

In the current study, the principal aim was to investigate the differential effects of chronological age and puberty status on brain activity during a mentalising task, and specifically a task exploring the emotional sensitivity to opinions and actions that characterise early adolescence (Sebastian et al., 2010). Therefore a 'social emotion' mentalising task was used that had been previously designed to investigate social brain development across age in females (Burnett et al., 2009), to investigate the impact of puberty on social emotion processing. Social emotions (e.g. guilt, embarrassment) are emotions that require mentalising about others and their reactions to one's actions; in contrast, basic emotions (e.g. disgust, fear) do not require mentalising. Adolescent females aged 11-13 years performed the task during fMRI. This age range incorporates females at all pubertal stages (see **Section 2.3**) and is characterised by steep gradients of gonadal hormone secretion (Rubin et al., 2009).

Three independent measures of puberty were obtained: salivary hormone assays for testosterone, oestradiol and DHEA); visual clinician assessment of Tanner stage (Marshall & Tanner, 1969); and a self-report measure of menarcheal status. It was

predicted that puberty measures would be related to Social>Basic activity within the ATC since this mentalising region is densely connected with steroid hormone receptor-rich limbic regions (Ahmed et al., 2008; Cooke & Woolley, 2005). In contrast, the dmPFC is thought to be sensitive to age effects, and not puberty (Casey, Duhoux, & Cohen, 2010). It was therefore hypothesized that chronological age would predict functional changes in dmPFC, independent of the effects of puberty.

5.2 Methods

5.2.1 Participants

The sample consisted of 42 female adolescents aged 11.1 to 13.7 years (mean 12.5, S.D. 0.7 years; see **Table 5.1**). For recruitment and inclusion/exclusion information, see **Section 2.2**). Participants assented to the study, and informed written consent was obtained from a parent/guardian. Subjects received £10/hour for their participation in data collection (max. 2 hours). The study was approved by the UCL National Hospital for Neurology and Neurosurgery Ethics Committee. Verbal IQ (vIQ) was measured using the British Picture Vocabulary Scale II (Dunn, Dunn, Whetton, Burley, & NFER-Nelson, 1997), which was administered individually to participants in a quiet testing room. BMI was calculated for each participant except one whose height was not measured (see **Table 5.1**).

5.2.2 Endocrine assessments

Three independent measures of pubertal development were taken from each participant: (1) salivary hormone assays for testosterone, oestradiol and DHEA (2) visual assessment of breast and pubic hair stage using established Tanner stages (Marshall & Tanner, 1969) (3) self-report of menarcheal status and timing. For full details, see **Section 2.4**. Participants were dichotomised into early-mid puberty (referred to as Early) and late-post puberty (referred to as Late) puberty groups, as outlined in **Section 2.4.3**. Early and Late puberty groups differed significantly on both age and vIQ (see **Table 5.1**); therefore, we included age and vIQ as covariates in all subsequent between group/hormone analyses.

	Whole group (N=42)		Puberty groups		
			Early (N=21)	Late (N=21)	Difference between group means
	Mean ± S.D. (Range)		Mean ± S.D. (Range)	Mean ± S.D. (Range)	
Age	12.5 ± 0.7 (11.1-13.7)		**12.2 ± 0.7 (11.1-13.6)**	**12.9 ± 0.6 (12.0-13.7)**	**t_{40} = 3.47 p < 0.001**
BMI^ (N=41)	19.2 ± 3.0 (13.5-27.3)		19.2 ± 3.5 (14.1-27.3)	19.1 ± 2.5 (13.5-24.6)	t_{39} = 0.039 p = 0.969
vIQ	120.9 ± 12.6 (89-155)		**116.6 ± 13.2 (89-139)**	**125.2 ± 10.6 (107-155)**	**t_{40} = 2.34 p = 0.024**
Oestradiol+ (N=39)	3.60 ± 1.74 (1.34-9.86)		3.11 ± 0.90 (1.39-4.98)	4.06 ± 2.20 (1.34–9.86)	t_{39} = 1.91 p = 0.067
Testosterone + (N=41)	60.8 ± 23.7 (28.1-148.3)		53.9 ± 12.1 (30.9-78.3)	67.4 ± 29.8 (28.1-148.3)	t_{37} = 1.78 p = 0.088
DHEA+ (N=40)	176.0 ± 107.1 (56.5-534.3)		150.5 ± 61.0 (56.5-304.3)	201.5 ± 135.8 (69.4-534.3)	t_{38} = 1.53 p = 0.134
Tanner stage Breast	3.3 ± 1.2 (1-5)		**2.3 ± 0.6 (1-3)**	**4.3 ± 0.7 (4-5)**	**t_{40} = 10.19 p<0.001**
Tanner stage Pubic Hair	3.1 ± 1.2 (1-5)		**2.1 ± 0.8 (1-3)**	**4.1 ± 0.7 (3-5)**	**t_{40} = 8.91 p<0.001**

Table 5.1: Participant demographics *including Mean, S.D. and Range of participants for age, BMI, vIQ, pubertal hormone levels and Tanner stage for the whole group (N=42) and for the Early and Late puberty groups separately. Significant differences (p<0.05) between puberty groups* ***in bold****.*

^ One girl in the Early puberty group was not measured for height, leaving N=41 for BMI for the whole group, and N=20 for the Early puberty group.

+ One subject did not produce a saliva sample. There was insufficient sample collected for one subject for analysis of either DHEA or oestradiol, and insufficient in a second participant for oestradiol only.

5.2.3 fMRI task

During the fMRI experiment, participants read scenarios designed to evoke one of four emotions: two social emotions (embarrassment and guilt) and two basic emotions (disgust and fear; see Burnett et al., 2009). Examples of social emotion scenarios are

''You were quietly picking your nose but your friend saw you'' (embarrassment) and ''You laughed at a quiet girl you know and it made her sad'' (guilt). Examples of basic emotion scenarios are ''Your dad told you that the fridge was infested with maggots'' (disgust) and ''Your friend screamed that there was a wasp inside your jumper'' (fear). Scenarios featured the protagonist ('you') plus one other person. Consequently, the crucial difference between social and basic emotion conditions was the requirement for mentalising, not the mere presence of another person in the scenario (Abraham et al., 2008). The mean (and range) word length, and the number of clauses, was equated across emotion conditions.

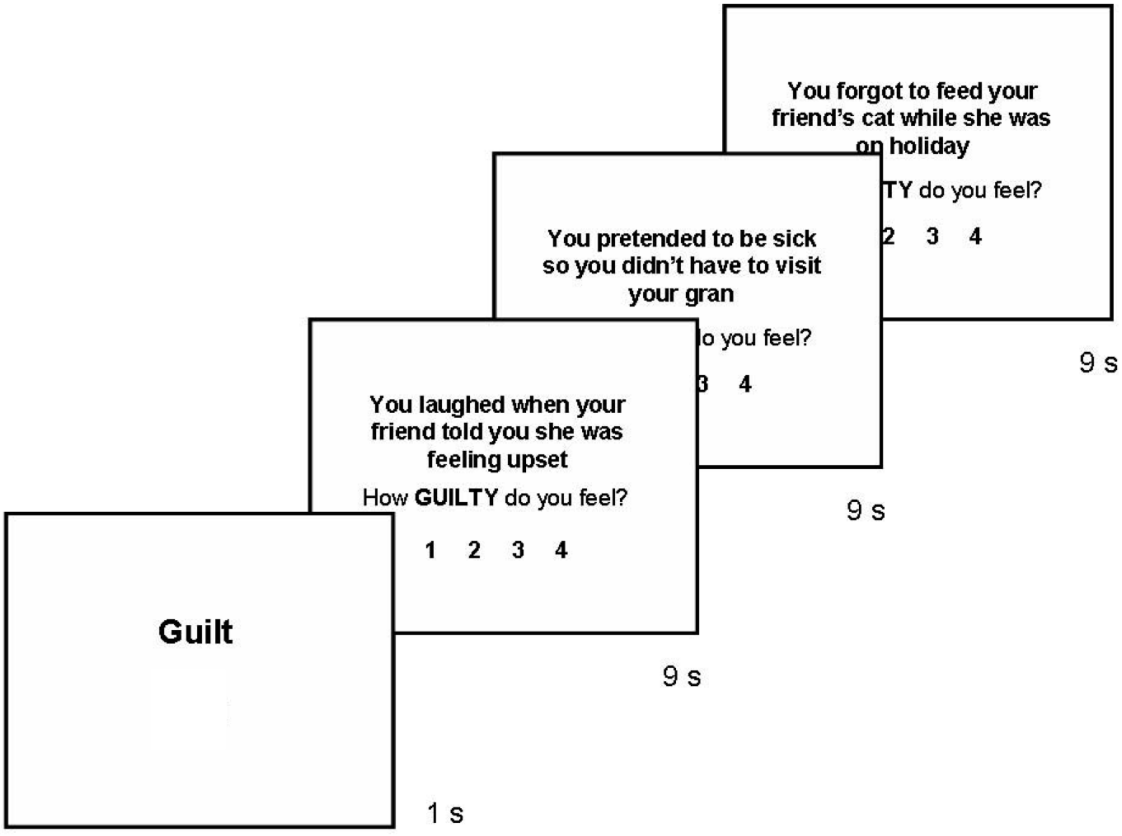


Figure 5.1: Social emotion processing fMRI task (guilt block). *Participants read scenarios designed to evoke one of four emotions: two social emotions (embarrassment and guilt) and two basic emotions. The scenarios were divided into blocks. Each block contained 3 emotion sentences, and was preceded by a 1 s emotion cue screen. Each emotion sentence was shown for 9 s.*

Participants had 9 s to read each scenario silently and imagine their response. After reading each scenario, participants rated to what extent they would feel the given

emotion, on a discrete rating scale from 1 (not at all) to 4 (very much), using a button box. There were 72 emotion scenarios in total, presented in blocks of three. In each block, all three scenarios featured the same emotion (disgust, embarrassment, fear or guilt). At the start of each block, a 1 s cue screen informed participants which emotion would be featured. Prior to scanning, all participants completed a guided practice session consisting of one example scenario from each of the emotions. The example scenarios did not appear inside the scanner.

The fMRI experiment was split into two 7 min sessions, each containing 12 emotion blocks, each lasting 28 s. Condition order was fully randomized. In addition there were four 7 s visual fixation blocks per session, occurring at regular intervals through both sessions. Stimulus presentation was programmed in Cogent (www.vislab.ucl.ac.uk/Cogent/index.html) running in Matlab 7.3.0, which recorded participant responses.

5.2.4 Data acquisition

A 1.5T Siemens Sonata head MRI scanner with 8-channel phased-array coil was used to acquire 3D T1-weighted fast-field echo structural images and multi-slice T2*-weighted echo-planar volumes with BOLD contrast. Each functional brain volume was composed of 45 3-mm axial slices with a 1.5 mm gap and in-plane resolution of 3*3 mm, with -30° slice tilt, zero z-shim and negative (down) phase encoding direction to minimize signal dropout in the orbital/rostral prefrontal and anterior temporal cortices. Repetition time was 4.05 s (90 ms per slice*45 slices). A total of 218 volumes were acquired over the two sessions, divided into 104 and 114 volumes.

Prior to functional scanning, an individual field map was acquired for each participant (scanning time 2mins) to correct for distortions in functional images (Weiskopf, Hutton, Josephs, & Deichmann, 2006). After functional scanning we acquired a 10-min T1-weighted anatomical image for each participant using a 3D modified driven equilibrium Fourier transform (MDEFT) sequence and an isotropic resolution of 1mm with the following parameters: echo time: 3.6 ms; repetition time: 12 ms; flip angle:

23°; acquisition matrix: 256 x 176; field of view: 25 cm, 176 slices. The total scanning duration was approximately 30 mins per participant.

5.2.5 Behavioural data analysis

Emotion ratings were analysed using 2x2 mixed model repeated measures ANOVA with between-subjects factor Group (Early vs. Late puberty) and within-subjects factor Emotion (Social vs. Basic). A regression analysis was performed to assess the relationship between mean emotion ratings and hormone levels.

5.2.6 Functional imaging data analysis

Imaging data were analysed using SPM5 (http://www.fil.ion.ucl.ac.uk/spm). The first six volumes from each run were discarded to allow for T1 equilibrium effects, leaving 206 image volumes per participant. Pre-processing included rigid-body transformation (realignment) and unwarping using individual field maps to correct for head movement. The images were then stereotactically normalized into the standard space defined by the Montreal Neurological Institute (MNI) template using the mean of the functional volumes, and smoothed with a Gaussian filter of 6mm full-width at half maximum to increase signal-to-noise ratio and facilitate group analysis. Time series for each participant were high-pass filtered at 128 s to remove low-frequency drifts.

The analysis of the functional imaging data entailed the creation of statistical parametric maps representing a statistical assessment of hypothesized condition-specific effects (Friston, Jezzard, & Turner, 1994), which were estimated with the General Linear Model (GLM). The effects of interest were the two scenario block types (Social and Basic emotion) and the visual fixation blocks. We modelled the six realignment parameters as effects of no interest to account for any group differences in head movement. Mean movement across the scans was 0.41mm (S.D. 0.21) for translation, and 0.40 degrees (S.D. 0.23). First-level contrast images ([Social>Fixation] vs. [Basic>Fixation]), from hereon referred to as (Social vs. Basic), were initially examined to look for main effects across the whole group, and then were entered into four second-level (random effects) multiple regression models examining: a) the

association between neural activity related to Social vs. Basic emotion processing and each puberty hormone (testosterone, oestradiol and DHEA), controlling for age and vIQ; and b) the relationship between neural activity related to Social vs. Basic emotion processing and age (controlling for each puberty hormone and vIQ). At the second level the interaction between condition (Social vs. Basic) and puberty group (Early vs. Late) was also modelled.

A priori regions of interest were investigated based on peaks reported in Burnett et al., (2009), which employed the same paradigm in a different sample of adolescents and adults, and showed main effects of the Social vs. Basic condition in the dmPFC ([-10 52 18]; [-4 52 -8]; [-18 42 16]; [-16 48 34]), precuneus [4 -56 28; -4 62 40], left pSTS/TPJ [-38 -66 42] and right pSTS/TPJ [44 -48 28]. In the second-level analysis, Burnett et al., (2009) showed age-related changes in social emotion processing in the left ATC [-40 -6 -26] and the left dmPFC [-16 42 20]. Small volume corrections (SVCs) on spheres with radius 6mm centred on these previously reported peak activations were conducted. Activations within these regions that survive family-wise error (FWE) SVC ($p<0.05$) are reported and, for completeness, activations that survive either cluster level FWE corrected threshold of $P<0.05$ or whole brain FWE height threshold at $P<0.05$. Brain mapping figures were made using Caret (Van Essen et al., 2001).

5.3 Results

Table 5.1 shows characteristics of subjects with respect to age, BMI, vIQ and salivary hormone levels, for the whole sample and for Early and Late puberty groups.

5.3.1 Pubertal data

Physician-assessed Tanner staging data were available for 40 participants, with self-reported Tanner stage data for the remaining two participants. Salivary hormone data were available on 42 participants. Mean levels were similar to previously reported norms for adolescents (Granger, Schwartz, Booth, Curran, et al., 1999; Matchock, Dorn, & Susman, 2007; Shirtcliff et al., 2009). There were significant correlations

between both oestradiol and testosterone and Tanner stage of breast development, and between testosterone and Tanner stage of pubic hair development (all p<0.05; see **Table 5.2** for all correlations). We found no association between hormone levels and either age, vIQ or BMI.

	TS pubic hair	TS breast	Oestradiol	Testosterone	DHEA
TS breast	**0.90*** **				
Oestradiol	0.31	**0.44** **			
Testosterone	**0.40** **	**0.36***	**0.50*** **		
DHEA	0.31	0.31	**0.61*** **	**0.69*** **	
Age	**0.68*** **	**0.58*** **	0.24	0.18	0.20
BMI	0.15	0.13	0.01	0.03	0.20
vIQ	0.26	**0.41** **	0.01	0.00	0.02
Mean Basic rating	-0.22	-0.28	-0.02	0.02	0.07
Mean Social rating	-0.16	-0.26	-0.14	-0.06	-0.01

Table 5.2: Pearson r correlation coefficients between pubertal measures and both participant demographics and behavioural ratings. ****P<0.005; **P<0.01; *P<0.05*

5.3.2 Behavioural data

Emotion rating data from four participants were not recorded by the stimulus computer, leaving N=38. There were no correlations between mean emotion ratings and hormone levels after controlling for age and vIQ (all p>0.5). Emotion ratings by scenario and puberty group can be seen in **Table 5.3**. There was a main effect of group: the Early puberty group gave higher ratings than the Late puberty group. This remained significant after co-varying out age and vIQ ($F(1,34) = 4.87$, $p = 0.034$). There was no significant effect of emotion and no interaction between puberty group and emotion ($F(1,36) = 0.055$; $p > 0.8$).

Emotion	**Puberty Group**	**Emotion rating** Mean (S.D.)
Basic	Early puberty (N=18)	3.34 (0.23)
	Late puberty (N=20)	3.07 (0.36)
Social	Early puberty (N=18)	3.22 (0.25)
	Late puberty (N=20)	2.97 (0.31)

Table 5.3: Mean emotion ratings by participants in Early and Late Puberty groups.

5.3.3 fMRI data

5.3.3.1 Main effect of Social vs. Basic emotion processing across participants (N=42)

Across the whole group, the main effect of Social vs. Basic emotion was associated with BOLD signal change in the dmPFC, bilateral pSTS/TPJ, precuneus and bilateral ATC, as shown in **Figure 5.2** (see **Table 5.4**).

Whole group (N=42)	Region of activation	MNI coordinates	Z	Size in voxels at P<0.001
Social>Basic Emotion	Precuneus	-2 -56 36	4.32	587 [a,b]
	Superior dmPFC	22 38 50	4.36	133 [b]
	Ventral mPFC	6 50 -10	4.23	261 [a,b]
	dmPFC	8 54 24	4.01	88 [a]
		-6 48 32	3.51	23 [a]
	Left pSTS/TPJ	-46 -60 38	4.09	214 [a,b]
	Right pSTS/TPJ	56 -64 26	3.73	98 [b]
	Left occipital cortex	-48 -82 6	3.81	94 [b]

Table 5.4: MNI co-ordinates, Z-values and cluster size for main effect of Social vs. Basic emotion. *We report activations that a) survive FWE SVC (p<0.05) within the a priori predicted regions or b) show cluster level corrected threshold of p<0.05. There were no clusters that survived whole brain FWE height threshold at P<0.05.*

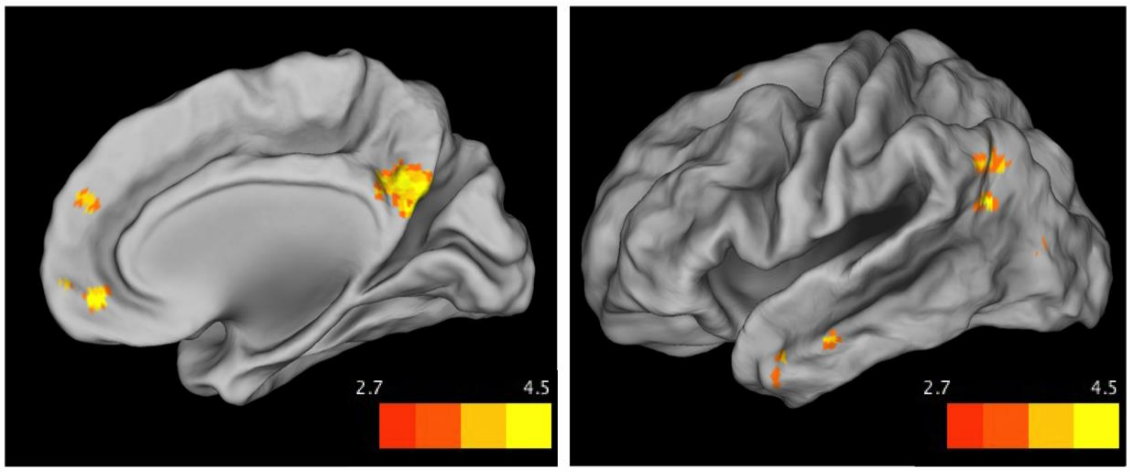


Figure 5.2: Main effect of Social vs. Basic emotion across all participants (N=42). *The left image shows the medial view, with activity in the dmPFC and precuneus, while the right hand image shows the lateral view with activity in the bilateral pSTS/TPJ and bilateral ATC. Significance voxel level p<0.001 unc., min. cluster size 15 voxels.*

5.3.3.2 Relationship between hormones and social emotion processing

Regression of whole-brain BOLD response for the contrast Social vs. Basic emotion against testosterone revealed a cluster in the left ATC [-42 -6 -22]. This activation remained significant [peak voxel -42 -8 -22] after co-varying vIQ and age (see **Figure 5.3 and Table 5.5**). Oestradiol and DHEA concentrations were also positively correlated with activity within the left ATC, both in a stand-alone model and when co-varying vIQ and age (note the correlations did not reach significance at SVC FWE correction; see **Table 5.5**). For all three hormones, incorporating the covariates vIQ and age did not change the level or significance of the clusters of interest, but did attenuate the size of the significant clusters (Results shown including covariates).

5.3.3.3 Interaction between puberty group and social emotion processing

There were no regions that survived the *a priori* significance threshold in these interaction contrasts. The analysis was also performed excluding the covariates (age, vIQ) from the model, and again there were no regions that survived the significance threshold. With a narrowed age range (11.5-13.5 years; N=30), there was no significant difference in age between Early and Late puberty groups. A repeated analysis with this subgroup again showed no regions that survived the significance threshold (Data not shown).

5.3.3.4 Relationship between age and social emotion processing

Whole brain linear regression analysis between age and BOLD signal change during Social vs. Basic emotion revealed a negative correlation with age within the left dmPFC [-16 48 20]. This cluster remained significant after co-varying out age and vIQ in the model (peak voxel [-16 50 22] with a small increase in cluster size (**Figure 5.4;** see **Table 5.5**).

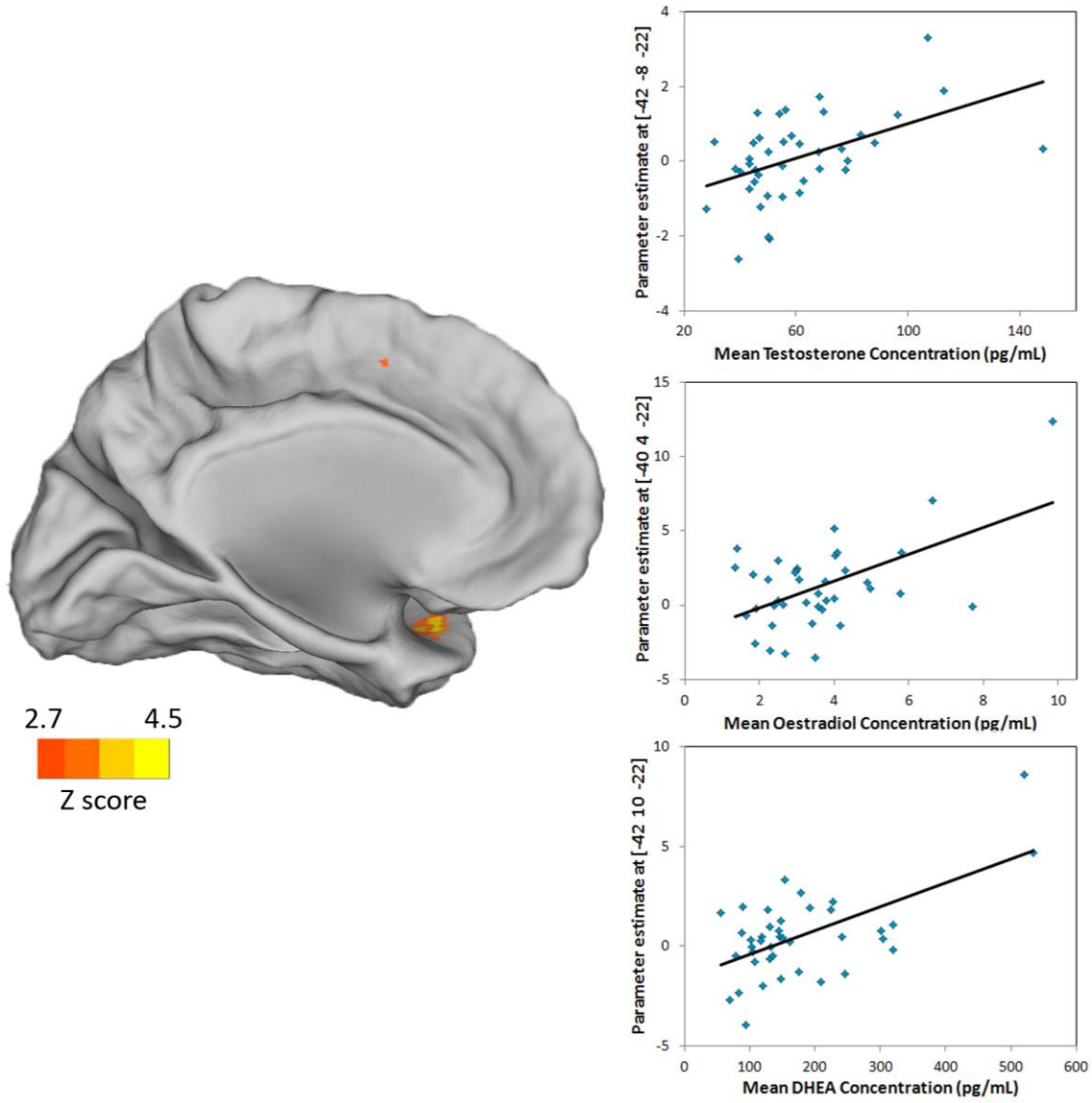


Figure 5.3: The association between puberty hormone level and BOLD signal during Social vs. Basic emotion (with age and vIQ covaried) in the left ATC *(peak voxels: testosterone [-42 -8 -22]; oestradiol [-40 4 -22]; DHEA [-42 10 -22]). The overlapping region of activation is shown at p<0.005. The graphs show the correlation between testosterone, oestradiol and DHEA and adjusted BOLD signal in the Social vs. Basic contrast at each peak left ATC voxel. The x-axis shows hormones concentration. The y-axis shows the difference in BOLD signal between [Social>Fixation] and [Basic>Fixation] at the peak voxel for each participant, shown as a parameter estimate.*

	Regressor	N	MNI coordinates	Z	Size in voxels at p<0.001
a.					
Positive regression	Testosterone*	41	-42 -8 -22	3.16	2
	Oestradiol[#]	39	-40 4 -22	3.38	6
	DHEA[#]	40	-42 10 -22	3.94	23
b.					
Negative regression	Age*	42	-16 50 22	3.83	34

Table 5.5: Significant results from regression analyses between BOLD signal during Social vs. Basic emotion processing and hormone and age variables.

a. *Positive regression between hormones and BOLD signal during Social vs. Basic emotion processing in left ATC, co-varying age and vIQ (Note: analysis without covariates shows no qualitative change in results).*

b. *Negative regression between age and BOLD signal during Social vs. Basic emotion processing in left dmPFC, co-varying for hormone levels (testosterone, oestradiol and DHEA) and vIQ.*

**survives SVC at P<0.05 FWE; [#]survives SVC at P<0.1 FWE.*

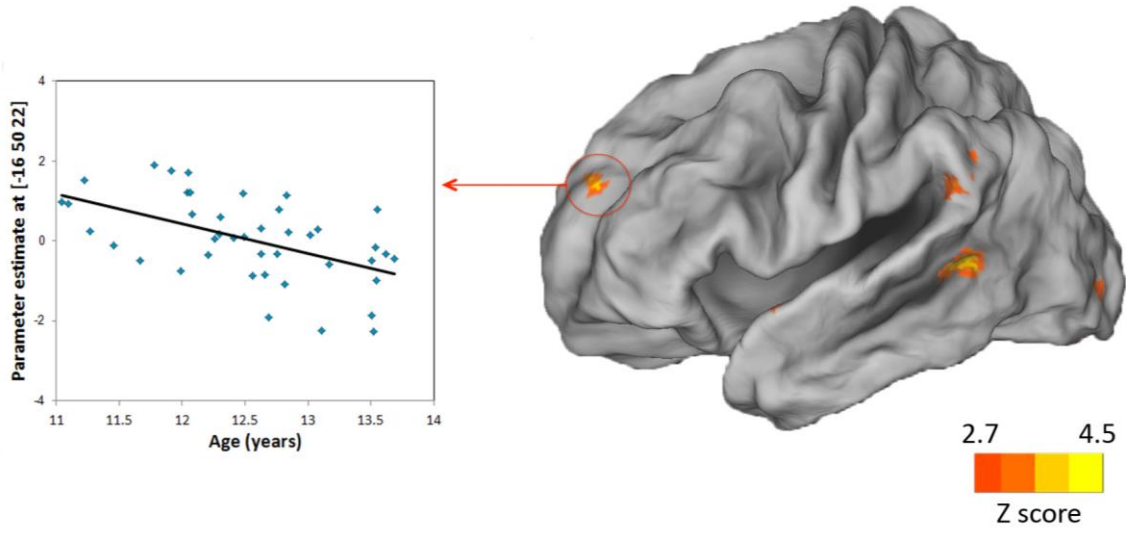


Figure 5.4: The negative association between age and BOLD signal during Social vs. Basic emotion (with puberty hormone level and vIQ covaried) in the left dmPFC *(peak voxel [-16 50 22]), shown here at p<0.005. The graph shows the negative correlation between age and adjusted BOLD signal at the peak voxel.*

5.4 Discussion

The current study used fMRI to investigate the relationship between puberty and the neural correlates of social emotion processing in females within a narrow age range from 11-13 years. The results provide evidence of functionally dissociable effects of puberty and age within the mentalising network. Across the whole sample, greater BOLD signal during social than during basic emotion processing in areas of the mentalising network (dmPFC and pSTS/TPJ) was observed. This result is consistent with a previous study using the same paradigm in adolescent participants aged 11-18 and adults aged 22-32 years (Burnett et al., 2009). In this previous study, an age-associated decrease in dmPFC activity during Social vs. Basic emotion processing was observed, as well as an age-associated increase in the ATC. The current results show puberty hormone-related, age-independent, increases in BOLD signal in the left ATC during social emotion processing. Conversely, chronological age-related decreases in BOLD signal within dmPFC were found that were not related to puberty hormone level.

5.4.1 Pubertal effects on behavioural ratings

Behaviourally, the Early puberty group gave higher emotion ratings for both Social and Basic emotion conditions than did the Late puberty group. A previous study from the same lab found that girls in late puberty report a wider combination ('mixedness') of emotions in response to social emotion scenarios than do girls in early puberty (Burnett, Thompson, Bird, & Blakemore, 2011), suggestive of an increasingly complex handling and understanding of emotions during puberty. As in the previous study using this task performed in the same lab (Burnett et al., 2009), across participants Basic emotion scenarios were given higher ratings than Social emotion scenarios, possibly reflecting the higher intensity of Basic emotions. There was no interaction between group and emotion for ratings, meaning that puberty stage did not have a greater influence over one type of emotion than the other.

5.4.2 Puberty-related fMRI effects

There were significant associations between levels of testosterone, oestradiol and DHEA and BOLD signal in the left ATC during Social relative to Basic emotion processing. These relationships were independent of chronological age. These findings are consistent with the hypothesis that pubertal hormones interact with the neurocognitive changes seen during this time (Blakemore et al., 2010; Sisk & Zehr, 2005). There are a number of developmental mechanisms that could underlie the pattern of findings in ATC, and the current fMRI study cannot distinguish among these. First, the ATC is a paralimbic region with direct connections with limbic regions (Olson, Plotzker, & Ezzyat, 2007), which contain large numbers of sex hormone receptors (Sar, Lubahn, French, & Wilson, 1990; Tabori et al., 2005). Thus, the increase in sex hormones at puberty might have a direct effect on activation of the ATC in social cognition tasks. Ernst et al., (2007), for example, demonstrated a relationship between adrenal hormone levels and amygdala activity during emotional face processing in females.

A second potential mechanism could be a developmental shift in cognitive strategy. The temporal poles, within the ATC, are thought to subserve semantic social knowledge (Lambon Ralph, Pobric, & Jefferies, 2008; Olson et al., 2007; Zahn et al., 2007). Transitions in social experience or cognitive strategy associated with increases in puberty hormone levels may cause a shift in the extent to which adolescents rely upon ATC representations during situations that provoke social emotions such as guilt and embarrassment. Previous studies have shown a relationship between puberty stage and emotion processing or more specifically, social emotion processing (Burnett, Thompson, et al., 2011; Spear, 2009; Sumter et al., 2010). We previously investigated how the ability to understand social emotional scenarios using mixed emotions varied across puberty in girls aged 9-16 (Burnett, Thompson, et al., 2011). There was a change between early and late puberty in the number of emotional responses that participants gave in social emotion scenarios, with girls in late puberty attributing a wider combination of emotions in social scenarios than their peers in early puberty.

Note that, in the current study, there was no significant difference between the puberty groups (as classified by Tanner stage and menarche) on social brain activity in the conventional analysis. This might be because Tanner staging is a noisier way of classifying individuals than measuring their puberty hormone levels. Nevertheless, the strong correlations between salivary pubertal hormone concentrations and physician-assessed Tanner staging suggest acceptable concurrent validity of the various methods used. These inter-correlations are hypothesised to reflect the underlying physiological processes whereby puberty hormones drive physical development corresponding to distinct Tanner stages. Pubic hair development, driven by serum androgen levels in puberty, correlated well with the measured salivary testosterone level, whilst breast development, primarily controlled by oestrogens during puberty, had the highest correlation with salivary oestradiol levels. These robust inter-correlations suggest that hormonal concentrations used as continuous regressors for BOLD signal are a valid index of pubertal development.

The region of left ATC that was positively related to puberty hormones in the current study is in close proximity to the region of ATC previously found to show an *age*-related increase in activity during Social vs. Basic emotion processing in a group of female participants aged between 11 and 32 years (peak voxels in the current study: testosterone [-42 -8 -22]; oestradiol [-40 4 -22] and [-36 -10 -22]; DHEA [-42 10 -22]; peak voxel in previous study [-40 -6 -26]; Burnett et al., 2009). In the current study, no age-effects within this region across the narrow age range of the sample (11-13 years) were found. This raises the possibility that the interaction between age and emotion in ATC found in the previous study reflects predominantly pubertal changes, or a combination of age-dependent and pubertal changes.

Some behavioural patterns usually associated with adolescence have been shown to correlate more closely with pubertal maturation than age, including parent-child conflict (Steinberg et al., 1988), sensation-seeking (Martin et al., 2002) and the development of romantic interests (Dahl, 2004). The current findings of puberty-related changes in neural activation, together with those shown in other recent fMRI studies using different 'social' tasks as described in the introduction, suggest that

aspects of functional brain development in adolescence, like these behavioural changes, may be more closely linked to the physical and hormonal changes of puberty than chronological age.

The study design focussed on comparing brain regions activated during the Social emotion condition versus the Basic emotion condition, and did not include a baseline condition with which to compare these conditions. Thus the increase in ATC activation seen with advancing puberty associated with the Social vs. Basic contrast corresponds to an increasing difference in BOLD signal between Social and Basic emotion conditions as hormone level increases. The regression could be driven by increased activation during Social emotion, decreased activation during Basic emotion, or a combination of the two. Future studies might consider including an appropriate baseline condition (e.g. reading non-emotional control sentences) to allow a comparison between each of the two emotion conditions (Social and Basic emotions) and baseline to further explore this question.

5.4.3 Age-related fMRI effects

There was an age-related decrease in activity within dmPFC during Social vs. Basic emotion processing that was unrelated to puberty hormone concentration. This finding replicates the results from the previous study using this paradigm, which also showed an age-related decrease in dmPFC activity during Social vs. Basic emotion processing at very similar coordinates (peak voxel in the current study [-16 50 22]; peak voxel in previous study [-16 42 20]; Burnett et al., 2009). It is notable that an effect of chronological age was observed in the current study despite the very narrow age (11–13 years) range of the current sample; in the previous study, the age range was much wider (11–32 years). This adolescent decrease in dmPFC activity during social cognition tasks seems to be a robust finding in the literature: an age-associated decrease in activity within dmPFC during adolescence has been reported in nine developmental fMRI studies that have used a variety of mentalising tasks (for meta-analyses see Blakemore, 2008, 2010). Recently, Gunther Moor et al. showed evidence that age-related differences in mPFC activity during mentalising were maximal in the transition from early adolescence (10-12 years) to mid-adolescence (14-16 years) in

males and females, stabilising thereafter (Gunther Moor et al., 2011). Therefore, it is possible that the early adolescent age range of the current sample maximized the study's power to detect differences within a narrow age range.

There are a number of developmental mechanisms that could underlie this pattern of findings, and the evidence presented here cannot distinguish amongst these. An age-related shift in the cognitive strategy for social emotion processing might underlie the differences seen in dmPFC, which is thought to represent the mental states of self and other (Amodio & Frith, 2006). A number of studies have shown development during adolescence in behaviour during on-line or strategic social cognition tasks where participants have to take into account another's mental state, either automatically or strategically (Dumontheil et al., 2010; Güroglu et al., 2009). Alternatively, or in addition, BOLD signal change in this region could be due to age-dependent neuroanatomical maturation or neurovascular change (Andersen et al., 2002; Attwell et al., 2010). Recent theoretical reviews have proposed that the cognitive operations subserved by the dorsal PFC mature with age, where age is shorthand for experience (Casey et al., 2010). According to this theory, more neurocognitive effort, requiring the recruitment of more extensive neural components, is needed to perform PFC-based cognitive operations at a younger age (Durston et al., 2006). The age-associated decrease within dmPFC that we observed was not related to puberty hormonal levels. Therefore, it is possible that decreases in dmPFC activity are driven by social experience, or the length of time an individual has been involved in social interactions. However, it should be noted that important life transitions such as puberty might cause abrupt changes in the rate of accumulation of social experience. Further work is needed to understand these complex relationships. The current set of findings suggests that changes during adolescence in social brain activity are not under the control of a single system. Instead, these changes may be differentially related to the effects of age and puberty, and may have multiply-specified biological and environmental drivers.

5.5 Conclusion

We found evidence for a relationship in the ATC between puberty and the neural correlates of social emotion processing, independent of chronological age. Age, independent of puberty, was associated with activity in the dmPFC during Social relative to Basic emotion processing. The current study presents the first evidence of a functional dissociation between puberty status and age in early adolescence on activity within the mentalising network.

5.6 Next chapter

The next chapter goes on to explore changes in functional connectivity with puberty and age using the social emotion processing task outlined in this chapter.

CHAPTER 6

Increased functional connectivity with puberty in the mentalising network involved in social emotion processing

There is growing evidence that puberty plays an important role in the structural and functional brain development seen in adolescence, but little is known of the pubertal influence on changes in functional connectivity. This chapter explores how pubertal indicators relate to functional connectivity between components of the mentalising network during a social emotion task. To investigate this, in this chapter, the fMRI data from Chapter 5 were reanalysed using psychophysiological interaction (PPI) analysis.

There was increased functional connectivity between the mPFC and the right pSTS and TPJ during Social relative to Basic emotion processing. Moreover, increasing oestradiol concentrations were associated with increased functional connectivity between the mPFC and the right TPJ during Social relative to Basic emotion processing, independent of age. Analysis of the PPI data by phenotypic pubertal status showed that more advanced puberty stage was associated with enhanced functional connectivity between the mPFC and left ATC during Social relative to Basic emotion processing, also independent of age. These results suggest increased functional maturation of the social brain network with the advancement of puberty in girls.

6.1 Introduction

Chapter 5 investigated the effects of puberty on the BOLD signal within the mentalising network and showed functionally dissociable effects of pubertal hormones and chronological age. Several neuroimaging studies of mentalising have found a similar *age-related* decrease in mPFC activity during adolescence into adulthood (Burnett et al., 2009; Gunther Moor et al., 2011; Pfeifer et al., 2009; Wang et al., 2006). These studies often report age-related increases in activity in other parts of the mentalising network (pSTS/TPJ and ATC) during the same period. Despite this literature detailing conventional fMRI analyses of mentalising in adolescence, and the evidence provided in Chapter 5 for role of puberty in the development of mentalising, no previous studies have investigated how puberty influences connectivity between brain regions within the social brain network. The aim of the current study was to examine the effects of puberty on *functional connectivity* within a mentalising network engaged in social emotion processing.

Developmental changes in functional connectivity – that is, correlated activity between brain regions during "resting state", or during one psychological task relative to another – have been reported in a growing number of studies. Time perception, spelling and scene retrieval studies show age-related increases between childhood and adulthood in functional connectivity between regions focally associated with task performance (Booth, Mehdiratta, Burman, & Bitan, 2008; Ofen, Chai, Schuil, Whitfield-Gabrieli, & Gabrieli, 2012; Smith et al., 2011). Successful response inhibition, as assessed using the Go/No-go task, shows patterns of both increasing and decreasing functional connectivity with age in adolescence, depending on the particular region and the functional network examined (Keulers et al., 2012; Stevens et al., 2007). In a study that employed a probabilistic learning task, increasing age during adolescence was associated with increased functional connectivity between the striatum and medial prefrontal cortex during positive relative to negative performance feedback (van den Bos, Cohen, Kahnt, & Crone, 2011).

In “resting-state” functional connectivity studies, which often examine large-scale connectivity patterns across the brain, a consistent finding is that functional connectivity between spatially distant, functionally-related brain regions increases between childhood and adulthood, while connectivity between more spatially proximal regions decreases (e.g. Dosenbach et al., 2010; Fair et al., 2008, 2009; Qin, Young, Supekar, Uddin, & Menon, 2012; for a review, see Vogel, Power, Petersen, & Schlaggar, 2010). Resting-state functional connectivity is modulated by serotonin transporter genotype (Wiggins et al., 2012), stress-induced activation of the hypothalamic-pituitary-adrenal axis (Thomason, Hamilton, & Gotlib, 2011)and oestrogen level in adult females (Ottowitz, Derro, et al., 2008; Ottowitz, Siedlecki, et al., 2008).

Relatively few developmental studies have examined functional connectivity during social cognition tasks. Studies investigating face processing show evidence for age-related increases in functional connectivity in the core face processing network between childhood and adulthood (Cohen Kadosh, Cohen Kadosh, Dick, & Johnson, 2011), and in networks mediating the impact of prior expectations on the processing of emotional faces between adolescence and adulthood (Barbalat, Bazargani, & Blakemore, 2013). A longitudinal fMRI study showed adolescent age-related increases in functional connectivity between action observation and social brain regions during observation of angry vs. neutral hand gestures in males but not in females (Shaw, Grosbras, Leonard, Pike, & Paus, 2011).

A previous developmental functional connectivity study conducted in the same laboratory as the current study showed an age-related *decrease* between adolescence and adulthood in task-dependent functional connectivity between the mPFC and pSTS⁄TPJ (Burnett & Blakemore, 2009). Neither this nor other developmental studies have explored potential relationships between puberty measures and functional connectivity during social cognitive tasks. In the previous developmental functional connectivity study outlined above (Burnett & Blakemore, 2009) no pubertal measures were acquired; thus it is unknown whether functional connectivity between regions within this mentalising network is influenced by pubertal development. However, it is

increasingly recognized that pubertal hormones organise structural brain connectivity in humans (Peper, van den Heuvel, Mandl, Pol, & van Honk, 2011). Given evidence for gender-specific patterns of adolescent functional connectivity during social cognition (Shaw et al., 2011), and evidence for an impact of female gonadal hormones on functional connectivity in adults, investigating this relationship could be fruitful.

The current study used a psycho-physiological interaction (PPI) analysis to explore functional connectivity between the mPFC and the other regions of a mentalising network identified to be engaged in social emotion processing by prior work from the same laboratory (Burnett & Blakemore, 2009), using the dataset described in **Chapter 5**. PPI analyses examine the association between BOLD signal in particular brain regions during a particular psychological context (experimental condition) and compared this to the association between BOLD signal in the same regions during a different psychological context (Friston et al., 1997; O'Reilly, Woolrich, Behrens, Smith, & Johansen-Berg, 2012). For this study, the mPFC served as the source region, based on its importance in a previous PPI analysis of the same task in a different sample (Burnett & Blakemore, 2009), and due to its acknowledged role in mentalising (Amodio & Frith, 2006). This study used a subset of the data described in **Chapter 5**, and assessed puberty based on hormonal measures and phenotypic pubertal stage, as described in this previous chapter. The analyses explored how functional connectivity within a well-defined mentalising network known to be engaged in social emotion processing was related to these measures of puberty.

6.2 Methods

6.2.1 Participants

The participants for this study are the same as those described in **Chapter 5**. Data are reported for 35 participants in the PPI analysis (mean age: 12.6 years; S.D. 0.7), after exclusion of 7 participants who showed no significant activation cluster in mPFC (see PPI methods below). Details of exclusion criteria, consent procedures and ethical approval are outlined in **Section 5.2.1**. See **Table 6.1** for participant demographics.

	Whole group		Puberty groups		
	(N=35)		Early (N=17)	Late (N=18)	Difference between group means
	Mean (S.D.) Range		Mean (S.D.) Range	Mean (S.D.) Range	
Age	12.6 (0.7) 11.1-13.7		**12.3 (0.7)** **11.1-13.5**	**12.8 (0.6)** **11.9-13.7**	**t_{34} = 2.57** **p=0.015**
BMI* (N=34)	19.3 (3.0) 13.5-27.3		19.7 (3.6) 15.4-27.3	19.0 (2.3) 13.5-23.9	t_{33} = 0.687 p=0.497
vIQ	120.4 (11.9) 89-143		117.4 (14.2) 89-139	123.3 (8.7) 107-143	t_{34} = 1.49 p=0.147
Testosterone (N=34)	61.2 (23.7) 28.1 –148.3		54.1 (12.5) 30.9 – 78.3	67.5 (29.3) 28.0 – 148.3	t_{33} = 1.77 p=0.090
Oestradiol (N=32)	3.78 (1.84) 1.34 – 9.86		3.37 (0.81) 2.24 – 4.98	4.13 (2.38) 1.34 – 9.86	t_{31} = 1.25 p=0.224
DHEA (N=33)	172.4 (96.7) 56.5 – 521.1		155.4 (63.0) 56.5 – 304.3	188.4 (120.1) 69.4 – 521.1	t_{32} = 1.00 p=0.329
Tanner stage Breast	3.4 (1.1) 2-5		**2.5 (0.5)** **2-3**	**4.2 (0.6)** **3-5**	**t_{34} = 8.83** **p<0.001**
Tanner stage Pubic Hair	3.2 (1.1) 1-5		**2.3 (0.8)** **1-3**	**4.1 (0.6)** **3-5**	**t_{34} = 7.37** **p<0.001**

Table 6.1: Participant demographics. *Included indices are Mean, S.D. and Range of participants for age, BMI, vIQ, pubertal hormone levels and Tanner stage for the whole group (N=35) and for the Early and Late puberty groups separately. Significant differences (p<0.05) between puberty groups* ***in bold****. Age was co-varied out of subsequent analyses. *One girl (Early puberty group) was not measured for height.*

6.2.2 Endocrine assessments

Full details of the endocrine data collected for the study participants can be found in **Section 5.2.2**. There was an insufficient sample collected for one subject for analysis of either DHEA or oestradiol, and an insufficient sample in a second participant for oestradiol only. These missing data from the saliva samples provided the following numbers for the PPI analyses with puberty hormone levels: testosterone N=34;

oestradiol N=32; DHEA N=33. The Early and Late puberty groups in this subgroup included in the PPI analysis differed significantly with age (see **Table 6.1**); therefore, we included age as a covariate in all analytical models.

6.2.3 Analysis of functional connectivity (PPI)

Details of the fMRI task used, together with the scanning parameters and data acquisition are described **Section 5.2**. Functional connectivity analyses are based on the principal that, if BOLD signal in one region (area A) correlates with BOLD signal in another region (area B), then the strength of the regression reflects functional coherence between the two areas. If the strength of the regression varies with the psychological context in which the physiological activity is measured then this is evidence for a psychophysiological interaction (PPI; Friston et al., 1997). In PPI analysis, a brain region of interest is defined as the physiological source. In this study, PPI analysis was used to estimate functional connectivity within a mentalising network identified to be engaged in social emotion processing by prior work from the same laboratory (Burnett & Blakemore, 2009) between mPFC (source region) and other social brain regions (pSTS, TPJ and ATC), during the Social vs. Basic emotion contrast. Consequently, activity within mPFC served as the physiological regressor in the PPI analysis, whilst emotion condition (Social vs. Basic) was the psychological regressor. A third regressor in the analysis represented the interaction between the first and second regressors.

The mPFC source region for the current study was defined based on the results of a previous study performed in the same laboratory (Burnett & Blakemore, 2009; see also Gilbert et al., 2006) and in order to include data from as many participants as possible in the analysis, the source region was increased in size in keeping with the mPFC definition used in a meta-analysis by Amodio & Frith (2006). This resulted in the following mPFC source region: -20 to +20 on the x-axis, +35 to +65 on the y-axis, and -10 to +40 on the z-axis. This source region also falls within the range of mPFC activity observed in meta-analysis of mentalising regions by Van Overwalle & Baetens (2009). In each single-subject t-contrast map for the emotion contrast (Social vs. Basic),

thresholded at $p<0.005$ uncorrected, the nearest local maximum to the centre of this volume was located. We created a spherical volume of interest (VOI) of radius 8mm centred on the single-subject peak. If there was no significantly active cluster within mPFC at this threshold (N=10), we lowered the threshold to $p<0.01$ uncorrected. Seven datasets that did not contain a peak within the defined mPFC volume at this significance level were excluded (four Early puberty, three Late puberty), leaving 17 Early puberty and 18 Late puberty participants (see **Table 6.1** for details) in subsequent PPI analyses. Individual participant peaks were distributed evenly around the centre of the mPFC source region and no differences in x, y, and z coordinates were observed between Early and Late puberty groups ($t<1$). Finally, we extracted the BOLD signal time series from each participant's VOI in the mPFC.

Voxel-wise PPI analysis was conducted at the combined group level (N=35), in order to identify target social brain regions of interest (pSTS/TPJ and ATC) that showed a significant increase in functional coupling with mPFC during Social vs. Basic emotion. Small volume corrections (SVC) were conducted on spheres with radius 12mm centred on peaks in pSTS/TPJ and ATC, using the same method as reported in the previous PPI study of social emotion processing from the same lab (Burnett & Blakemore, 2009). A partial correlation was then performed between puberty hormone levels and the PPI between mPFC activity and Social vs. Basic emotion, co-varying for participant age (N=32). PPIs for Social vs. Basic emotions between the two puberty groups were then compared, controlling for participant age. Finally, age related changes in PPI were analysed, incorporating puberty status as a covariate of no interest (N=35).

6.2.4 Control analysis: head motion

Since head motion can result in spurious activation patterns in (resting state) functional connectivity studies (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012), an analysis was performed to ascertain whether motion was related to age, puberty grouping or hormone level. There was no significant difference between mean movement during the Social and Basic conditions for either translational or rotational movement (translation: $t=0.09$, $p>0.9$; rotation: $t=0.19$, $p>0.8$)). In addition, there was

no significant correlation between Social vs. Basic mean movement and age (for translation, r=0.22, p>0.2; for rotation, r=0.20, p>0.2). There was no significant interaction between puberty group and movement in the Social vs. Basic condition (translation: t=0.09, p>0.6; rotation t=-0.02, p>0.9), and no significant correlation between hormones and movement in the Social vs. Basic condition (for translation: Testosterone, r=0.03, p>0.8; Oestradiol, r=0.14, p>0.4; DHEA, r=-0.09, p>0.6. for rotation: Testosterone, r=-0.04, p>0.8; Oestradiol, r=0.10, p>0.5; DHEA, r=-0.06, p>0.7).

6.3 Results

6.3.1 PPI across all participants

Across the entire group of participants, PPI analysis revealed that the right pSTS and right TPJ showed greater functional connectivity with the mPFC during Social vs. Basic emotions (**Table 6.4** and **Figure 6.1**). Whole brain analyses with family wise error correction thresholded at p<0.05 revealed one significant cluster outside the *a priori* regions of interest in the left superior occipital gyrus (see **Table 6.4**).

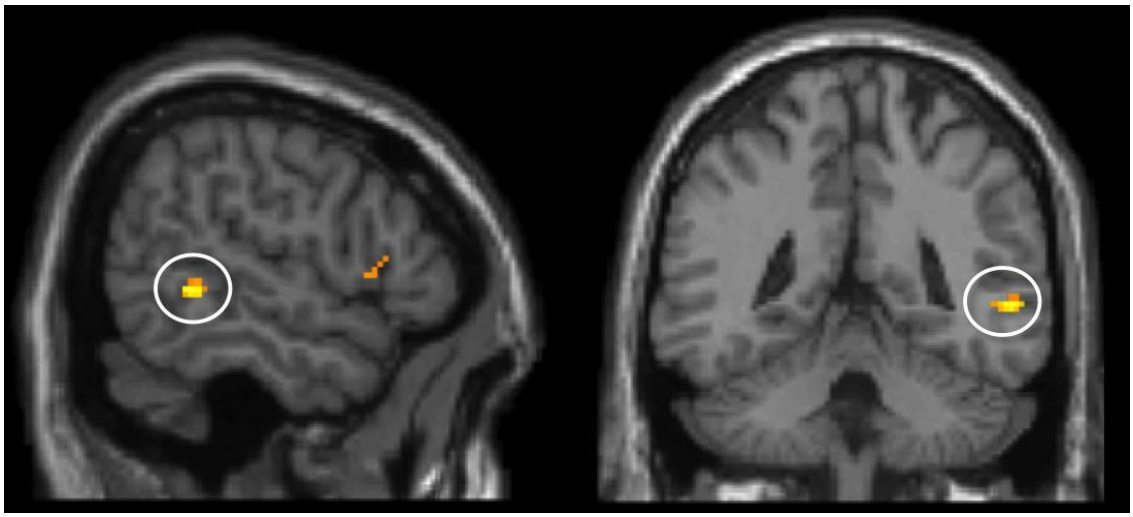


Figure 6.1: PPI results for all participants. *Significant interaction between emotion (Social vs. Basic) and mPFC BOLD signal in the right pSTS [56, -44, 0], shown at p< 0.001 projected onto sagittal and coronal T1 images (at x = 56 and y = -44).*

6.3.2 PPI between hormones and emotion

There was increasing connectivity between the mPFC and the right TPJ for Social vs. Basic emotions with increasing levels of oestradiol (see **Table 6.4** and **Figure 6.2**). Repeating the analysis without the two participants with the highest oestradiol levels (potential outliers) did not change the significance of the results.

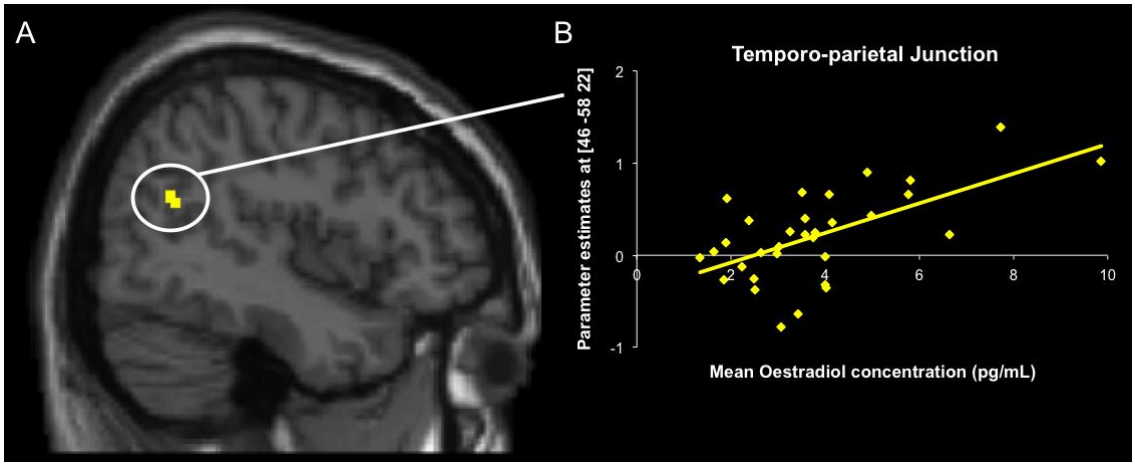


Figure 6.2: Interaction between oestradiol levels, emotion and mPFC BOLD signal in the right TPJ (peak MNI coordinate: [46 -58 22]), co-varying for age. *(a) Significant cluster shown at p< 0.001 projected onto sagittal T1 image at x = 46. (b) Graph showing PPI β weights extracted for the peak voxel (based on the mPFC timeseries x Social vs. Basic emotion; y-axis) as a function of oestradiol levels (x-axis).*

6.3.3 PPI between puberty group and emotion

There was a significant interaction between puberty group (Late vs. Early puberty) and the PPI between mPFC and left ATC in Social vs. Basic emotion (**Table 6.4** and **Figure 6.3**). The Late puberty group showed increased functional connectivity between the mPFC and the left ATC compared with the Early puberty group, independent of age (see **Table 6.4**). For the reverse contrast (Early vs. Late puberty), there were no significant interactions in pSTS/TPJ or ATC.

For all PPI analyses, the results did not change after controlling for vIQ, except for one minor change in the size and position of the left ATC cluster in the Late vs. Early puberty analysis (6 voxels centred around [-50, 12, -28], instead of 4 voxels at [-50, 14, -28]).

Interaction	Region	MNI cords.	Z	No. of voxels
a. Whole group				
Across all participants	R pSTS	56 -44 0	4.75	55
		48 -40 0	3.88	
	R TPJ	44 -52 22	3.25	2
	L superior occipital gyrus*	-12 -94 8	5.48	9
b. Hormonal regression				
Oestradiol	R TPJ	46 -58 22	3.51	16
c. Puberty groups				
Late puberty vs. Early puberty	L ATC	-50 14 -28	3.25	4

Table 6.2: Summary of significant PPI results. *MNI coordinates, Z-values and cluster size for brain regions expressing a psychophysiological interaction between activity within the mPFC and Social vs. Basic emotion condition: (a) across all subjects (N=35); (b) with oestradiol, after co-varying for age; (c) with puberty group (Late puberty vs. Early puberty), co-varying for age.*

All results thresholded at p<0.001, and all reported regions survive SVC (12mm sphere) except (*) which survives whole-brain FWE correction thresholded at p<0.05.

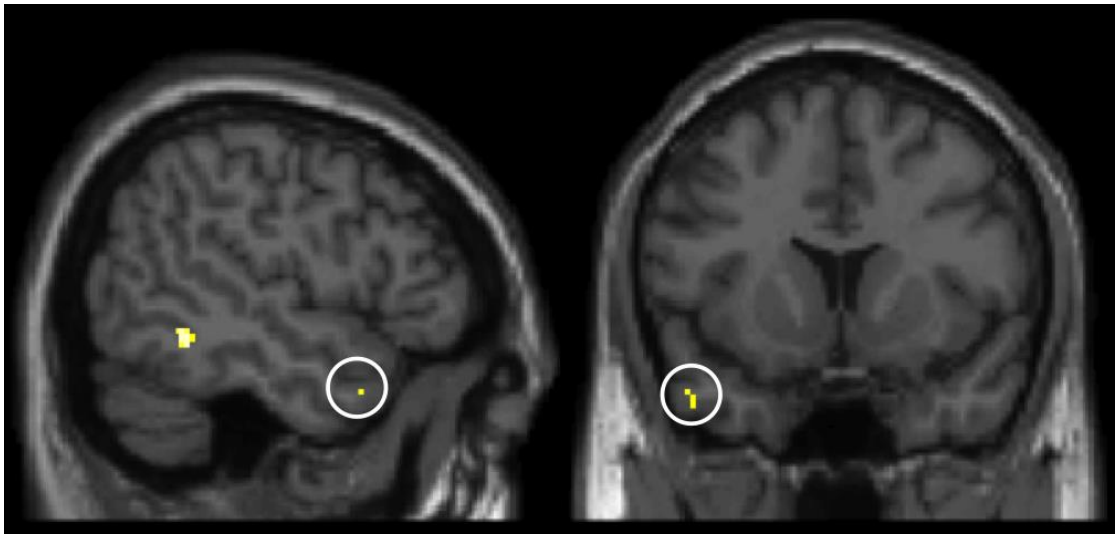


Figure 6.3: Significant PPI results for Late vs. Early puberty group*. A cluster in the left ATC [-50, 14, -28] showed a significant interaction between group (Early vs. Late puberty), emotion (Social vs. Basic) and BOLD signal in the mPFC (after co-varying for age). Shown at p<0.001.*

6.3.4 PPI between age and emotion

No interactions between age and connectivity between mPFC and the *a priori* regions of interest were found. No significant interactions were observed in any brain region at FWE corrected thresholds.

6.4 Discussion

The current study investigated how functional connectivity between regions within a mentalising network identified to be engaged in social emotion processing by prior work (Burnett & Blakemore, 2009) is influenced by pubertal stage and puberty hormone levels in girls. Increasing oestradiol concentrations were associated with enhanced functional connectivity between the mPFC and the right TPJ during Social vs. Basic emotion processing, independent of age. When the PPI data were analysed by phenotypic pubertal status, more advanced puberty stage was associated with enhanced functional connectivity between the mPFC and left ATC during Social vs. Basic emotion processing, also independent of age.

The PPI analysis performed in this study found evidence of functional connectivity between mPFC and parts of the mentalising network during social emotion processing. Specifically, there was increased functional connectivity between mPFC and the right pSTS and right TPJ during Social vs. Basic emotion processing. Furthermore, the analysis found that with increasing pubertal indicators (independent of age), connectivity between the mPFC and the left ATC (with development in phenotypic puberty stage), and between the mPFC and right TPJ (with increasing oestradiol) increased during Social vs. Basic emotion processing. The mechanism by which this increase in connectivity might emerge is not yet clear. Direct anatomical connections between brain regions of the social brain network might account for the observed functional connectivity. In the macaque brain, direct connections exist between mPFC and both STS/TPJ and ATC (Bachevalier, Meunier, Lu, & Ungerleider, 1997; Barbas, Ghashghaei, Dombrowski, & Rempel-Clower, 1999). In human adolescence, fronto-temporal white matter integrity has been found to be associated with self-reported

puberty stage (Asato et al., 2010; see **Chapter 4**), and the relationship between functional and structural (i.e. anatomical) connectivity increases from childhood into adolescence (Hagmann et al., 2010). Therefore, the pubertal and puberty hormone-related increases in functional connectivity observed in the current study might result from hormonal effects on white matter structure and function (cf. Peper, Hulshoff Pol, et al., 2011).

Whilst changes in white matter interregional connections could contribute to changes in functional connectivity, previous studies have shown that this direct mechanism is unlikely to account for the complete picture (Hagmann et al., 2010). Indeed, neurochemical modulation and changes in synaptic physiology may also play a significant role (Uhlhaas et al., 2009). Oestrogens and other steroid hormones have been shown to exhibit neuromodulatory properties (Saldanha, Remage-Healey, & Schlinger, 2011; Srivastava & Penzes, 2011; Zehr et al., 2008), influencing synaptic plasticity and connectivity in cortical and subcortical brain regions. Changes in circulating oestradiol, as measured in the current study, might therefore affect functional connectivity via changes in synaptic physiology. Consistent with this account, a previous study revealed an inverse relationship between circulating oestradiol and prefrontal, parietal and middle temporal grey matter volume in adolescent females (Peper et al., 2009).

Besides the influence of pubertal changes in grey and white matter on the observed increases in functional connectivity within the mentalising network, the connectivity in the current study may have been mediated by other regions beyond this network (Friston et al., 1997). Since we restricted the analysis to the mentalising network, we might have missed such a common input to the mPFC, TPJ and ATC. Furthermore, we stress that the results of the PPI analysis differ fundamentally from both structural connectivity analysis and conventional task-related fMRI analysis. PPI analysis provides information regarding task-dependent changes in the relationship between two brain regions (Friston et al., 1997; O'Reilly et al., 2012). This relationship does not have to correspond to structural connections between these regions or be restricted to regions found active in conventional task-related analysis.

In this study, there was no demonstrable relationship between levels of circulating testosterone or DHEA and functional connectivity during the social emotion task. Whilst increases in sex hormones during puberty are correlated, they have demonstrably different effects throughout the body depending on the location and role of specific hormone receptors. A recent review (Peper, van den Heuvel, et al., 2011) highlighted the differential effects of steroid hormones on connectivity in the brain, which may relate to receptor distribution, enzyme location or differences in hormone properties.

The results of this study describe differential correlations between oestradiol and phenotypic puberty status and functional connectivity. Whilst both hormone level and puberty status are measures of pubertal development, they focus on different aspects of the developmental process. Phenotypic puberty stage (Tanner stage) provides an estimation of the time since the activation of the HPG axis in an individual (Bordini & Rosenfield, 2011), and therefore the cumulative exposure to sex steroid hormone levels above the pre-pubertal baseline. In contrast, salivary hormonal measurements provide an accurate indication of systemic concentrations at the time of sampling and therefore salivary hormones give an indication of current exposure. The contrasting results emphasise the need to consider differing views of pubertal development, since absolute and relative hormone exposure may have differing, perhaps complementary, effects on brain development.

6.4.1 Limitations

Several limitations of the study should be noted. First, not all participants from the original sample could be included in the PPI analyses (Chapter 5), due to the lack of a significant activation cluster in the mPFC or missing hormonal data. The initial threshold for the individual mPFC activations was lowered and the search space increased, which resulted in the maximum number of eligible PPI participants from the original sample. Although all reported analyses included a minimum of 32 participants, using a larger sample size might reveal relationships between levels of testosterone or DHEA and functional connectivity during the social emotion task that were not

observed in the current study. The comparison of the two puberty groups in particular needs replication with larger sample sizes, given the small size of the cluster observed in the ATC. Furthermore, the absence of significant activation in the mPFC in some of the participants suggests that tasks with more robust activation patterns should be considered for future studies using task-related connectivity analyses.

Second, this study was limited to investigation of pubertal effects on functional connectivity in the social brain in girls. Only female participants were included because sex differences are observed in pubertal timing (Spear, 2000) in neural maturation of grey matter in early adolescence (Giedd et al., 1999), including in the social brain (Mills, Lalonde, Clasen, Giedd, & Blakemore, 2012), and in activation during social and emotional tasks (Schulte-Rüther, Markowitsch, Shah, Fink, & Piefke, 2008; Whittle, Yücel, Yap, & Allen, 2011). It would be interesting for future studies to investigate the effect of puberty stage and puberty hormones on functional connectivity during social emotion processing in boys.

Third, there was no attempt to control for menstrual cycle phase in the current study. It is complex to accurately assess menstrual cycle phase in young people who are undergoing puberty, and who therefore may have recently become menarcheal, or may be about to experience menarche. Cyclical hormone release begins prior to menarche, and many girls have irregular cycles for up to two years following menarche, with many of these cycles being anovulatory (Bordini & Rosenfield, 2011; WHO Task Force on Adolescent Reproductive Health, 1986). Therefore, using self-report of menstrual cycle may not correlate with hormonal patterns well in this population, precluding us from controlling for cycling of hormone levels in the participants within a cross-sectional design.

Finally, this study focused on functional connectivity within a mentalising network identified as being engaged in social emotion processing by prior work from the same laboratory. Future studies are needed to see whether similar increases in functional connectivity with puberty are also observed during other (non-emotional) mentalising tasks or during tasks designed to study basic instead of social emotion processing.

6.5 Conclusion

This study found that with increasing pubertal stage (independent of age), connectivity between the mPFC and the left ATC increased during Social vs. Basic emotion processing, and that with increasing oestradiol concentrations (also independent of age) connectivity increased between the mPFC and the right TPJ during Social vs. Basic emotion processing. Together, these results can be interpreted to show increasing long distance connectivity between regions of a mentalising network identified to be engaged in social emotion processing with advancing puberty in girls.

6.6 Next Chapter

Chapters 5 and 6 have focussed on social emotion processing and its functional development in girls during puberty. The next chapter explores a different behaviour typically associated with adolescence, risky decision-making, and investigates how developmental changes in brain function when performing a risk-taking task were related to puberty, independent of chronological age in boys.

CHAPTER 7

The relationship between pubertal status and neural activity in reward processing and cognitive control regions during risky decision-making

During adolescence, risk-taking emerges as an important behaviour. It has been hypothesised that this behaviour results from a dissociation in the developmental timing of the two different networks in the brain involved in reward-processing and cognitive control. The aim of the fMRI study described in this Chapter was to explore how developmental changes in brain function when performing a risk-taking task were related to puberty, independently of chronological age.

50 male participants aged 12-14 years underwent fMRI scanning whilst performing a risky decision-making task. Self-reported pubertal stage was used to assign boys to Early and Late puberty groups, and salivary sex steroid hormone levels were assessed. Oestradiol levels were positively correlated with neural activity in the ventromedial prefrontal cortex and orbitofrontal cortex when deciding not to make a risky choice. Testosterone levels and puberty grouping (Early vs. Late) were correlated with prefrontal cortex activation during the processing of outcomes of a risky decision. This study highlights the complexity of the decision-making process, and the extensive network of brain regions involved. It suggests that various aspects of the process of deciding to make a risky decision are related to distinct elements of pubertal development, and that some of the brain changes seen during this time may not be directly related to puberty.

7.1 Introduction

As outlined in Chapter 1, the increased propensity for adolescents to take risks is one of the key elements of stereotypical adolescent behaviour. With the advancement of the field of adolescent cognitive neuroscience, a prominent neurobiological theory has been put forward to explain the emergence of this increased tendency towards risky behaviour in adolescence (Casey et al., 2008; Steinberg, 2008). This 'dual systems' model of adolescent brain development hypothesises that the propensity for risk-taking results from a mismatch in the developmental timing of the different neural systems involved in decision-making (see **Section 1.3.2**). The model is based on the premise that the brain regions involved in cognitive control develop gradually throughout adolescence, while the brain regions responsible for reward processing mature relatively early in adolescence, in tandem with puberty. The relative 'mismatch' in maturational timing is hypothesised to underlie the risk-taking behaviour that is seen in adolescents (see **Figure 1.9**), since they are therefore more biased towards reward-seeking choices (Casey et al., 2008; Steinberg, 2008). This model has proved appealing across a variety of disciplines, and there has been widespread use of this model as a means of explaining adolescent risk-taking behaviour. Importantly, the neuroimaging evidence for this model is still limited and conflicting (see **Section 1.8.2**), and recent reviews of the literature have cautioned that the data suggest a more complex picture of development than the dual systems model proposes (Crone & Dahl, 2012; Pfeifer & Allen, 2012), and call for further studies to better describe the developmental trajectories and relationships of the various brain regions.

One proposed explanation behind this hypothesised differential timing of development of the reward processing and cognitive control regions lies in the role of puberty in brain maturation (Smith, Chein, & Steinberg, 2013). The development of reward salience and sensation-seeking have been linked to pubertal maturation, both in terms of behaviour (Forbes & Dahl, 2010; Martin et al., 2002; Smith et al., 2013; Steinberg et al., 2008) and in terms of brain development (Blakemore et al., 2010; Geier, 2013; Ladouceur et al., 2012; Sisk & Foster, 2004; Sisk & Zehr, 2005; Teicher,

Andersen, Polcari, Anderson, & Navalta, 2002). In contrast, the gradual development of the PFC and markers of cognitive control have been described as continuing throughout adolescence (Asato, Sweeney, & Luna, 2006; Bunge & Wright, 2007; Crone & Dahl, 2012; Geier, 2013; Luna & Sweeney, 2001; Pfeifer & Allen, 2012; Smith et al., 2013).

At the time of designing this study, only two studies had set out to specifically address this theory by investigating the differences in reward processing with puberty (Forbes et al., 2010; Op de Macks et al., 2011; see **Section 1.8.3**). Both studies found significant correlations between testosterone and BOLD signal within the reward network, although the specific regions and relationships differed. Forbes and colleagues (2010) found that adolescents aged 11-13 years at more advanced stages of puberty showed less caudate activation and greater rostral mPFC activation when processing reward outcome compared to their less mature, age-matched peers (Forbes et al., 2010). They additionally reported correlations between levels of BOLD signal in the VS and testosterone levels, whereby testosterone was positively correlated with BOLD signal during reward anticipation in males, and negatively correlated with BOLD signal during reward outcome in males and females (Forbes et al., 2010). Contrastingly, Op de Macks and colleagues (2011) showed a positive correlation between testosterone levels and VS activation during reward outcome processing for both males and females (Op de Macks et al., 2011). To date, no published studies have looked at the relationship between puberty and cognitive control processing during a risky decision-making task (Smith et al., 2013).

7.1.1 The current study

The aim of the current study was to investigate the effects of puberty on brain activity during a risky decision-making task in regions identified as being involved in reward processing or cognitive control in the dual systems model of adolescent risk-taking. The task used was the Balloon Analog Risk-taking Task (BART; adapted from Lejuez et al., 2002), which has been shown to correlate to real-life risk-taking behaviours in adolescent populations (Lejuez et al., 2007; MacPherson, Magidson, Reynolds, Kahler,

& Lejuez, 2010; Vaca et al., 2013). As with the cross-sectional studies in previous chapters, to minimise the co-linearity between age and pubertal development in our sample, participants were recruited from a narrow age range (12.7-14.3 years) during which individuals can be at an stage of puberty as part of natural variation (see **Figure 2.2**). As noted in **Section 2.1.2**, including both male and female participants in studies of pubertal development can result in confounding results due to the different hormonal regulation of puberty between the sexes, as well as the different chronological ages at which puberty occurs. This study investigated only male participants, since many of the health risk behaviours associated with adolescence including alcohol and drug use, violence and crime have been shown to be more prevalent in males.

In accordance with the dual systems model, the principal hypothesis for this study was that pubertal status would be related to levels of BOLD signal activity during the BART task in *a priori* regions associated with reward processing, but not in regions associated with cognitive control (Smith et al., 2013; Steinberg, 2008). Identifying *a priori* ROIs for reward processing and cognitive control is complex since there is a lack of consensus regarding specific brain regions in the published literature. This is likely due to the different types of tasks used, differential focus of the analyses and different populations of interest. To account for this, a priori ROIs were divided as follows:

a. Subcortical reward processing areas: the ventral striatum (VS), consisting of the NA, caudate and putamen. The VS is consistently activated in adolescents when receiving rewards (for review, see (Crone & Dahl, 2012), and is known to underpin affective processing and motivational salience.

b. Cortical regions associated with reward value: ventromedial PFC (vmPFC) and OFC. The position of these regions within the dual systems model is unclear since they have been interpreted as being part of the reward processing network in some studies (Chein et al., 2011; Forbes et al., 2010; Van Leijenhorst, Gunther Moor, et al., 2010), whilst others have limited reward regions to subcortical regions and incorporated all of the PFC into cognitive control regions (Casey et al., 2011; Paulsen et al., 2011; Rodrigo,

Padrón, de Vega, & Ferstl, 2014; Spear, 2013). Both the vmPFC and the OFC have been shown to be important in representing current reward values and determining the current incentive value of a behavioural outcome (Kennerley, Behrens, & Wallis, 2011; Kennerley & Walton, 2011). They are closely functionally linked to the VS (van den Bos et al., 2011), and are demonstrably important in reward processing (Gläscher et al., 2012; Kennerley & Walton, 2011), supporting their inclusion in the reward-processing 'system' of the dual systems model (Forbes et al., 2010). However, the role of the OFC and vmPFC in value interpretation to potentially influence behaviour (Eshel et al., 2007; Rushworth, Noonan, Boorman, Walton, & Behrens, 2011; Shad et al., 2011), together with the location of these regions within the PFC, has been used to categorise these regions as part of the cognitive control 'system' of the dual systems model. For the purposes of this paper, the OFC and vmPFC have been considered part of the reward-processing system, but the implications of this discordance are considered in the discussion.

c. Cortical regions associated with cognitive control: the dACC and the lateral PFC. The dACC has been implicated in the higher-level integration of information about a decision, combining information about a decision's value with information about an action's value to establish overall value (Cai & Padoa-Schioppa, 2012; Kennerley et al., 2011; Shenhav, Botvinick, & Cohen, 2013; Wallis & Kennerley, 2011). The lateral PFC is known to be involved in a number of executive control processes including response inhibition (Aron, Behrens, Smith, Frank, & Poldrack, 2007; Rubia et al., 2001) and representation of rules (Reverberi, Görgen, & Haynes, 2012). Together the ACC and the lateral PFC appear to track the history of choices and outcomes to influence future decisions and behaviour (Ridderinkhof, van den Wildenberg, Segalowitz, & Carter, 2004; Rushworth et al., 2011). The lateral PFC represents a large, functionally and anatomically heterogenous area. Previously published literature investigating decision-making tasks have reported activation across a range of lateral PFC regions with inconsistent developmental patterns (Mills, Goddings, Clasen, et al., 2014).

There were three specific hypotheses concerning brain activation during the risky decision-making task and the relationship with pubertal status:

1. During a period of actively making a decision in a risky context, there would be differences with pubertal status in the levels of BOLD signal in reward-processing regions but not in cognitive control regions of the brain.
2. There would be differences with pubertal status in the levels of neural activation when processing reward vs. loss outcomes in reward-processing but not cognitive control regions.
3. Differences would be seen with pubertal status in BOLD signal during the processing of each of the outcomes (reward, loss and choosing not to take a risk).

In addition to the fMRI predictions, additional hypotheses were made concerning behavioural differences associated with puberty. Specifically, it was predicted that pubertal status would be related to levels of risk-taking behaviour, such that boys at more advanced stages of puberty would have higher levels of sensation-seeking and risk-taking behaviour, measured both by the self-reported levels of real life behaviours by questionnaire and by behavioural performance on the BART lab-based task.

7.2 Methods

7.2.1 Participants

The sample consisted of 50 male participants aged 12.7 to 14.3 years (mean 13.6 years, S.D. 0.4 years; see **Table 7.2**). Recruitment information, together with inclusion and exclusion criteria can be found in **Chapter 2**. Participants assented to the study, and written informed consent was obtained from a parent or legal guardian. Subjects received £10 for attending the testing session, and earned a further £5-10 depending on their performance in the task (see below for details). The study was approved by the UCL Research Ethics Committee.

IQ was measured using a two subtest version of the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999). BMI was calculated for participants, except for a subset (N=13) whose height was not measured (see **Table 7.1**).

7.2.2 Endocrine assessments

Two independent indicators of pubertal development were obtained for each participant: a self-reported assessment of phenotypic pubertal stage; and salivary hormone assays for testosterone, DHEA and oestradiol; see **Chapter 2** for further details. A subset of participants (N=4) did not provide saliva samples on the day of scanning; for these participants, saliva samples were collected within a week of the MRI scanning session. As outlined in **Section 2.4.3**, participants were dichotomised into early-mid puberty (referred to as Early) and late-post puberty (referred to as Late) puberty groups on the basis of the self-report. Early and Late puberty groups differed significantly in age (see **Table 7.2**), although the difference in mean age was small at just 4 months. Therefore age was included as a covariate in subsequent between group/hormone analyses.

7.2.3 Behavioural measures

Participants completed in private three questionnaires related to their real-life behaviours and preferences. Participants were informed that the questionnaires would be anonymised and that no information would be shared with their parents, families, friends or schools. The questionnaires included the Cognitive Appraisal of Risk Events (CARE) questionnaire (Fromme, Katz, & Rivet, 1997), the Barratt Impulsiveness Scale (Patton, Stanford, & Barratt, 1995) and the Sensation-seeking scale (Zuckerman, Kolin, Price, & Zoob, 1964). Each have been previously used and validated for use in adolescent populations. The results of each questionnaire were regressed against measures of pubertal development, and against the measured performance on the BART task. All statistical analyses were performed using R (http://www.r-project.org/).

7.2.3.1 Cognitive Appraisal of Risky Events (CARE) questionnaire

Self-reported levels of real life risk-taking behaviour were assessed using an adapted version of the CARE questionnaire (Fromme et al., 1997). The questionnaire contained 31 questions covering six risk-related themes including sexual risk-taking, illicit drug use, alcohol use, aggressive or illegal behaviours, academic risk-taking and risky sports (for full questionnaire, see **Appendix 1**). Each subscale was made up of 4-8 questions.

The risky sports theme was excluded from the study since the questions were potentially biased by availability and opportunity e.g. skiing, rock-climbing. For each question, participants were asked to rate on a 7-point scale the likelihood that they would participate in the activity in the next 6 months, where 1 represents not at all likely and 7 represents extremely likely (Fromme et al., 1997). Answers were averaged for each of the five subscales. Three of the subscales (relating to questions on risky sexual activity, illicit drug use and alcohol use) showed very little variability in the study sample, with very few participants reporting any significant 'risky' activity (risky sexual activity median score 1.0, IQR 1.0-1.3; illicit drug use median score 1.0, IQR 1.0-1.2; alcohol use median score 1.2, IQR 1.0-2.0). These subscales were excluded from further analyses, leaving the aggressive or illegal behaviours and academic risk-taking subscales. The scores on these two subscales were correlated with each other ($r=0.57$, 95% C.I. 0.34, 0.74, $p<0.001$).

7.2.3.2 Barratt Impulsiveness Scale (BIS)

Impulsivity was measured using the BIS (Patton et al., 1995). The questionnaire contained 27 items, and participants were asked to rate how often they acted the way described in the statements using a 4-point scale, where 1 represented Rarely/Never, and 4 represented Usually/Always (Patton et al., 1995; for full questionnaire, see **Appendix 1**). The questions were divided into three subscales representing attentional impulsivity, motor impulsivity and non-planning impulsivity, and scores were averaged for each subscale (Patton et al., 1995).

7.2.3.3 Zuckerman Sensation-Seeking Scale (SSS)

Sensation-seeking was measured using a modified version of the SSS (Zuckerman et al., 1964, modified by Steinberg et al., 2008). The modified SSS is a six-item questionnaire with Yes/No responses e.g. 'I like doing things just for the thrill of it'; 'I'll try anything once' (for full questionnaire, see **Appendix 1**). The responses were averaged to provide a single value of sensation-seeking between 0 and 1 for each participant, where 1 is high sensation-seeking (Steinberg et al., 2008).

7.2.4 fMRI task

During the fMRI experiment, participants completed a modified version of the Balloon Analog Risk-taking Task (BART task, adapted from Lejuez et al., 2002). In this task, the participants see a series of balloons on a screen in the fMRI scanner. This task consists of two trial-types:

1. **Active decision trials**: 50% of the balloons are 'active decision' trials. In each active decision trial, the participant sees a coloured balloon on the screen that is worth £0.50 (see **Figure 7.1**). Participants choose whether to inflate the balloon or not by pressing one of two buttons. If they decide to inflate the balloon (*risky choice*), it could increase in size and value, or it could pop and the money for that balloon would be lost (see **Figure 7.1**). If the balloon successfully inflates, the participant is again given the choice to inflate it further or not. With each successful inflation, the balloon's value increases by £0.50. If the participant chooses to stop inflating the balloon, they see a tick on the screen, and the balloon's final value is saved towards the total earnings for the session.
2. **Passive decision trials**: 50% of the trials are control 'passive decision' trials. In each passive decision trial, the participant sees a balloon on the screen of a different colour to their active decision balloons (see **Figure 7.1**). These balloons are not worth any money. Participants press one of two buttons as indicated on the screen by a button labelled PRESS, and observe the outcome. They have no control over whether the balloon is stopped or inflated, nor whether, if inflated, it pops or successfully inflates.

The active and passive balloons were identifiable by colour (either yellow or green), and remained the same colour throughout the task for each participant. The balloon colours were counterbalanced between participants. The participants were trained in the task outside of the scanner, and practised before the scan.

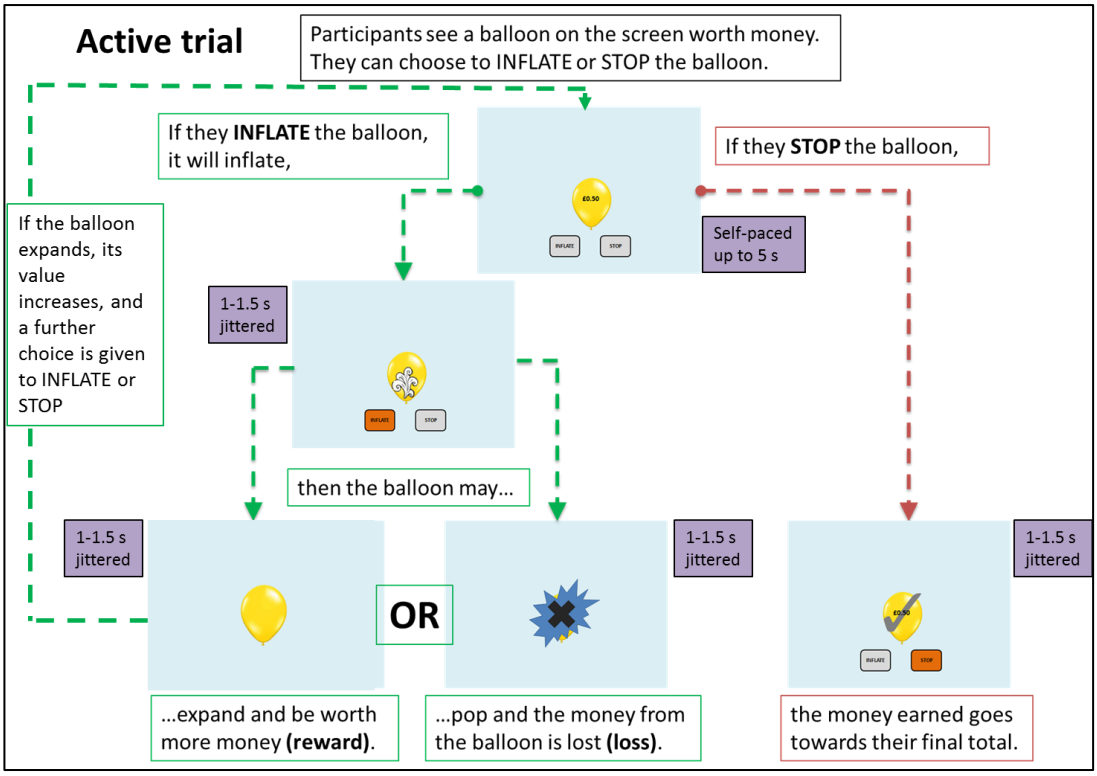
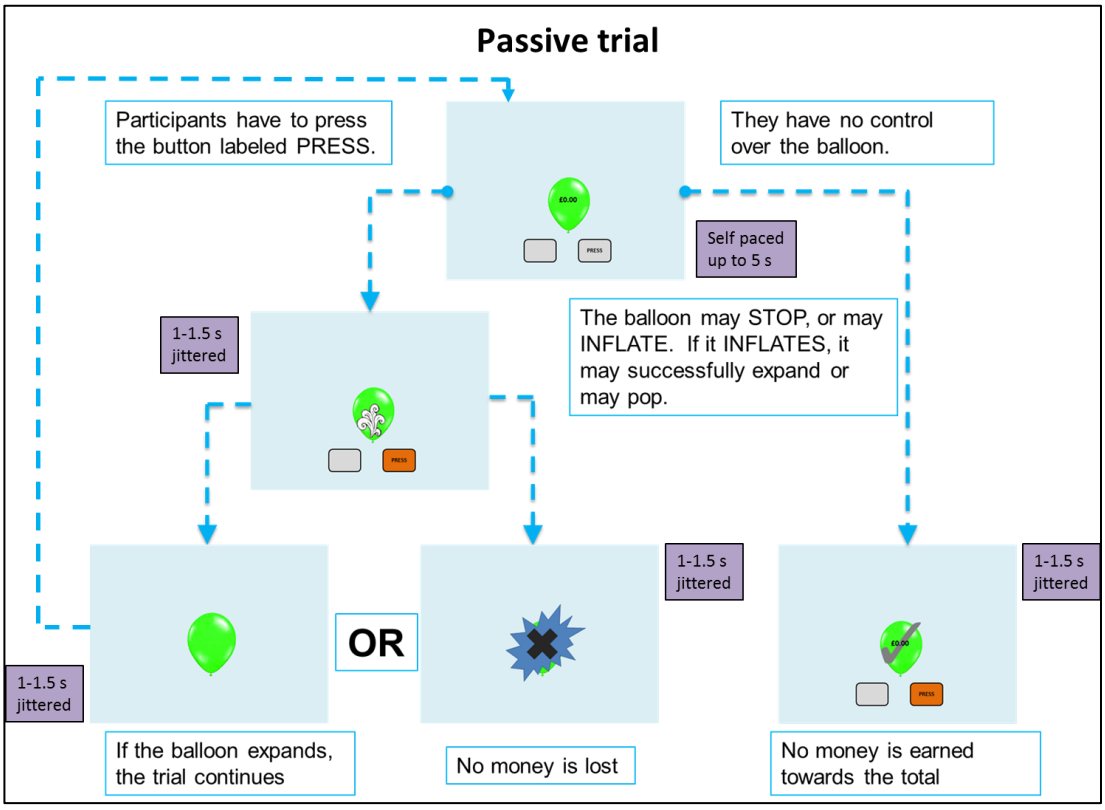


***Figure 7.1: The modified BART task**. Participants see active (above) or passive (below) trials. The decision making phase is self-paced (up to 5 s) and the inflation and outcome stages were displayed for jittered intervals of 1-1.5s. See **Section 7.2.4** for full details of task. Balloon colour was counterbalanced between participants.*



Participants completed 4 6-minute runs of the BART task. The decision phase was self-paced (up to 5 s). The outcome screens were shown for between 1.0 and 1.5 s (jittered). If the participant chose to inflate the balloon, they were shown an 'inflation' screen (1.0-1.5 s, jittered) and then were shown the outcome (a larger, intact balloon, or a popped balloon; each 1.0-1.5 s, jittered). If they chose to stop, they saw the balloon, with its value, on the screen (1.0-1.5 s, jittered). The active decision balloons were programmed to pop after 1-12 inflations (popping point) using a block randomisation method, such that within a block of 12 successive active balloon trials, there would be a trial programmed to pop at each of the 12 popping points. The passive decision balloons were programmed to end (stop or pop) when they were the same size as the active decision balloon end-point two trials earlier in the run, in order that the active and passive balloons were approximately matched in duration of trial and size of balloon seen.

7.2.5 Data acquisition

MRI scanning was performed using a 1.5T Siemens Avanto head MRI scanner with a 32-channel head coil. Head movement and scanner noise were minimised using soft cushions to support the participant's head within the head coil. The visual stimuli for the task were projected onto a screen inside the MRI scanner so that the participant could view them during the scan. T2*-weighted echo-planar (EPI) volumes with blood oxygenation level dependent (BOLD) contrast were obtained while participants performed the BART task described above. The following scanning parameters were used to acquire 127 volumes per session for a total of four sessions: TR = 2.975s; TE =50msec; 35 axial slices; in plane resolution 3x3x3mm; sequential acquisition. A 3D T1-weighted anatomical scan was also collected for reference, with the following parameters: TR = 2.73sec, TE = 3.57ms, flip angle=7^{o}, 176 slices, field of view 224x256x176mm^3. Two runs of an EPI sequence with diffusion weighted images were also acquired (see **Chapter 4** for details of the scanning parameters and data analysis). Maximum scanning duration was 50 mins per participant.

7.2.6 fMRI pre-processing

fMRI data were analysed with SPM8 (Wellcome Trust Centre for Neuroimaging; http://www.fil.ion.ucl.ac.uk/spm/). The first four volumes were discarded to allow for T1 equilibration effects. Pre-processing included rigid-body transformation (realignment) to the first analysed volume, with a second degree B-spline interpolation to correct for movement during the session, followed by slice time correction. The bias-field corrected structural image for each participant was co-registered to the mean realigned functional image and segmented using MNI registered International Consortium for Brain Mapping tissue probability maps. The spatial normalisation parameters were applied to the realigned images to obtain normalised functional images with a $3mm^3$ voxel size. These images were smoothed with a Gaussian filter of 8mm full-width at half maximum (FWHM). fMRI data were excluded for 2 participants after preprocessing due to inadequate spatial coverage for the ROIs (N=1; 13.6 years, Early puberty) and a failure in co-registration and segmentation (N=1; 13.1 years, Early puberty).

Movement was not correlated with age in our sample, but was different between Early and Late puberty groups, such that the Early puberty group moved on average more than the Late puberty group. Any sessions that had more than $3mm/3^o$ movement in any direction were removed from the analysis. If 3 or more sessions were removed for a participant, they were removed from the analysis completely (N=1; 13.3 years, Late puberty). Further movement correction was included by censoring any volumes in the GLM where the scan-to-scan movement exceeded 0.9mm across all directions (Siegel et al., 2014).

7.2.7 fMRI data analysis

Statistical analyses were performed on individual subjects' data using the general linear model (GLM) in SPM8. The four scanning sessions were treated as a single time series with covariates incorporated to model session effects. Separate GLMs were modelled for the decision-making phase of the task and the outcome phase, since these were necessarily not independent events. In the decision-making GLM, events

were modelled as events of interest with the duration from trial onset to time of making a button press. In the outcome GLM, outcomes were models as events of interest with no duration. Given that there was a significant difference in age between the puberty groups, age was modelled as a covariate in the GLMs. Behavioural performance (as measured by proportion of active balloons popped) was included as a covariate in *post hoc* GLM analyses, to ascertain whether differences in performance on the BART task was driving any significant results. Trials in which participants did not make a response were modelled as covariates of no interest. Time series for each participant were time filtered at 128 secs to remove low frequency drifts. The resulting contrast images for each participant were then submitted for second level group and regression analyses. Between-subject analyses were selected *a priori* to address the three hypotheses outlined in the Introduction (**Section 7.1.1**). The analyses were performed separately for puberty group and each of the puberty hormones.

7.2.7.1 Analysis of BOLD activation and pubertal group

To investigate the link between pubertal group and functional brain development, the following between-subject analyses were performed based on the three *a priori* hypotheses outlined in the Introduction:

1. During a period of actively making a decision in a risky context, there would be differences with pubertal status in the levels of neural activation in reward-processing regions but not in regions associated with cognitive control.

The regions involved in active decision-making were analysed using a 2x2x2 ANOVA with two within-subject factors (decision to inflate vs. decision to stop, and active vs. passive decision trial) and one between-subject factor (Early vs. Late puberty group).

2. There would be differences with pubertal status in the levels of neural activation when processing reward vs. loss outcomes in reward-processing but not cognitive control regions after a decision to take a risk.

The brain regions involved in processing outcomes after making an active decision to take a risk were analysed using a 2x2 ANOVA where the within-subjects factor was reward outcome after taking a risk (the active decision balloon successfully inflating) vs. loss outcome after taking a risk (the active balloon popping), with puberty group again as the between-subjects factor.

3. Differences would be seen in reward-processing but not cognitive control regions during the processing of each of the outcomes (reward, loss and choosing not to take a risk) with pubertal status.

Each of the outcomes in the active decision trials were compared to the same outcome in the passive decision trials in 3 separate 2x2 ANOVAs (within-subject factor active vs. passive trial; between-subject factor puberty group) to identify differences between puberty groups.

7.2.7.2 Analysis of BOLD activation and hormone levels

First level contrast images for each participant were entered into a series of second-level (random effects) multiple regression models examining the association between neural activation in each of the contrasts outlined in **Section 7.2.7.1** and each pubertal hormone (testosterone, DHEA and oestradiol), controlling for chronological age.

7.2.7.3 *A priori* regions of interest

The ROIs were based on the theoretical dual systems model, and included reward-processing and cognitive control regions. Reward-processing ROIs included the ventral striatum (consisting of the NA, caudate and putamen), the OFC and the vmPFC. The cognitive control ROIs included the lateral PFC and the dACC. The MNI coordinates of each cortical region were defined *a priori* (see **Table 7.1**), and subcortical regions were determined using an anatomical mask obtained from the Harvard-Oxford subcortical structural atlas in FSL. Task-related responses were considered significant if they exceeded a threshold of $p<0.001$ uncorrected, minimum cluster size of 15 contiguous voxels.

ROI	MNI boundaries			Approx. Brodmann areas
	x	y	z	
vmPFC	$<\|20\|$	>40	>0 z <15	medial 10
OFC	any	>10	<0	11, 12, 25, 47
dACC	$<\|20\|$	0<y<40	>0	24, 32, 33
Lateral PFC	$>\|20\|$	>20	>0	lateral 9 and 10, 46

Table 7.1: A priori determined MNI coordinates for each ROI, *and the approximate Brodmann areas included in these coordinates.*

7.3 Results

7.3.1 Participant demographics

50 participants were recruited to the study. Demographic details including age, BMI, IQ and pubertal measures are described in **Table 7.2**.

	Whole group N=50		Early puberty N=23	Late puberty N=26	Difference between groups
	Mean ± S.D.		Mean ± S.D.		p value
Age (years)	**13.56 ± 0.4**		**13.33 ± 0.4**	**13.75 ± 0.4**	**<0.001**
BMI (N=36)	19.9 ± 3.2		20.8 ± 4.1	19.2 ± 2.0	0.16
IQ	112 ± 12		109 ± 14	114 ± 10	0.23
Testosterone pg/ml (N=47)	**89.5 ± 36.1**		**67.4 ± 24.6**	**107.3 ± 34.3**	**<0.001**
	Median (IQR)*		Median (IQR)*		
DHEA pg/ml (N=47)	111.4 (81.4 - 177.2)		116.2 (86.3 - 166.9)	107.2 (80.6 - 170.1)	0.9
Oestradiol pg/ml (N=45)	1.57 (1.11 - 2.09)		1.53 (1.01 - 2.31)	1.70 (1.36 - 1.95)	0.6

Table 7.2: Participant demographics including mean and S.D. for age, BMI, IQ and pubertal hormone levels *for the whole group (N=50) and for Early and Late puberty groups separately. One participant did not complete the pubertal self-assessment. Significant differences between puberty groups are highlighted* ***in bold****.*

**Since DHEA and oestradiol were significantly positively skewed, median and interquartile ranges (IQR) are presented, and Mann-Whitney tests were performed to assess between group differences.*

7.3.2 Pubertal data

Self-reported pubertal status was provided by 49 participants. Genitalia and pubic hair ratings were highly correlated (r=0.83, p<0.001; see **Table 7.3**). 23 participants were categorised as Early puberty and 26 participants were categorised as Late puberty (see **Section 2.4.3** for pubertal grouping method). The two groups were significantly different in age, with a difference of 4.8 months between the mean ages (see **Table 7.2**). Age was included as a covariate in all of the fMRI GLM analyses.

Salivary levels of testosterone and DHEA were available on 46 participants. Oestradiol levels were available for 44 participants; two participants had insufficient volume samples for analysis (aged 13.5 years, Late puberty group and 13.7 years, Early puberty group). Testosterone levels were normally distributed, while the DHEA and oestradiol data were positively skewed (see **Figure 7.2** for hormone distributions). Within the oestradiol data, there was one clear outlier who had an oestradiol level of 6.37pg/ml. This participant was excluded from analyses of oestradiol, but was included in other analyses.

Testosterone levels were significantly correlated with Tanner stage (TS) development and age (see **Table 7.3**). In contrast, neither DHEA nor oestradiol were correlated with TS, testosterone or age, but were significantly correlated with each other (see **Table 7.3**).

	Tanner stage pubic hair	Tanner stage genitalia	Testosterone	DHEA	Oestradiol
Tanner stage genitalia	**0.83*** **				
Testosterone	**0.55*** **	**0.45** **			
DHEA	0.06	0.05	0.25		
Oestradiol	-0.09	-0.16	0.10	**0.45** **	
Age	**0.45** **	**0.47*** **	**0.31***	-0.06	0.04

Table 7.3: Pearson r correlation coefficients between pubertal measures.
*Significant results are highlighted in **bold**: *** p<0.001; ** p<0.01; p<0.05*

7.3.3 Self-reported behavioural questionnaires

50 participants completed the CARE and SSS questionnaires, and 49 participants completed the BIS questionnaire. The summary responses for the whole group and for each puberty group are outlined in **Table 7.4**. Academic risk behaviour differed between the Early and Late puberty groups (U=157.5; p=0.03; see **Table 7.4**), with boys in Late puberty reporting greater levels of academic risk-taking than those in Early puberty. There were no other significant differences between puberty groups.

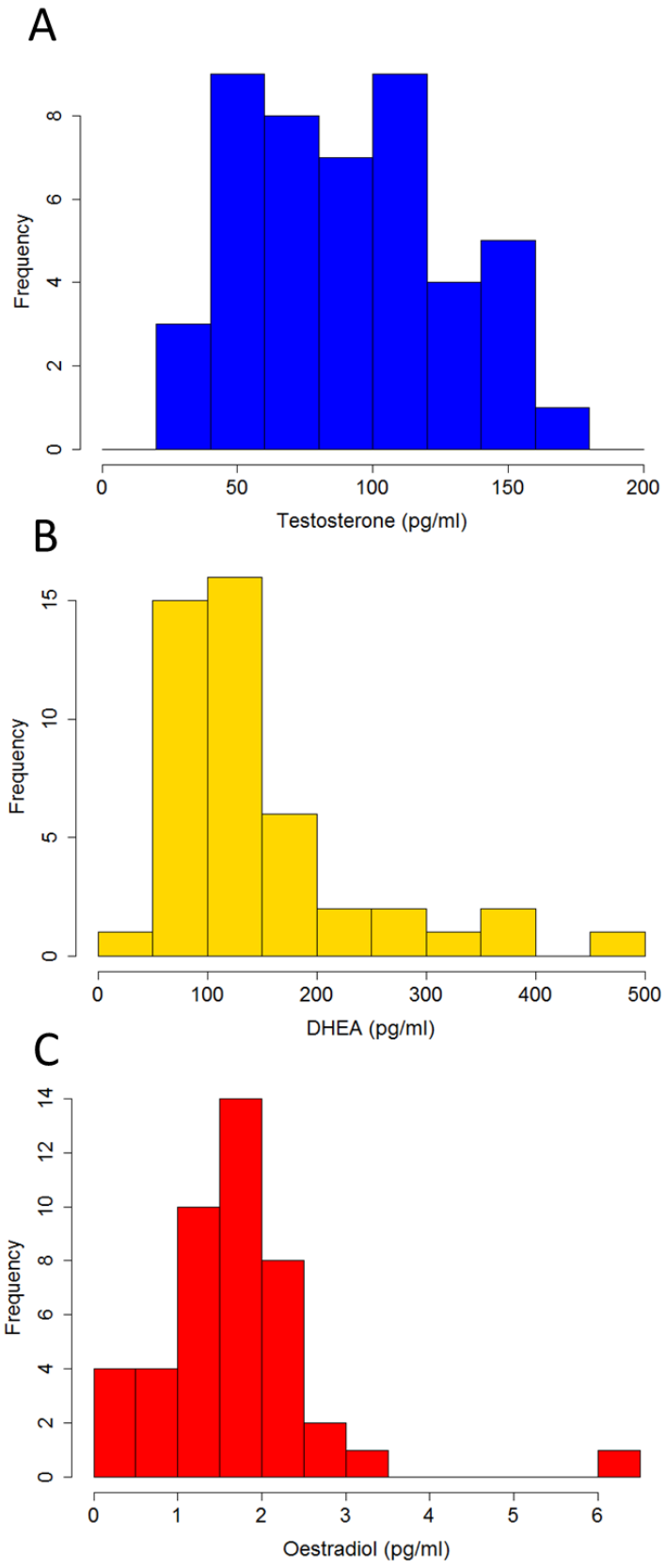


Figure 7.2: Frequency histograms for testosterone (Panel A, in blue; N=46), DHEA (Panel B, in yellow; N=46) and oestradiol (Panel C, in red; N=44).

Questionnaire	Score range	Whole group		Early puberty	Late puberty	Group difference
		Median (range)		Median (range)		p value
CARE Aggressive/ Illegal behaviour	1.0-7.0	2.5 (1.0-6.5)		2.1(1.0-6.5)	2.5(1.0-4.0)	0.13
CARE Academic		**3.1(1.0-7.0)**		**2.8(1.0-7.0)**	**3.8(1.0-5.8)**	**0.03**
BIS attention	1.0-4.0	2.1(1.3-3.3)		2.2(1.4-3.1)	2.1(1.3-2.9)	0.4
BIS motor		2.2(1.6-3.2)		2.2(1.9-2.8)	2.1(1.6-3.2)	0.6
BIS non-planning		2.4(1.0-3.6)		2.2(1.0-3.6)	2.4(1.1-3.4)	0.4
Sensation-seeking	1.0-2.0	1.3(1.0-1.8)		1.3(1.0-1.8)	1.3(1.0-1.8)	0.5

Table 7.4: Summary of self-report questionnaire responses *for the whole group, and by puberty group, including median and range.*

There was a significant correlation between testosterone and the CARE academic risk score (r=0.43; p=0.003). No other correlations between questionnaire scores and hormone measures survived a Bonferroni-corrected significance level of p<0.017 (α=0.05 and 3 tests of pubertal hormones).

	Testosterone	DHEA	Oestradiol
CARE Aggressive/Illegal behaviour risk	0.21	0.28	0.25
CARE Academic risk	**0.43*** **	0.35	0.06
BIS attention	0.29	0.23	0.09
BIS motor	0.09	0.05	0.05
BIS non-planning	0.29	-0.08	-0.18
Sensation seeking	0.12	-0.16	0.00

Table 7.5: Correlation between the behavioural questionnaire scores and puberty hormones. ****p<0.005*

7.3.4 Behaviour on the BART task

All participants completed at least three runs of the BART task. There was significant variability in the levels of risk-taking behaviour demonstrated on the task, with participants popping from 4% to 64% of active decision balloons (mean 32.3%; see **Table 7.5**). There were no significant differences in levels of risky decision-making between puberty groups as assessed by average number of inflations before stopping or percentage of active balloons popped, and no differences in success on the task as

determined by the amount of money banked or earned over the task (see **Table 7.5**). There were no significant correlations between any of the three pubertal hormone levels and the behavioural outcomes on the BART task (all p>0.1).

7.3.4.1 Relationship between self-reported behaviours and BART task outcomes

There was a significant negative correlation between self-reported sensation-seeking using the SSS and the average number of inflations on the BART task before choosing to stop (r=-0.46; p=0.001). There were no significant correlations between self-reported risk-taking or impulsivity behaviour and behaviour on the BART task (all *p value*s >0.01, the modified Bonferroni corrected level for α=0.05 and 4 tests of performance on the BART task for each behaviour).

Behavioural outcome	Whole group N=50		Early puberty	Late puberty	Difference between groups
	Mean (S.D.)		Mean (S.D.)		p value
Average no. of inflations before stopping	4.7 (1.4)		4.3 (1.2)	5.1 (1.6)	0.07
% active balloons popped	32.3 (14.1)		28.1(13.0)	35.1(14.1)	0.06
Average money banked per run (£)	15.21 (2.28)		15.44 (2.43)	15.15 (2.12)	0.7
Average money earned per run (£)	1.45 (0.28)		1.43 (0.28)	1.49 (0.29)	0.5

Table 7.6: Summary of behavioural performance during active decision-making in the BART task *for the whole group, and by puberty group, including means and S.D.*

7.3.5 fMRI results

fMRI data were available for 47 participants for the analysis (see Methods **Section 7.2.6** for exclusion reasons).

7.3.5.1 Differences in BOLD signal during active decision-making with puberty

Between the Early and Late puberty groups, there were no regions showing significant differences in neural activation in the [Go decision (active-passive) – Stop decision

(active-passive)] contrast. Regression of BOLD response against each of the three pubertal hormones revealed two significant clusters in *a priori* specified ROIs when regressed against oestradiol. These were in the left lateral PFC and the right OFC (see **Table 7.7**). Both clusters showed a positive relationship between oestradiol level and neural activation in the [Go decision (active-passive) – Stop decision (active-passive)] contrast (see **Figure 7.3**).

			No behavioural covariate			**With behavioural covariate**		
Puberty variable	peak voxel (MNI)	ROI	No. of voxels	Z score	p value	No. of voxels	Z score	p value
Oestradiol positive correlation	-21 38 1	L lateral PFC	21	3.85	0.0001	Not significant		
	12 62 -2	R OFC	19	3.57	0.0004	15	3.44	0.0006

Table 7.7: Regions showing significant relationships between activation during the [Go decision (active-passive)>Stop decision (active-passive)] condition and pubertal status*, thresholded at p<0.001 unc., min. cluster size 15 voxels.*

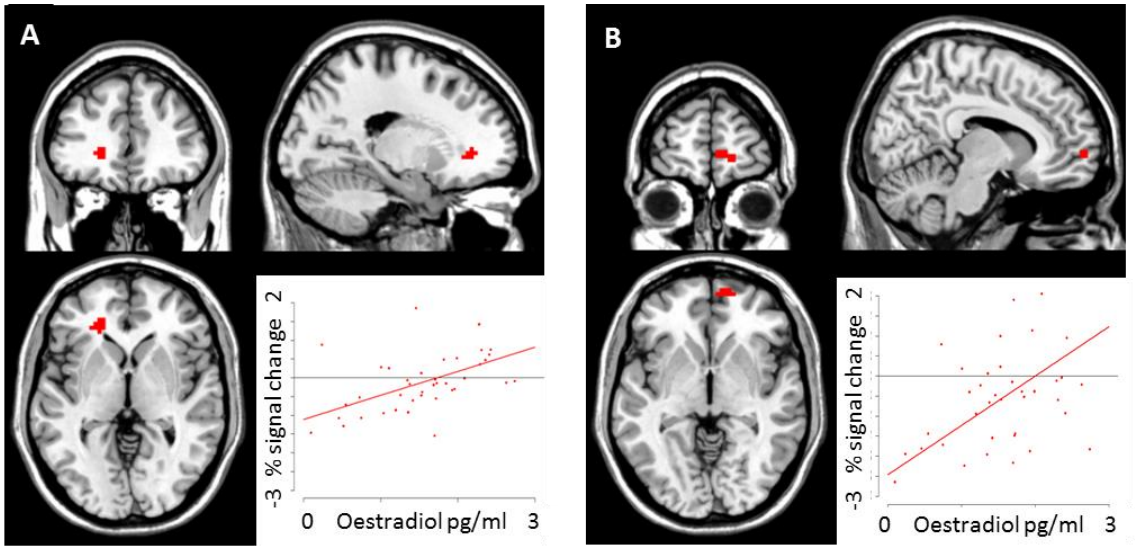


Figure 7.3: The association between oestradiol level and BOLD signal during the [Go decision (active-passive)>Stop decision (active-passive)] contrast (covarying for age). *(A) left lateral PFC cluster (peak voxel -21 38 1); (B) right OFC cluster (peak voxel 12 62 -2). The graphs show the correlation between salivary oestradiol level (x-axis) and mean difference in BOLD signal in the [Go decision (active-passive) > Stop decision (active-passive)] condition for each participant (y-axis), shown as % signal change for the whole cluster.*

No regions survived regression against either testosterone or DHEA. Incorporating behaviour (percentage of active balloons popped) as a covariate in the oestradiol regression attenuated the size of the OFC cluster, while the left lateral PFC cluster became non-significant (see **Table 7.7**).

7.3.5.2 Differences with puberty in BOLD signal while processing reward vs. loss outcomes following an active risky choice

Three regions showed significant differences in BOLD signal in the [Active decision (Inflate outcome>Pop outcome)] contrast between Early and Late puberty groups including clusters in the bilateral vmPFC, and right lateral PFC (see **Table 7.8 and Figure 7.4**). Only the two large bilateral vmPFC clusters survived the *post hoc* analysis including behaviour as a covariate. Regression of whole brain BOLD response for the [Active decision (Inflate outcome>Pop outcome)] contrast against each of the three pubertal hormones highlighted one significant cluster when regressed against testosterone (see **Table 7.8**) in the right lateral PFC, which showed a positive correlation between BOLD signal and testosterone level (see **Table 7.8 and Figure 7.5**). Including behaviour as a covariate in the *post hoc* analysis did not change the significance of this cluster. No regions survived regression against either DHEA or oestradiol.

			No behavioural covariate			**With behavioural covariate**		
Puberty variable	peak voxel (MNI)	ROI	No. of voxels	Z score	p value	No. of voxels	Z score	p value
Puberty group Late>Early	-15 41 13	L vmPFC	124	4.14	<0.0001	74	3.90	0.0001
	18 53 7	R vmPFC	96	3.98	0.0001	71	3.87	0.0001
	42 32 1	R lateral PFC	17	3.91	0.0001	Not significant		
Testosterone positive correlation	30 41 1	R lateral PFC	24	3.83	0.0001	22	3.80	0.0001

Table 7.8: Regions showing significant relationships between activation during the [Active decision (Inflate outcome>Pop outcome)] condition and pubertal status, *thresholded at p<0.001 unc., min cluster size 15 voxels.*

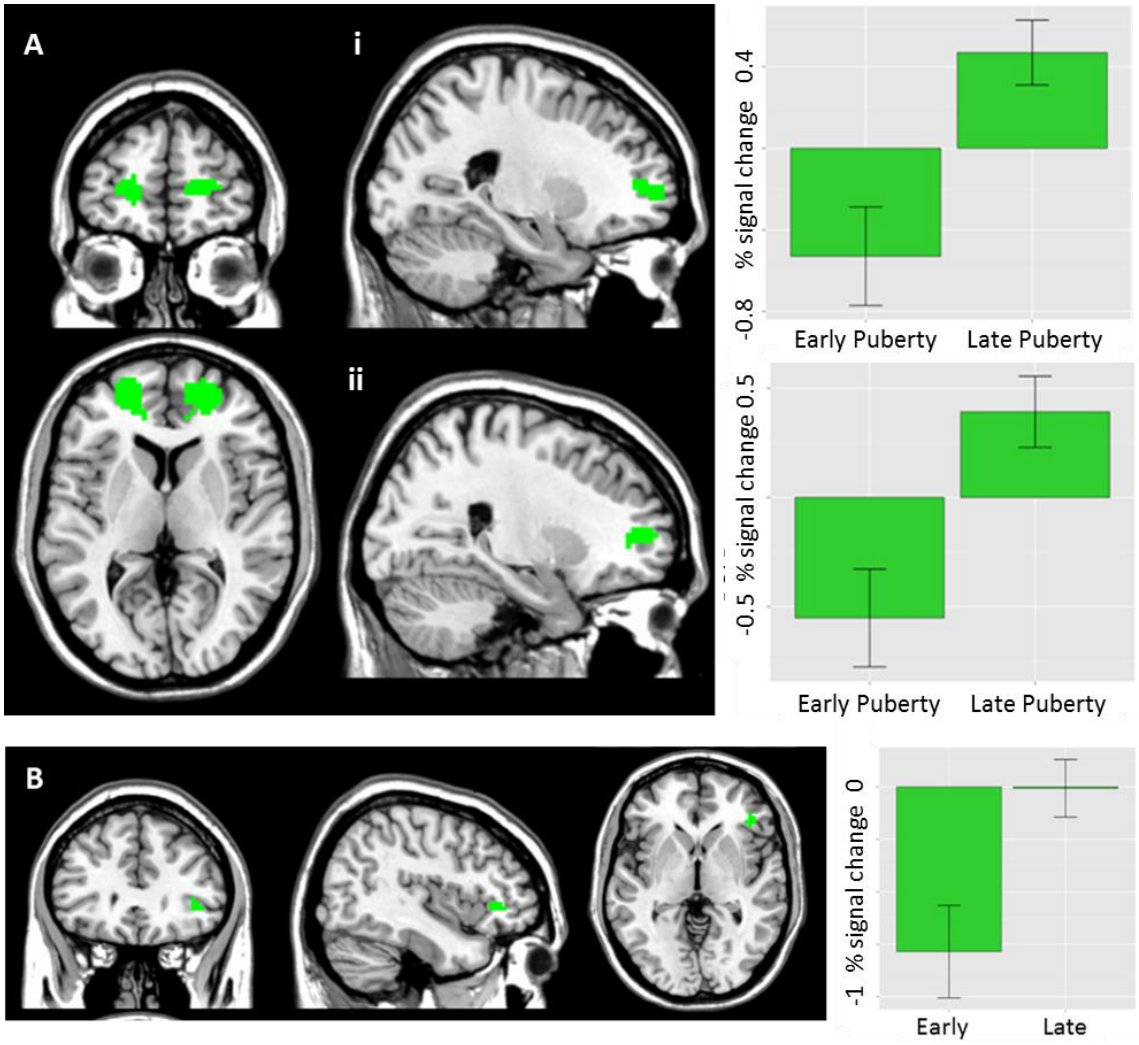


Figure 7.4: The association between puberty group and BOLD signal during the [Active decision (Inflate outcome>Pop outcome)] contrast *(after covarying for age). Associated bar graphs show mean difference in BOLD signal during the (Inflate outcome – Pop outcome) condition for active trials for the Early and Late puberty groups separately. The y-axis represents % BOLD signal change. (A) bilateral vmPFC clusters with the L vmPFC cluster above (i; peak voxel -15 41 13) and R vmPFC cluster below (ii; peak voxel 18 53 7); (B) R lateral PFC cluster (peak voxel 42 32 1).*

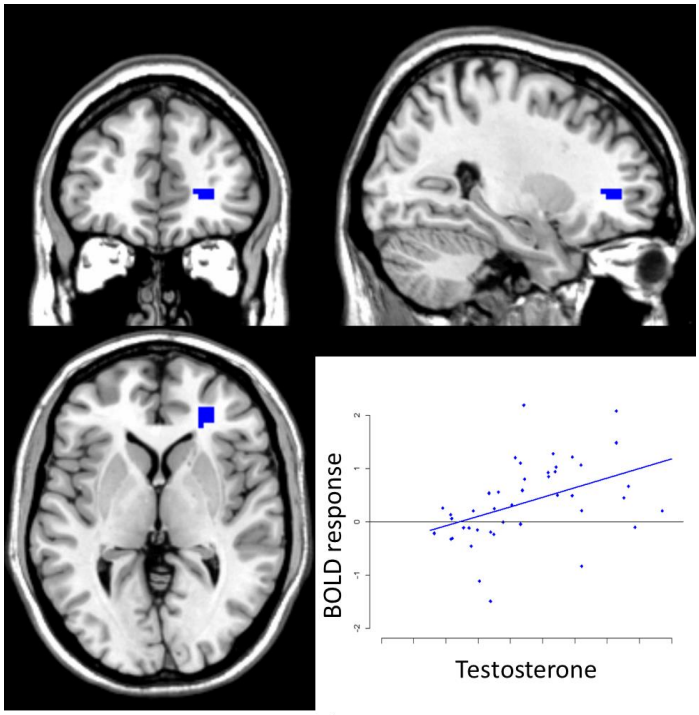


Figure 7.5: The association between testosterone level and BOLD signal during [Active decision (Inflate outcome>Pop outcome)] *(after covarying for age) in the R lateral PFC cluster (peak voxel 30 41 1).*

The graph shows the correlation between testosterone level (x-axis) and mean difference in BOLD during (Inflate outcome – Pop outcome) for active trials (y-axis), shown as % signal change.

7.3.5.3 Differences in BOLD signal while processing active outcomes vs. passive outcomes with puberty

For the [Active decision Inflate outcome – Passive decision Inflate outcome] contrast, one cluster in the OFC showed a positive relationship with testosterone, where increasing testosterone levels were related to BOLD signal in the [Active decision Inflate outcome – Passive decision Inflate outcome] contrast (see **Table 7.8**). This relationship was not affected by the addition of behaviour as a covariate in the GLMs. There were no significant relationships between the [Active decision Inflate outcome – Passive decision Inflate outcome] contrast and puberty group, DHEA or oestradiol.

There were significant differences in activation in the [Active decision Pop outcome>Passive decision pop outcome] contrast in the dACC between Early and Late puberty groups (see **Table 7.8**). Only the right-sided cluster remained significant when behaviour was added to the GLM as a covariate. Regression of the [Active decision Pop outcome>Passive decision pop outcome] contrast against salivary testosterone produced one significant cluster in the left lateral PFC, which showed a significant negative correlation between hormone level and activation (see **Table 7.8**). The

significance of this was not affected by incorporating behaviour as a covariate in the GLMs. There were no significant results for either the DHEA or oestradiol regressions.

			No behavioural covariate			With behavioural covariate		
Puberty variable	peak voxel (MNI)	ROI	No. of voxels	Z score	p value	No. of voxels	Z score	p value
A. [Active decision Inflate outcome>Passive decision inflate outcome]								
Testosterone positive correlation	-6 23 -5	L OFC	19	3.76	0.0001	21	3.89	0.0001
B. [Active decision Pop outcome>Passive decision pop outcome]								
Puberty group Early>Late	-3 32 46	L dACC	43	3.90	<0.0001	Not significant		
	6 14 49	R dACC	64	3.84	0.0001	43	3.75	0.0002
Testosterone negative correlation	-27 23 19	L lateral PFC	21	3.62	0.0003	23	3.62	0.0003
C. [Active decision Stop outcome>Passive decision Stop outcome]								
Oestradiol negative correlation	9 35 -5	R OFC	79	4.08	<0.0001	84	4.07	<0.0001

Table 7.9: Regions showing significant relationships between activation during the active decision outcome and the passive decision outcome for each of the inflate, pop and stop outcomes and pubertal status, *thresholded at p<0.001 unc., min cluster size 15 voxels.*

For the [Active decision Stop outcome>Passive decision Stop outcome] contrast, there were no regions that showed significant differences in neural activation between the Early and Late puberty groups. Regression against each of the three pubertal hormones revealed one significant cluster related to oestradiol in the right OFC (see **Table 7.8**), which survived the addition of behaviour as a covariate in the GLM. There were no significant relationships between the [Active decision Stop outcome > Passive decision Stop outcome] and either testosterone or DHEA.

7.4 Discussion

The current study examined the relationship between pubertal status and BOLD signal in reward-processing and cognitive control regions during a risky decision-making task in a group of 12-14 year old boys. During risky decision-making, oestradiol was positively correlated with BOLD signal in the right OFC (reward-processing ROI) as well as the left lateral PFC (cognitive control ROI). When being presented with the reward outcomes compared to loss outcomes of a risky choice, boys in the Late puberty group exhibited greater BOLD signal in a reward-processing region (bilateral vmPFC), as well as a cognitive control region (right lateral PFC), than boys in the Early puberty group. There were no differences in the VS for any of the contrasts with any measure of pubertal development. These results provide some tentative evidence for changes in regions of the reward-processing network with pubertal development, but in the current study these are limited to the cortical regions involved in this network. The results also indicate that there may be changes in the cognitive control regions associated with puberty, suggesting that the mechanisms of brain development may be more complex than those proposed by the dual systems model.

7.4.1 Relationship between oestradiol and risky decision-making

Oestradiol levels were significantly related to BOLD signal in the right OFC when deciding to take a risk compared to when deciding to stop. Furthermore, oestradiol levels were negatively correlated with BOLD signal in the same areas when processing the stop outcome in the active condition compared to the passive condition. The OFC has been shown to be functionally involved in the representation of the reward value of potential outcomes (Gläscher et al., 2012; Kennerley et al., 2011; Kennerley & Walton, 2011). Changes in BOLD signal in this region during decision-making may relate to changes in reward value representation, which in turn could impact on decision-making. Thus, lower levels of BOLD signal during the stop outcome for participants with higher oestradiol level could equate to a lesser sense of reward for successfully stopping and banking the money earned in the trial. Any such process is likely to be context-dependent, since the OFC is thought to be easily adaptable in its

reward representation function by recent experience (Kennerley & Walton, 2011). It is not possible to infer a cause or mechanism of the significant relationship between BOLD signal and pubertal status seen in the study, as the current study can only demonstrate associations. Of note, in the current study the decision to stop (not take a risk) was not independent of seeing the stop outcome. Therefore it is not possible from these data to distinguish whether changes in BOLD signal with oestradiol relate to differences in activation during the decision-making or the outcome phase of the trial.

Oestradiol is a product of androgen metabolism, predominantly synthesised in the male by the aromatisation of testosterone both in the testes and the peripheral tissues e.g. muscles and adipocytes (see **Section 1.4.3** and **Figure 1.3**). Levels of oestradiol increase across male puberty, but the pattern of increase differs from that of DHEA and testosterone (Ankarberg-Lindgren & Norjavaara, 2008), with the rise in oestradiol occurring in the later stages of puberty. This may result from relatively low aromatisation capacity in boys at early stages of puberty (Cuttler et al., 1993), resulting in a low rate of metabolism of testosterone to oestradiol. The observation that BOLD signal during decision-making is related to oestradiol level but not to other pubertal markers could indicate a non-linear change in brain activity that specifically occurs late in puberty, concomitant with the rise in oestradiol. This could reflect a direct relationship, with change in OFC function directly related to aromatase activity and oestradiol levels in the brain and circulation, or may be indirect, with oestradiol serving as a proxy marker of a specific late pubertal change.

A previous study looking at neural activation during risky decision-making with age also found significant results in the OFC (Eshel et al., 2007). Eshel and colleagues (2007) found that adults (aged 20-40 years) showed greater activation in the left OFC [peak voxel: -44 14 6] than adolescents (aged 9-17 years) when making a risky decision. Of note, the significant cluster found in this previous study is located in a more lateral position than the significant OFC clusters in the current study.

7.4.2 Relationship between pubertal group and the processing of outcomes after a risky choice

With maturing pubertal stage, there was greater vmPFC activation when processing the reward vs. loss outcome of a risky decision. The vmPFC, together with the OFC, is involved in representation of reward value (Hare, Camerer, & Rangel, 2009) and determining the current incentive value of a behavioural outcome (Kennerley & Walton, 2011). One possible interpretation of this change in vmPFC activity is that changing activity with puberty could result in changes in the perception of reward value for a decision's outcome, and therefore influence an individual's tendency to take a risk. However, the vmPFC has many functions, and the current study cannot determine whether changing BOLD signal influences behaviour, or the mechanism by which this might occur. Furthermore, in the current study there was no significant difference in behaviour on the task with pubertal group in this study despite the differences in vmPFC activation.

One previous study investigating the dual systems model found changes in the vmPFC with age. Van Leijenhorst and colleagues (2010) reported a peak in vmPFC activation during risky decision-making in adolescents compared to children and adults (see **Table 1.1** for details of study participants), but reported no differences with age during outcome processing (Van Leijenhorst, Gunther Moor, et al., 2010). The findings in this previous study and the current one are not directly comparable since the independent variables differ (chronological age vs. pubertal stage); in addition, the two studies employed different tasks and analysis methods.

7.4.3 Relationship between pubertal development and maturation of cognitive control regions

BOLD signal in cognitive control regions of the dACC and the lateral PFC was related to a variety of pubertal measures including oestradiol, testosterone and pubertal group during the different contrasts analysed in this study (see **Tables 7.7, 7.8 and 7.9**). Previous studies specifically exploring the dual systems model with age in adolescence have found no evidence of age-related change in lateral PFC during risky decision-

making or outcome processing (Eshel et al., 2007; Van Leijenhorst, Zanolie, et al., 2010), and conflicting results in the dACC, with studies showing highest levels of BOLD signal in childhood (Van Leijenhorst, Gunther Moor, et al., 2010 during risky decision-making), adolescence (Geier et al., 2010 during reward anticipation) and adulthood (Eshel et al., 2007 during risky decision-making). The findings of the current study, which is the first to look for changes in cognitive control regions associated with puberty during a risky decision-making task, suggest that pubertal maturation may influence cognitive control development during adolescence, as well as reward-processing.

7.4.4 Evidence for the role of puberty in a dual systems model of brain development

The dual systems model of adolescent brain development proposes that there is a mismatch in developmental timing between the network of brain regions involved in reward-processing and the regions involved in cognitive control. The earlier maturation of the reward-processing regions has been associated with the timing of puberty, while cognitive control regions are thought to develop over a more prolonged period, not temporally related to puberty. The results of the current study show that there are puberty-related changes in functional activity in reward-processing regions, particularly the vmPFC and the OFC. However, in contrast to the dual systems model, the current results also suggest that there are changes in activity in cognitive control regions during a risk-taking task that are associated with puberty.

Furthermore, no pubertal differences in activity were seen in the subcortical reward processing region of the VS. Previous studies investigating changing neural activation with pubertal status that have found significant differences in the VS have focussed on reward outcome (Forbes et al., 2010; Op de Macks et al., 2011), and reward anticipation (Forbes et al., 2010). Op de Macks and colleagues (2011) found that, in boys aged 10-16 years (N=14), activation in the left putamen (part of the VS) during the reward>loss contrast of a gambling task was positively correlated with salivary testosterone levels (Op de Macks et al., 2011). As highlighted by the authors of this

study, the sample size and age range of the participants meant that they were unable to control for age in their analysis. This may account for the differing results from the current study, as might the differences between the risk-taking tasks used. Forbes and colleagues (2010) reported reduced activation of the caudate for participants in mid/late puberty compared to those in pre/early puberty during reward outcome (N=77; mixed females aged 11-12 years and males aged 12-13 years; pre/early puberty=26, mid/late puberty=51; Forbes et al., 2010). The authors suggest that this reduction in VS activation may relate to the low intensity of the rewards used in their study, again highlighting the potential for task-specific activation.

7.4.5 Methodological considerations

The findings of this study add to our current understanding of the potential role of puberty in the functional development of the adolescent brain. As with all studies, there are a number of limitations that must be taken into consideration when interpreting the results. Many of these are common across the studies in this thesis, are discussed in **Chapter 8**. In the current study, ROIs for reward-processing and cognitive control regions were defined *a priori* (see **Section 7.2.7.3**). There is, however, some overlap between regions considered to be primarily involved in reward processing and those considered to be primarily involved in cognitive control, as the regions are contiguous, and their functional relationships undoubtedly overlap. Thus the division between the dACC and the vmPFC, the dACC and the OFC, and the vmPFC and lateral PFC are all subject to interpretation. Some of the clusters described in the results of this study overlap these regions, and can therefore be interpreted differently depending on the assigned labels. This may in part reflect the significant functional interplay between differing regions and networks, in contrast to the notion of discrete networks described in the dual systems model. In order to further study further the development of cortical regions associated with decision-making in adolescence, it is crucial to establish clear, evidence-based regions of interest, ideally with the benefit of localiser tasks specific to the functions being investigated.

7.5 Conclusion

The current study found evidence for a relationship between pubertal status and BOLD signal within both reward-processing and cognitive control ROIs during a risky decision-making task, independent of chronological age. The results were limited to cortical regions within the ROIs, and no puberty-related differences in subcortical function were seen during the task. These results provide evidence of a possible role of puberty in cortical functional development of both reward-processing and cognitive control regions. They do not support the hypothesis that any functional differences between reward-processing and cognitive control regions proposed by the dual systems model are driven by a differential influence of puberty on these regions.

7.6 Next chapter

The next, and final, chapter of the thesis will summarise the main findings and will relate these to the research questions set out in the introduction.

CHAPTER 8

Discussion

In the introduction of this thesis, I laid out three key research questions, and the principal aim of the studies described has been to investigate these and build on the published evidence for each of these questions. Chapters 3 and 4 focussed on changes in brain structure with pubertal development, and therefore relate to the first research question, 'What is the relationship between pubertal status and structural brain development?' Chapters 5, 6 and 7 focussed on task-related changes in brain function with puberty, and sought to address the second research question, 'How does the functional activation of the brain during tasks incorporating key adolescent behaviours differ in relation to pubertal status?' Each of the chapters within the thesis fed into the third research question, 'To what extent can chronological age and pubertal status be disentangled when looking at brain development in human adolescents?' The main findings will now be summarised for each chapter in the context of the key questions that they address. Outstanding issues are described, and possible future directions for further research are considered.

8.1 Research Question 1: What is the relationship between pubertal status and structural brain development?

Whilst many studies and reviews have hypothesised a relationship between the structural brain development in adolescence and puberty (Blakemore et al., 2010; Lenroot et al., 2007; Peper, Hulshoff Pol, et al., 2011; Smith et al., 2013; Sowell et al., 2002), only a small number of studies have directly investigated this hypothesis. **Chapter 3** investigated whether subcortical structural development during adolescence is correlated with changing pubertal status, using a large longitudinal dataset of 711 structural MRI scans. The analysis used mixed effects modelling, and incorporated repeated measures of self-reported Tanner stage, chronological age and subcortical structural volumes from the same participants.

The results showed that pubertal development was related to grey matter development in all six of the subcortical regions analysed, for both males and females. In both sexes, amygdala and hippocampal volumes increased across puberty, while the volumes of nucleus accumbens, caudate, putamen and globus pallidus decreased. Incorporating both pubertal stage and chronological age improved the fit of the models for the amygdala and putamen in both sexes, as well as the hippocampus and caudate in females. In males, the hippocampus and globus pallidus were best modelled using only chronological age as an explanatory variable.

The analysis in **Chapter 3** demonstrated significant relationships between developmental trajectory and each of puberty and age for some subcortical structures, as well as an interactive puberty-by-age term. This suggests that, during adolescence, puberty and age could have dissociable effects on brain development, as well as a potential interactive effect (although this may also reflect the high co-linearity of the variables), and emphasises the need to consider puberty in studies of age-related change, and vice versa. Previous studies investigating changes in subcortical volumes with puberty have had conflicting results, with both increases (Neufang et al., 2009) and decreases (Bramen et al., 2011) in amygdala volume reported with pubertal

measures. These two studies used cross-sectional designs sampled from different age ranges, and one potential reason for the differing results could relate to differential age effects within their samples. Similarly, many age-related studies have not collected pubertal measures and are therefore limited in their ability to distinguish between potential age and pubertal effects.

Whilst previous studies investigating pubertal effects on subcortical brain structure have been cross-sectional (Bramen et al., 2011; Neufang et al., 2009), the study in **Chapter 3** is the first to explore this question in an unstructured multiple cohort longitudinal design (Thompson, Hallmayer, O'Hara, & Alzheimer's Disease Neuroimaging Initiative, 2011) to show changing developmental trajectories in subcortical structural volumes with pubertal development. Given the substantial variation in individual total and regional brain volumes, longitudinal data analysis significantly improves the power of a study to detect differences between groups (Steen, Hamer, & Lieberman, 2007). Furthermore, inter-individual differences in developmental trajectories have themselves been linked to functional performance (Ordaz, Foran, Velanova, & Luna, 2013), and further emphasis on differential trajectories of brain development is likely to emerge with the increasing availability of longitudinal data (Mills & Tamnes, 2014).

To date, few studies have investigated white matter development with puberty (Peters et al., 2012), and the study described in **Chapter 4** aimed to investigate this relationship. Mean diffusivity (MD) and fractional anisotropy (FA) were analysed against measures of pubertal development for a group of 61 adolescent boys. There was a significant relationship between MD and puberty group in a diverse set of white matter regions such that males in late stages of puberty had lower MD values than those in early stages of puberty. In contrast, there was no relationship between FA and pubertal stage. *Post hoc* regression modelling of MD in the significant regions against puberty stage and age found that the best-fitting model to explain the differences in MD incorporated both age and puberty measures. The lack of significant association between FA and pubertal development in the current study could reflect that the changes in white matter microstructure seen relate more to reduction in overall

magnitude of diffusion, reflecting changes in cells and cell components within the white matter (Blumenfeld-Katzir et al., 2011; Sagi et al., 2012), than in changes of directionality of diffusion (see **Section 4.4.4**). *In vivo* human studies cannot, however, be used to establish the exact nature of these microscopic changes, and further work across disciplines is needed to investigate further.

The study in **Chapter 4** builds on previously published literature investigating the link between white matter microstructural development and puberty (Asato et al., 2010; Herting et al., 2012). Compared to these earlier studies, the current analysis focussed on a narrower age-range (3 years compared to 20 years (Asato et al., 2010) and 6 years (Herting et al., 2012)) to try to minimise the potentially confounding effects of age, and on a single sex. Whilst there were some broad similarities in the results reported across the three studies, with continued decreases in white matter diffusion measures (Asato et al., 2010) and relationships between testosterone and white matter microstructural changes (Herting et al., 2012), there were also some discrepancies between the results of the different studies (see **Section 4.4.2**). This is likely to reflect a number of the methodological differences across the studies, each of which are cross-sectional in nature, and emphasises the need for further studies in this area, ideally of longitudinal design incorporating multiple measures of pubertal development (Peters et al., 2012).

In summary, despite our increasing knowledge of the on-going structural development of the adolescent brain, the mechanisms underlying this development remain largely unknown. The findings in **Chapters 3 and 4** support the hypothesis that puberty influences a diverse range of structural brain development indices during adolescence. The close relationship between age and puberty during adolescence is highlighted, and emphasise the need for future work to account for both pubertal development and chronological age in explanatory models of developmental trajectories.

8.2 Research Question 2: How does the functional activation of the brain during tasks incorporating key adolescent behaviours differ in relation to pubertal status?

Two key behaviours - social emotion processing and risky decision-making - were investigated. **Chapters 5 and 6** focussed on social brain development network by analysing the neural activation of female participants aged 11-13 years while imagining social emotions scenarios compared to basic emotion scenarios. Using fMRI, **Chapter 5** showed an association between increased sex steroid hormone levels and higher levels of BOLD signal activation in the left ATC, a key part of the social brain network, during social emotion processing, independent of chronological age. In contrast, there was a significant relationship between chronological age and levels of dmPFC activity during social emotion processing, independent of hormone level, such that older participants showed lower levels of dmPFC activity than their younger peers.

The study in **Chapter 5** is the first fMRI study to focus on the development of social emotion processing using the mentalising network with puberty. The task used had previously been used to investigate age-related change in social emotion processing (between adolescents and adults; Burnett et al., 2009). This previous study (which did not assess pubertal development) found age-related changes in the ATC. Based on the results of the current study, it is possible that this previous result could reflect puberty-related maturation (which would have been correlated with age). The age-related decrease in dmPFC activity seen in the current study with increasing age is in keeping with the previous study looking at age-related change using the same task (Burnett et al., 2009) as well as many other studies involving mentalising tasks (Blakemore et al., 2007; Gunther Moor et al., 2011; Overgaauw, van Duijvenvoorde, Gunther Moor, & Crone, 2014; Pfeifer et al., 2009; Wang et al., 2006 see Blakemore, 2010 for review), and suggests that this change in functional activation is not directly related to pubertal maturation. Distinguishing between age-related and puberty-related changes in functional activation is crucial in order to determine the possible mechanisms underlying functional maturation of the adolescent brain. **Chapter 5** emphasises that

changes occurring concurrently may not be under the control of a single driver, and may be differentially related to age and puberty.

In **Chapter 6**, fMRI data from the study described in Chapter 5 were re-analysed to investigate functional connectivity within the mentalising network. Using a PPI analysis, **Chapter 6** showed that increasing oestradiol concentrations were associated with increased functional connectivity between the mPFC and the right TPJ during Social relative to Basic emotion processing, independent of age. Analysis of the PPI data by phenotypic pubertal status showed that more advanced puberty stage was associated with enhanced functional connectivity between the mPFC and left ATC during social relative to basic emotion processing, also independent of age.

Developmental changes in functional connectivity with age-related increases between regions focally associated with task performance have been reported in a growing number of studies (Barbalat, Bazargani, & Blakemore, 2013; Booth et al., 2008; Cohen Kadosh et al., 2011; Ofen et al., 2012; Smith et al., 2011; van den Bos et al., 2011; see **Section 6.1**). None of these studies included any measures of pubertal development, and the relationship between functional connectivity and puberty remains poorly described, although there is evidence for the differential effects of steroid hormones on connectivity in the brain (Peper, van den Heuvel, et al., 2011). In particular, this review highlights a relationship between higher levels of oestrogens (exogenous and endogenous) and increased functional connectivity both within the cortex and between the cortex and subcortical regions (Peper, van den Heuvel, et al., 2011), a finding that is in keeping with the results of the current study. Further studies of the relationship between both task-based and resting state functional connectivity are needed to expand up on this area of research, especially in the light of an increasing understanding of the importance of functional connectivity in the brain over the past decade (Castellanos, Cortese, & Proal, 2014).

Chapter 7 explored the role of puberty in the development of risky decision-making during adolescence. The dual systems model of adolescent brain development proposes a mismatch in developmental timing between the reward-processing regions

of the brain, which mature in early adolescence in tandem with puberty, and cognitive control regions, which mature more slowly throughout adolescence (Casey et al., 2008; L Steinberg, 2008). **Chapter 7** investigated the relationship between puberty and BOLD signal within reward-processing and cognitive control ROI during risky decision-making in a group of boys aged 12-14 years. There were significant relationships between BOLD signal and pubertal measures in the vmPFC and the OFC (regions within the reward-processing network) as well as the dACC and lateral PFC (regions within the cognitive control network) for different aspects of risky decision-making (see **Section 7.3.5**). There were no differences in the ventral striatum for any of the contrasts with any measure of pubertal development.

While many papers have suggested a link between puberty and functional development of the reward-processing network of the brain (e.g. Blakemore et al., 2010; Geier, 2013; Smith et al., 2013), few studies have directly investigated this in humans. The two previously published studies investigating BOLD signal during reward processing with pubertal development (Forbes et al., 2010; Op de Macks et al., 2011) have found differing patterns of activation. Op de Macks et al. (2011) found a positive correlation between ventral striatal activity during reward outcome and testosterone level. Importantly, this study was unable to control of age in their analysis (age range 10-16 years; Op de Macks et al., 2011). In contrast, Forbes et al. (2010) found that BOLD signal in the ventral striatum was negatively correlated with testosterone activity during reward outcome, but positively correlated during reward anticipation (Forbes et al., 2010). In the current study, no puberty-related change in ventral striatal activity was seen in any part of the risky decision-making task. These discrepant results may reflext the differing tasks used, the small sample sizes and the age ranges of the participants involved. Nevertheless, based on these studies there is currently insufficient evidence to support the role of puberty in modulating ventral striatal response to reward.

The current study shows a correlation between activation in cortical reward-processing regions (the OFC and the vmPFC) and measures of pubertal development. Previous studies investigating age-related changes based on the dual systems model have

shown changes in the OFC (Eshel et al., 2007) and vmPFC activation (Van Leijenhorst, Gunther Moor, et al., 2010) with age. These studies did not report measures of pubertal development, so direct comparison of the results is not possible. Furthermore, the current study shows puberty-related changes in BOLD signal in the lateral PFC and the dACC, both cognitive control regions. No previous studies have examined a relationship between puberty and BOLD signal activation in cognitive control regions during a risk-taking task, and further studies are needed to investigate this relationship further.

In summary, the results of Chapters 5-7 are strongly suggestive of a role of puberty in functional brain development during adolescence. Potential mechanisms underlying the link between puberty and function brain development could include a direct action of sex steroid hormones on receptors in target regions during specific tasks (Zehr et al., 2008), or a shift in cognitive strategy with pubertal development, which resulting in differential BOLD signal during the task. In addition, the functional differences seen in these studies may be driven by pubertal effects on structure, which then lead to changes in measurable BOLD signal (Ladouceur et al., 2012; Peper, Hulshoff Pol, et al., 2011; Peper, van den Heuvel, et al., 2011). Each of the studies is cross-sectional and involves relatively small numbers of individuals, so cannot determine a causal relationship between puberty and functional brain development.

8.3 Research Question 3: To what extent can chronological age and pubertal status be disentangled when looking at brain development in human adolescents?

Since puberty, once triggered, progresses in a temporal pattern over 3-5 years in healthy individuals, age and puberty are necessarily correlated during development within an individual. However, across a population there is significant inter-individual variation in the timing and tempo of puberty (Marshall & Tanner, 1969, 1970; see **Section 2.3**) and the studies presented in this thesis used two different methods to distinguish between pubertal and age effects on brain development by taking

advantage of this natural variation. In **Chapter 3**, a large, longitudinal sample (obtained using an unstructured multi-cohort design) was used which allowed the use of mixed effects modelling analyses to tease apart age and pubertal effects. In **Chapters 4-7**, where cross-sectional samples were used, participants were selected from a narrow age range, within which typically developing individuals can be at any stage of puberty. This latter method aimed to maximise pubertal variation while minimising age differences. Despite the narrow age ranges of these samples, in each study there continued to be significant differences in the average age of the participants in the Early and Late puberty groups. The size of this difference was small, with a 4-9 month difference in average age of the two groups across the studies.

The results of the studies included in this thesis show that it is possible to design studies that disentangle pubertal effects from those of chronological age, but that this requires a narrow, targeted age range and therefore a specific focus of a study design on pubertal effects. This has implications for studies intending to analyse puberty as one of a number of potential independent variables. Furthermore, while this method works to focus on pubertal effects, it is also possible but perhaps more complex to do the reverse and focus on age-effects independent of puberty. For this, populations would need to be recruited that are at similar stages of puberty while at different chronological ages. While the studies in this thesis have focussed on the relationship between pubertal variables and brain development, the results have also highlighted age-related changes that are not linked to puberty. Chronological age can be considered as a composite measure of development that incorporates a multitude of different social, physiological and psychological exposures, and it is important to recognise that the potential mechanisms underlying age-related brain changes are numerous. Relating changes to age therefore challenges future research to understand what aspect of aging during adolescence may trigger and drive these changes.

8.4 Methodological considerations

The studies included in this thesis are subject to a number of limitations.

8.4.1 Cross-sectional study design

Many of the studies in this thesis employed cross-sectional study designs (**Chapters 4-7**). Whilst these are a valuable design method to demonstrate significant relationships between variables, they cannot be used to infer causality, and are unable to provide information about the developmental trajectory of an individual. Longitudinal studies may be of particular importance for future work looking at the potential mechanisms for pubertal effects on brain development, since pubertal status impacts on social interactions with family, peers and wider society. Thus, teasing apart the direct influences of hormones on the brain as opposed to influences via other systemic physical or psychological mechanisms provides an immense challenge in human subjects. However, longitudinal studies are costly and time-consuming to undertake, and need to be hypothesis-driven (Kuller, Bracken, Ogino, Prentice, & Tracy, 2013). **Chapter 3** used an unstructured multi-cohort longitudinal design (Thompson et al., 2011). While this has the advantage of allowing within-individual variation over time to be measured, the random nature of age of recruitment and duration of follow-up can result in age cohort effects that can be difficult to assess, and can lead to unequal sample sizes across different ages (Thompson et al., 2011).

8.4.2 Measurement of puberty

A number of different methods have been used to assess pubertal development in the course of this thesis. Each has methodological limitations that affect the interpretation of the results of the studies. Self-reported Tanner stage, as used in **Chapters 3, 4 and 7**, is the most widely used and clinically validated measure of pubertal development. However, as discussed in **Section 2.3.2.1**, it subjectively categorises puberty into broad developmental stages, and has been shown to be variable in its accuracy (See **Table 2.2**). Self-reported Tanner stage is therefore limited in its capacity to document accurately small developmental changes, and has significantly poorer resolution than

age as an explanatory variable. This may have resulted in some misclassification bias, and an underestimation of the puberty-related effects on developmental trajectories.

For each of **Chapters 4-7**, the participants were grouped into Early and Late puberty categories based on Tanner stage assessments (either physician-assessed or self-assessed; see **Section 2.4.3**). Grouping participants was necessary to ensure sufficient numbers of participants within the analysis. However, categorising participants in this way further reduces the statistical power to detect a relation between puberty and brain development measures, and may introduce misclassification bias. Follow-up studies with additional resources could recruit larger sample sizes and would allow Tanner stage to be analysed as separate stages, as with the sample used in **Chapter 3**, instead of as a grouped variable, which would increase the power of the analyses. It may however, also increase misclassification, and could introduce further bias if this were systematic within a population.

Four of the studies (**Chapters 4-7**) measured hormone levels using single salivary hormone assays. Salivary hormone sampling is a validated approach that is non-invasive and thereby more acceptable to young people than a blood test. However, precise information regarding how peripheral hormone levels in either saliva or blood relate directly to hormone concentrations that cross the blood-brain barrier, and how *in situ* enzymes, e.g. aromatase, affect androgen receptor exposure to circulating androgens *in vivo* is not yet available. Using a single sample salivary assay restricts any investigation into the impact of variation in hormonal level both diurnally and over longer time periods within the study participants, and how this might relate to brain development. Further exploration of hormonal variation is being conducted by other groups at present (e.g. Mundy et al. 2013), and better understanding of this normal variation within and between individuals during adolescence is needed.

8.4.3 Correlation between age and puberty

Despite significant efforts to recruit age-matched samples at varying stages of pubertal development, in each of the studies there continued to be a significant correlation

between puberty stage and age (as well as with hormonal measures) and age was therefore included as a covariate in the analytical models. Statistically controlling for this co-linearity may result in masking possible significant effects of puberty. Studies recruiting young people with constitutional delay of puberty, or conversely precocious puberty, would allow the comparison of individuals who were exactly age-matched but at contrasting stages of puberty. However, since aberrations in pubertal timing may themselves be related to additional clinical pathology such as the presence of chronic disease or an endocrine disorder, this approach may prove problematic in understanding the normal process of pubertal development.

8.4.4 Application of the findings to the general population

Participants for each of these studies volunteered to participate after seeing the publicity and advertising, and had parental support to take part in the study. This self-selection may limit the extent to which the results are applicable to the general population, where differences in IQ and educational background, socioeconomic status, ethnicity, cultural background and parent-child relationships may all impact significantly on brain development. Many MRI studies necessarily cannot address these issues due to their sample size and the cross-sectional design, but as a result the findings of these studies should be interpreted with caution. Furthermore, each of **Chapters 4-7** included participants of only one sex. Males and females are exposed to different levels of different sex steroid hormones at different chronological ages in adolescence. Therefore it is not possible to extrapolate these results between sexes, and further work would need to be done to see whether the sex-specific findings can be replicated in the opposite sex.

8.5 Future directions

8.5.1 Exploring the possible mechanisms underlying pubertal influence on brain development

The studies presented in this thesis provide evidence for a relationship between puberty and brain development during adolescence. As discussed in **Section 8.2**, our

understanding of the mechanisms underlying these changes in brain structure and function with puberty during adolescence is limited.

8.5.1.1 Direct influence of puberty hormones on sex steroid receptors

Structural and functional brain development may result from the direct influence of pubertal hormones on the brain via sex steroid hormone receptors. Both androgen and oestrogen receptors are known to exist in multiple key regions of the brain including subcortical and preoptic regions (see **Section 1.6.1**). There are different types of ER including classic nuclear receptors (α and β) and membrane receptors e.g. GPR30 and ER-X, and each are thought to have differing effects on the functioning of the brain (Cui et al., 2013). While oestrogens are thought to predominantly bind to oestrogen receptors, androgens (including testosterone and DHEA) work both directly on androgen but also on oestrogen receptors, after being converted into oestrogens locally via the enzyme aromatase (Kawata, 1995). Both androgen and oestrogen receptors are found in multiple regions of the brain in varying concentrations, with high levels in subcortical regions, particularly the hippocampus and amygdala, both in animal species and in humans (Abdelgadir et al., 1999; Clark et al., 1988; González et al., 2007; Sholl & Kim, 1989; Shughrue et al., 1997). The distribution of receptors in prefrontal cortical regions is less well described, and investigation in this area is hindered by a paucity of appropriate animal models. Mechanisms by which sex hormones exert such control include their action as transcription factors for gene expression and cellular proliferation (Melcangi et al., 2001), and activation of signalling cascades (Nguyen et al., 2005). Sex steroids can also directly impact glial cells and thereby influence myelination (Garcia-Segura & Melcangi, 2006). There is very little data describing relationship between serum/saliva levels of sex steroid hormones and levels in the local environment around these sex steroid hormone receptors. The presence of aromatase at the site of oestrogen receptors may, for example, dramatically increase the levels of oestrogen at the receptor site compared to systemic levels (Kawata, 1995). Improved understanding of the role of puberty in human adolescent brain development is reliant on *in vitro* and animal model studies in this area, and future work in human participants needs to ensure that it incorporates our

developing understanding of receptor action into our interpretation of imaging and behavioural studies.

8.5.1.2 Indirect influence of puberty by altering neurocognitive strategy

An alternative way in which puberty may indirectly influence brain development could be through altering neurocognitive strategies, resulting in changing patterns of structural development and functional activation (Durston et al., 2006). Transitions in social experience or cognitive strategy associated with pubertal advances may cause a shift in how a young person navigates a situation, by influencing the extent to which adolescents rely upon anterior temporal cortex representations during situations that provoke social emotions such as guilt and embarrassment (see **Chapter 5**) or the relative value attributed to a rewarding stimulus within the OFC and vmPFC (see **Chapter 7**). This potential shift in strategy may influence the relative levels of functional activation of different areas, as well as their structures and the connections between different regions that form part of a functional network.

This hypothesis of changing neurocognitive strategies with pubertal development is consistent with the known roles of different brain regions identified across the empirical chapters in this thesis. However, to date there are very few behavioural tasks designed to investigate neurocognitive strategies, making the hypothesis challenging to test. Development of new tasks and testing procedures to investigate this key question would undoubtedly shed new light on the changing behaviour and patterns of brain maturation seen in adolescence.

8.5.1.3 Relationship between structural and functional and connectivity changes in the brain

Each of the studies presented in this thesis addressed a different aspect of brain development. However, it is highly likely changes in the different modalities of brain structure and function, and the associated structural and functional connectivity across brain regions, are not independent, but are instead all highly related. Teasing apart how these different variables all affect one another will be crucial if we are to understand adolescent brain maturation and the potentially causal role of puberty in

this. Advancing analytic techniques and software for MRI analysis, together with the emergence of additional large-scale longitudinal MRI databases will allow the interplay between structure, function and connectivity to be investigated.

Using automated surface-based reconstruction tools like Freesurfer, the anatomy of target structures can be defined for individual participants e.g. ventral striatum, and individual fMRI data can be extracted from each defined region. This can provide directly comparable fMRI data for equivalent anatomical structures within the same individual over time, as well as across different individuals. In the same way, the reverse can be performed using localiser tasks, whereby specific regions of functional activation during specific tasks can be linked to specific anatomical regions within different individuals. Combining these different MRI techniques in future studies may lead to more accurate evaluation of task-related fMRI data, and will also allow the relationship between brain structure and function within and between individuals to be interrogated. Studies using longitudinal datasets and combining structural, functional and connectivity data in this way may be able to track the process of change between these modalities, and relate them to changes, in participant behaviour. The future use of longitudinal datasets will allow individual level analysis, both looking at intra-individual change over time, and comparing trajectories of growth and development between individuals. This more sensitive analysis would allow a more nuanced interpretation of data than the current group-based analyses traditionally used, and may help in understanding more about the key variables driving brain development.

8.5.2 Improving understanding of the physiological changes of puberty

The publication of Tanner's influential work in the late 1960s transformed our ability to describe and assess pubertal development in a standardised way. In the intervening 50 years, many studies of pubertal development have been undertaken in different populations around the world. However, despite this body of work, there are significant areas where our understanding of puberty remains lacking. While it is known that there are many physiological changes that occur in tandem with puberty

e.g. cardiovascular changes, the mechanisms behind these changes are poorly understood. By considering how pubertal development influences other physiological systems, we can improve our understanding of the impact on the brain.

There continues to be relatively little data documenting the natural variation in pubertal tempo, and whether the speed at which an individual progresses through puberty is related to the age at which they start. Having a clearer understanding of this progression through longitudinal data analysis would provide useful information to share with patients, and parents of patients, who experience early or late pubertal onset, and may improve our awareness of how this variation might affect a young person's successful progression into adolescence.

Use of circulating sex steroid hormone levels, through serum sampling or salivary sampling, have long been used as a proxy measure for approximate pubertal development across a range of disciplines. Although hormonal levels are frequently used to estimate pubertal development, the data available describing the diurnal variation and cyclical patterns of these hormones in teenagers is limited. This is particularly the case for oestradiol in males, and testosterone in females, which have had less research focus. Many studies using hormonal sampling are cross-sectional and rely on a single hormone measure, and future longitudinal descriptive work outlining the ranges and variation of salivary hormone levels across different ethnic populations, and how these relate to other physiological and psychological aspects of development, would be invaluable.

One key area for understanding the hormonal influence on the brain lies in the role of the enzyme aromatase. This converts testosterone into oestradiol in peripheral tissues, and is known to be present at the site of oestradiol receptors in the brain. Understanding the development of aromatase action during adolescence will be important to grasp the role of testosterone in activating oestradiol receptors in the brain.

8.5.3 Consideration of the implications for wider society including education, health and the law

Adolescence is a key period in the human life course and crucial for a successful transition into adulthood. The choices and decisions that are made during this time impact significantly on the behavioural, social and health trajectories for an individual. The work in this thesis highlights the relationship between pubertal development and the structural and functional maturation of the brain, and supports the notion that puberty may have biological as well as psychosocial impacts on behaviour, which could have implications for education, health and the law.

8.5.3.1 Education

Increasing knowledge of adolescent brain development has many implications for education, and the findings in this thesis may help to further understand the reported associations between puberty and educational attainment during adolescence and beyond. Studies in girls have reported that girls undergoing early puberty are more likely to have poor academic attainment in secondary school than on-time or late maturing peers (Mendle, Turkheimer, & Emery, 2007) and more problems within school (Cavanagh, Riegle-Crumb, & Crosnoe, 2007). It has been suggested that this could relate a link between early puberty and problem behaviour including getting into trouble at school, absenteeism and truancy (Cavanagh, 2004; Graber, Lewinsohn, Seeley, & Brooks-Gunn, 1997; Haynie, 2003; Simmons & Blyth, 1987; Stattin & Magnusson, 1990), resulting in a negative impact on school performance. An alternative theory suggests that the relative better performance in girls who experience puberty later relates to the differing impact of puberty on social interactions, whereby girls who experience early puberty are more distracted by peer and romantic relationships, and focus less on their academic work, while girls who experience puberty at an older age have fewer social distractions (Simmons & Blyth, 1987). In contrast, studies in males have shown an association between late pubertal development and poor academic achievement (Koivusilta & Rimpelä, 2004), which may relate to similar a similar trend with socio-emotional development that late puberty in boys can negatively impact on their successful navigation of an increasingly complex

social environment (Graber et al., 1997; Laitinen-Krispijn et al., 1999; Nottelmann et al., 1987).

The studies in this thesis relate pubertal development to both continuing social brain development and the on-going maturation of decision-making and risk-taking behaviours. Improved understanding of the relationship between puberty and the developing brain amongst adolescents, their parents and teachers, as well as increased recognition of the potential impact this may have on behaviour, could lead to greater awareness of at-risk individuals and novel strategies to prevent the negative consequences outlined above. The findings in this thesis emphasise that, during adolescence, the brain is continuing to change in both its structure and its function, suggesting that continues to be malleable and sensitive to change (Burnett, Thompson, et al., 2011). Furthermore, this thesis illustrates that some aspects of brain maturation relate better to pubertal development than to chronological age alone. The majority of educational systems rely on age-based methods to judge the appropriate curriculum, necessary levels of attainment, and timing of assessments. The evidence in this thesis that the timing of puberty may play an influential role in brain maturation challenge this model, and support a more multi-faceted view of adolescent development. This may be of particular importance where pubertal timing overlaps with significant educational transitions. For example, changing schools at 11 years of age may disproportionately impact on girls who are simultaneously experiencing early puberty (Koenig & Gladstone, 1998; Simmons & Blyth, 1987); whereas the pressure and social stress of making career choices and completing major examinations may be particularly taxing for 16 year old boys who are still experiencing pubertal changes in their bodies and their brains.

8.5.3.2 Health

During adolescence, individuals are expected to be healthy, being faster, stronger and more resistant to disease than earlier in childhood, and compared to much of adulthood (Dahl, 2004; Patton & Viner, 2007). Despite this perception, there are specific health challenges that particularly affect adolescents: management of long-term health conditions; the emergence of mental health conditions; and the rise in

mortality and morbidity associated with accidents and injuries. The studies in this thesis, as part of a multidisciplinary body of emerging research, may help understand and inform some of these key health issues.

Young people with long-term health conditions must transition during adolescence from paediatric to adult care settings, and from parental/carer management of their disease to self-management, and success of this transition significantly impacts on their short and long-term disease outcomes (McDonagh, 2005; Stam, Hartman, Deurloo, Groothoff, & Grootenhuis, 2006). Successful transition involves an awareness of socio-emotional and cognitive maturation of an individual, and an understanding of the priorities and perceptions of each patient, to tailor a service and a treatment plan to their needs (McDonagh, 2005). Adolescent neuroscience can been used to help develop transition services, and to advocate for young people's needs (Colver & Longwell, 2013). The studies in this thesis build on our understanding of adolescent neuroscience, and can be used to highlight that services need to incorporate factors aside from chronological age alone that may influence maturation, and an individual's preparedness to take responsibility for their disease management.

As outlined in the introduction, an estimated 75% of mental health disorders have their onset before the age of 25 years (Kessler et al., 2007), and the prevalence of some of these conditions, particularly depression and anxiety disorders, have been associated with puberty (Angold & Costello, 2006; Joinson et al., 2012). Numbers of structural and functional MRI studies investigating adolescent mental health pathology are rapidly increasing, and by demonstrating an association between puberty and the structural and functional development of the brain, this thesis raises the need for future research to incorporate markers of pubertal development in their study design.

In both England and the US there are increases across the second decade of life in the numbers of admissions to hospital with unintentional injuries (Hargreaves & Viner, 2014; Willoughby et al., 2013), and the leading causes of death in adolescence are road traffic accidents and injuries (GHDx, 2014). Furthermore, during adolescence, many risk-taking behaviours (e.g. smoking, alcohol and substance use) that adversely affect

future health emerge (Dahl, 2004; Steinberg, 2008). To date, interventions to reduce adverse health risk behaviours have had limited success, and improved understanding of adolescent brain development, and the potential mechanisms driving these behaviours including puberty, may lead to innovative preventative methods and help to highlight young people who potentially may be at higher risk of these adverse behaviours.

8.5.3.3 Legal implications

Laws regarding rights and responsibilities tend to rely on age cut-offs. These are often based on historical precedents, and vary both between countries, and depending on the right or responsibility being bestowed. Evidence from adolescent neuroscience has been used to challenge some of these precedents, particularly in the US, where evidence of on-going brain development has been used to successfully argue against life imprisonment or death sentences individuals under 18 years of age (*Graham vs. Florida*, 2010, *Miller vs. Alabama*, 2012, *Roper vs, Simmons*, 2005; see Steinberg, 2013).

The results of the studies in this thesis suggest that the maturation of the brain is related not just to age but also to pubertal development. For laws affecting adolescents, such at the age of criminal responsibility (10 years in UK; 8-16 years in other European countries) or minimum driving age (17 years in UK; 14-18 years in other countries), these findings are relevant but challenging to incorporate within a legal framework. Importantly, adolescent neuroscience does not currently provide any specific evidence regarding an age at which the brain is 'mature', or a point at which an individual is 'capable'. Neuroscientific findings are limited to demonstrating that the brain continues to developed into adolescent in terms of both structure and function, and shows greater flexibility and potential for change than is seen in the adult brain.

Some areas of the law have evolved the ability to assess an individual's maturity in bestowing their rights. One example is the assumption of capacity to consent to a medical treatment. In the UK, young people aged 16 and over are presumed to be capable of consenting to medical treatment as for adults (but not to refusal of

treatment). However, children under the age of 16 years can be judged to be capable of consenting to a treatment if they meet particular criteria. This judgement of capacity is limited to a specific decision on a specific occasion. Such an individual approach to other areas of the law would not necessarily be practical, but this thesis highlights the limitations of relying solely on chronological age as an indicator of maturity in adolescence.

8.6 Concluding remarks

This thesis uses a combination of sMRI and fMRI, DTI and PPI analyses to explore the role of puberty in adolescent brain development. It adds to our current understanding of the relationship between puberty and brain maturation by focusing on pubertal influences independent of chronological age. The studies in this thesis demonstrate that puberty relates to a number of different aspects of brain development during adolescence, but that other variables are also important in this process. By using a variety of different methods to assess pubertal status, the studies in the thesis provide an indication of the complexities of pubertal development, and the results raise many further questions about potential mechanisms underlying the key findings.

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APPENDIX

Sensation-Seeking Scale (SSS)

*For each of the statements below, please circle whether you would say that **the statement is true or false for you**. Do not leave any items blank.*

*We are interested only in **your** likes or feelings, not in how others feel about these things or how one is supposed to feel. There are no right or wrong answers. **Please be frank and give your honest appraisal of yourself**.*

1. I like to have new and exciting experiences and sensations even if they are a little frightening.
    a. Yes
    b. No

2. I like doing things just for the thrill of it.
    a. Yes
    b. No

3. I sometimes like to do things that are a little frightening.
    a. Yes
    b. No

4. I'll try anything once.
    a. Yes
    b. No

5. I sometimes do 'crazy' things just for fun.
    a. Yes
    b. No

6. I like wild and uninhibited parties.
    a. Yes
    b. No

Barratt Impulsivity Scale (BIS)

*People differ in the ways they act and think in different situations. This is a test to measure some of the ways in which **you tend to think and act**. Read each statement and **circle the appropriate number on the right side of this page**. Please answer all the statements and do not leave any of the statements blank.*

		Rarely/ Never	Occa-sionally	Often	Usually/ Always
1.	I plan tasks carefully.	1	2	3	4
2.	I do things without thinking.	1	2	3	4
3.	I make up my mind quickly.	1	2	3	4
4.	I am happy-go-lucky.	1	2	3	4
5.	I don't "pay attention."	1	2	3	4
6.	I have "racing" thoughts.	1	2	3	4
7.	I plan trips well ahead of time.	1	2	3	4
8.	I am self-controlled.	1	2	3	4
9.	I can concentrate easily.	1	2	3	4
10.	I save my money regularly.	1	2	3	4
11.	I can't keep still in school lessons.	1	2	3	4
12.	I am a careful thinker.	1	2	3	4
13.	I plan my career.	1	2	3	4
14.	I say things without thinking.	1	2	3	4
15.	I like to think about complex problems.	1	2	3	4
16.	I change interests or hobbies frequently.	1	2	3	4
17.	I act "on impulse."	1	2	3	4
18.	I get easily bored when solving thought problems.	1	2	3	4
19.	I act on the spur of the moment.	1	2	3	4
20.	I am a steady thinker.	1	2	3	4
21.	I buy things on impulse.	1	2	3	4
22.	I can only think about one thing at a time.	1	2	3	4
23.	I often have unrelated thoughts while thinking.	1	2	3	4
24.	I am more interested in the present than the future.	1	2	3	4
25.	I am restless at the theatre.	1	2	3	4
26.	I like puzzles.	1	2	3	4
27.	I am future-orientated.	1	2	3	4

Critical Appraisal of Risky Events (CARE) questionnaire

For each of the following activities, HOW LIKELY IT IS THAT:

(A) You would experience some **positive consequence** (e.g. pleasure, win money, feel good about yourself etc.) if you engaged in this activity?
(B) You would experience some **negative consequence** (e.g. become sick, be injured, embarrassed, lose money, suffer legal consequences, fail in a class, or feel bad about yourself) if you engaged in this activity?
(C) **You will engage in this activity** in the next 6 months?

Mark your answers below, using a scale of 1 (not at all likely) to 7 (extremely likely):

	Not at all likely			**Moderately likely**			**Extremely likely**
Missing class or work:							
Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7
Grabbing, pushing or shoving someone:							
Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7
Drinking alcohol:							
Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7
Playing non-contact team sports, for example football, cricket:							
Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7
Smoking cigarettes:							
Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Snow or water skiing:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Getting into a fight or argument:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Having sex:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Making a scene in public:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Smoking marijuana:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Not studying for an exam or quiz:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Driving after drinking alcohol:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Damaging/destroying public property:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Leaving tasks or homework till the last minute:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Hitting someone with a weapon or object:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Rock or mountain climbing:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Playing drinking games:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Trying/using drugs other than alcohol or marijuana:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Having sex without protection against pregnancy and sexually transmitted infections:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Failing to do an assignment or homework:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Slapping someone:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Having sex with someone you have just met or don't know very well:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Mixing drugs and alcohol:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Not studying or working hard enough:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Punching or hitting someone with your fist:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Sex with multiple partners:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Playing individual sports:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Drinking more than 5 alcoholic beverages:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Disturbing the peace:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Drinking alcohol too quickly:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7

Leaving a social event with someone you have just met:

Likelihood of positive consequence	1	2	3	4	5	6	7
Likelihood of negative consequence	1	2	3	4	5	6	7
Likelihood of doing this activity in the next 6 months	1	2	3	4	5	6	7