Research and Perspectives
in Endocrine Interactions

J.-P. Bourguignon J.-C. Carel
Y. Christen (Eds.)

Brain Crosstalk in Puberty and Adolescence

FONDATION IPSEN
POUR LA RECHERCHE THERAPEUTIQUE

Springer

Research and Perspectives in Endocrine Interactions

More information about this series at
http://www.springer.com/series/5241

Jean-Pierre Bourguignon • Jean-Claude Carel • Yves Christen
Editors

Brain Crosstalk in Puberty and Adolescence

Springer

Editors
Jean-Pierre Bourguignon
Developmental Neuroendocrinology Unit,
G GIGA Neurosciences
University of Liège
Liège
Belgium

Jean-Claude Carel
Serv. d'endocrinologie diabetol. pédiatr
Hopital Robert Debre
Paris
Paris
France

Yves Christen
Fondation IPSEN
Boulogne-Billancourt Cedex
France

ISSN 1861-2253
ISSN 1863-0685 (electronic)
ISBN 978-3-319-09167-9
ISBN 978-3-319-09168-6 (eBook)
DOI 10.1007/978-3-319-09168-6
Springer Cham Heidelberg New York Dordrecht London

Library of Congress Control Number: 2014956674

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Contents

Volume Editors

Bourguignon, Jean-Pierre
Department of Pediatrics, CHU de Liège, and Developmental Neuroendocrinology unit, GIGA Neurosciences, University of Liège, Liège, Belgium

Carel, Jean-Claude
Endocrinologie Diab.Ped. & Inserm U690, Université Paris 7 Denis Diderot, Hôpital Robert Debré, 48, Boulevard Sérurier, 75019 Paris, France

Christen, Yves
Fondation IPSEN, 65 Quai Georges Gorse, 92100 Boulogne-Billancourt, France

List of Contributors

Julie Bakker Center of Expertise on Gender Dysphoria, Department of Medical Psychology, Neuroscience Campus Amsterdam, VU University Medical Center, Amsterdam, The Netherlands

Netherlands Institute for Neuroscience, Amsterdam, The Netherlands

GIGA Neuroscience, University of Liege, Liege, Belgium

Jean-Pierre Bourguignon Department of Pediatrics, CHU de Liège, Site ND Bruyères, Liège, Belgium

Developmental Neuroendocrinology Unit, GIGA Neurosciences, University of Liège, Liège, Belgium

S.M. Burke Center of Expertise on Gender Dysphoria, Department of Medical Psychology, Neuroscience Campus Amsterdam, VU University Medical Center, Amsterdam, The Netherlands

Netherlands Institute for Neuroscience, Amsterdam, The Netherlands

Jean-Claude Carel Endocrinologie Diab.Ped. & Inserm U690, Université Paris 7 Denis Diderot, Hôpital Robert Debré, Paris, France

Juan Manuel Castellano Division of Neuroscience, Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, OR, USA

Jerome Clasadonte Jean-Pierre Aubert Research Center, U837, Development and Plasticity of the postnatal Brain, Inserm, Lille, France

School of Medicine, UDSL, Lille, France

Nicolas de Roux Inserm U1141, Université Paris Diderot. Hôpital Robert Debré, Paris, France

Alexander H. Denker Child Psychiatry Branch, National Institute of Mental Health, Bethesda, MD, USA

Françoise Domine Department of Pediatrics, CHU de Liège, Site CHR de la Citadelle, Liège, Belgium

Paul W. Frankland Program in Neurosciences and Mental Health, Hospital for Sick Children, Toronto, ON, Canada

Jay N. Giedd Child Psychiatry Branch, National Institute of Mental Health, Bethesda, MD, USA

Fabienne Glowacz Clinic of Delinquency, Social Maladjustment and Integration Processes, University of Liège, Liège, Belgium

Anne-Lise Goddings MRC Clinical Training Research Fellow, UCL Institute of Child Health, London, UK

Lukas Huijbregts Inserm U1141, Université Paris Diderot. Hôpital Robert Debré, Paris, France

Marie-Christine Lebrethon Department of Pediatrics, CHU de Liège, Site ND Bruyères, Liège, Belgium

Developmental Neuroendocrinology Unit, GIGA Neurosciences, University of Liège, Liège, Belgium

Alejandro Lomniczi Division of Neuroscience, Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, OR, USA

Pierre-André Michaud Multidisciplinary Unit for Adolescent Health, Lausanne University Hospital – CHUV, Lausanne, Switzerland

Sergio R. Ojeda Division of Neuroscience, Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, OR, USA

Anne-Simone Parent Department of Pediatrics, CHU de Liège, Site ND Bruyères, Liège, Belgium

Developmental Neuroendocrinology Unit, GIGA Neurosciences, University of Liège, Liège, Belgium

Vincent Prevot Jean-Pierre Aubert Research Center, U837, Development and Plasticity of the postnatal Brain, Inserm, Lille, France

School of Medicine, UDSL, Lille, France

Basak Selcuk Division of Neuroscience, Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, OR, USA

Cheryl L. Sisk Department of Psychology and Neuroscience Program, Michigan State University, East Lansing, MI, USA

Kemal Sonmez Division of Neuroscience, Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, OR, USA

Brooke Tata Inserm U1141, Université Paris Diderot. Hôpital Robert Debré, Paris, France

Manuel Tena-Sempere Department of Cell Biology, Physiology and Immunology, University of Córdoba; CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Madrid, Spain

Instituto Maimónides de Investigaciones Biomédicas (IMIBIC), Hospital Universitario Reina Sofia, Córdoba, Spain

Russell Viner General and Adolescent Paediatrics Unit, UCL Institute of Child Health, London, UK

Anne L. Wheeler Kimel Family Translational Imaging Genetics Laboratory, Research Imaging Centre, Centre for Addiction and Mental Health, Toronto, ON, Canada

Hollis Wright Division of Neuroscience, Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, OR, USA

Introduction

Puberty and adolescence are key developmental processes occurring in the transition period between childhood and adulthood. They involve profound physical and behavioral changes that share dependency on maturational events in the central nervous system (CNS). The neurobiology of puberty and adolescence has made important progress during the past decade through finely tuned studies on behavior, CNS imaging, and molecular neurobiology.

The aim of the symposium on which these proceedings are based was to provide the attendants with a pathophysiological perspective on the role of CNS in puberty and adolescence, starting from genetic/molecular aspects, going through structural/imaging changes, and leading to physical/behavioral characteristics. Renowned investigators involved in both animal and human research convened and shared with the participants their recent data as well as overall appraisals of relevant questions around CNS control of puberty and adolescence.

Among other findings, some contributors have underscored that adolescence is a critical phase for risk of addiction and mental illnesses in adulthood. Such a critical period may be longer than commonly expected, since brain imaging studies indicate that the final maturational changes associated with adolescence can occur as late as between 25 and 30 years.

Others have emphasized the importance of the prepubertal period that is crucial for the effect of testosterone on male sexual behavior, the gender dimorphic androgen effects on executive functioning, the epigenetic control of transcriptional repression of the neuroendocrine regulators of the onset of puberty, and the interactions between leptin- and kisspeptidergic-permissive effects on the onset of puberty.

Finally, some factors arising in the fetal and perinatal periods have emerged as possible new players in the early control of the onset of puberty. They involve microRNAs, rabconnectin-3alpha, and endocrine-disrupting chemicals.

No doubt the present book will inspire those involved in either scientific research or clinical practice or both in the fascinating field of puberty and adolescence.

Liège, Belgium — Jean-Pierre Bourguignon
Paris, France — Jean-Claude Carel
Boulogne-Billancourt, France — Yves Christen

Drugs and the Adolescent Brain

Anne L. Wheeler and Paul W. Frankland

Abstract Adolescence is a developmental stage that is associated with increased risk-taking, novelty-seeking and greater vulnerability to peer pressure. Given this, it is perhaps not surprising that adolescence is also a time that is associated with drug experimentation. In some cases, drug experimentation progresses to drug dependence, a state commonly referred to as addiction. As the adolescent brain is still developing, how does drug dependence impact brain development? Imaging studies suggest that cocaine dependence is associated with abnormalities in brain structure in humans. However, it is unclear whether these differences in brain structure predispose an individual to drug use or are a result of cocaine's action on the brain. We have addressed this issue by studying the impact of chronic cocaine exposure on brain structure and drug-related behavior in mice. In our studies, mice were exposed to cocaine at two developmental time periods: adolescence (27–46 days-old) and young adulthood (60–79 days-old). Following 30 days of abstinence, either fixed brain T2 weighted MRIs were acquired on a 7T scanner at 32 μm isotropic voxel dimensions or mice were assessed for sensitization to the locomotor stimulant effects of cocaine. Three automated techniques (deformation-based morphometry, striatum shape analysis and cortical thickness assessment) were used to identify population differences in brain structure in cocaine vs. saline-exposed mice. We found that cocaine induced changes in brain structure, and these were most pronounced in mice exposed to cocaine during adolescence. Many of these changes occurred in brain regions previously implicated in addiction, including the nucleus accumbens, striatum, insular cortex, orbital frontal cortex and medial forebrain bundle. Furthermore, exposure to the same cocaine regimen caused sensitization to the locomotor stimulant effects of cocaine, and these effects were again more pronounced in mice exposed to cocaine during adolescence. These results suggest that altered brain structure following one month of abstinence may

A.L. Wheeler
Kimel Family Translational Imaging Genetics Laboratory, Research Imaging Centre, Centre for Addiction and Mental Health, 250 College St., Toronto, ON M5T 1R8, Canada

P.W. Frankland (✉)
Program in Neurosciences and Mental Health, Hospital for Sick Children, 555 University Ave., Toronto, ON M5G 1X8, Canada
e-mail: Paul.frankland@sickkids.ca

J.-P. Bourguignon et al. (eds.), *Brain Crosstalk in Puberty and Adolescence*,
Research and Perspectives in Endocrine Interactions 13,
DOI 10.1007/978-3-319-09168-6_1

contribute to these persistent drug-related behaviors and may identify cocaine exposure as the cause of these morphological changes.

Introduction

An estimated 205 million people in the world use illicit drugs, and around 25 million of these can be described as having a drug use disorder (UNODC 2009). Of course, these numbers balloon if legal drugs such as alcohol and nicotine are additionally considered. At the outset, people take these drugs of abuse to benefit from their short-term effects, which include pleasurable sensation, the alteration of mental state and, in some instances, improved performance (Volkow and Li 2004). These acute effects are mediated by relatively short-lived changes in brain chemistry and physiology that are largely related to the molecular pharmacology of the drugs themselves (Nestler 2005). While the five major classes of abused drugs – psychostimulants, opiates, alcohol, cannabinoids and nicotine – have different mechanisms of action in the brain, ultimately they all act either directly or indirectly on dopamine function in the mesolimbic pathway. The mesolimbic circuitry consists of dopaminergic neurons that project from the ventral tegmental area (VTA) in the midbrain to the limbic system via the nucleus accumbens (NAc) to produce reinforcing effects (Pierce and Kumaresan 2006).

A portion of individuals who take drugs will progress to a pattern of repeated drug use that is typified by an intense desire for the drug and impaired ability to control urges to take that drug, even at the expense of serious adverse health and social consequences. This state is known as substance dependence and is often referred to as addiction. Substance dependence is not due solely to the physical dependence that underlies the withdrawal symptoms following cessation of drug use because, even after withdrawal symptoms subside, the risk of relapse remains very high (Milton and Everitt 2012). This increased relapse risk suggests that repeated drug use permanently alters the brain, producing persistent changes in brain circuits that favor learned drug-associated behaviors at the expense of adaptive responding for natural rewards (Kalivas and O'Brien 2008).

Repeated Drug Use Has a Long-Term Impact on Brain Circuitry

How do drugs of abuse permanently alter the brain? Pre-clinical and clinical research suggest that repeated use of drugs changes the brain of the user at the molecular, cellular and circuit organizational levels. These changes likely underlie a shift toward behaviors that are more reflexive and, consequently, much less

amenable to cognitive interference. Brain alterations have been studied in most detail in the neurocircuits responsible for reward, motivation, cognitive control and memory. These circuits are directly or indirectly modulated by dopamine and are highly connected to each other through glutamatergic and GABA-ergic projections that allow them to interact to generate behavioral output in response to reinforcing stimuli (Baler and Volkow 2006).

The rewarding properties of drugs are mediated by dopamine, and enduring alterations in dopaminergic signaling following repeated drug use are thought to contribute to drug dependence (Diana 2011). For example, dopamine release in the NAc is decreased in drug-dependent rodents (Diana et al. 1999; Rossetti et al. 1992), and human imaging studies have shown a reduction in dopamine receptors (Volkow et al. 1996) accompanied by reduced release of endogenous dopamine in the ventral striatum of drug-dependent subjects (Martinez et al. 2005).

Motivation is thought to be regulated by the orbital frontal cortex (OFC), which is responsible for processing the motivational value of rewarding stimuli (Tremblay and Schultz 1999). For example, the OFC is activated upon presentation of cocaine-associated stimuli in humans (Garavan et al. 2000) and rodents (Thomas et al. 2003). Furthermore, OFC dysfunction persists even following long periods of drug abstinence in drug-dependent individuals (Goldstein and Volkow 2002).

The frontal cortex is responsible for cognitive control. The compromised ability of cocaine users to reign in uncontrollable urges has been linked to reduced activity in the anterior cingulate gyrus (ACG) and in the prefrontal cortex (PFC; Hester and Garavan 2004). Additionally, a significant aspect of drug dependence is the pathological narrowing of goal selection to those that are drug-related. The representation of goals, assignment of value to them, and selection of actions based on the resulting valuation depend on the PFC (Hyman 2005).

Neuroadaptations in learning and memory processes have been proposed to play an essential role in drug dependence as they can account for its persistence. First, chronic drug exposure causes strong associations to develop between drugs and the environmental stimuli and contexts in which they are encountered. Exposure to these drug-associated cues often precedes drug relapse even after long periods of abstinence (Torregrossa et al. 2011). This is consistent with drug-associated cues producing limbic activation and craving (Childress et al. 1999), with associated dopamine release (Volkow et al. 2006) in cocaine addicts. Second, changes in the dorsal striatum underlie the learning of specific motor actions to receive rewards that are linked to the rituals of drug consumption (Porrino et al. 2004). Shifting from voluntary drug use to more habitual and compulsive drug use represents a transition at the neural level from prefrontal cortical to striatal control over drug-seeking and drug-taking behavior, as well as a progression from ventral to more dorsal domains of the striatum (Everitt and Robbins 2005).

Initiation of Drug Use Often Occurs During Adolescence, Which May Increase Vulnerability to Drug Dependence

The onset of drug use often occurs during adolescence, a developmental transition from childhood to adulthood during which the brain continues to develop and change. Indeed, in Canada, 60 % of illicit drug users are between the ages of 15 and 24 (Statistics Canada 2003). Adolescence is thought of as a period when risk-taking and novelty-seeking are typical and individuals are hyper-responsive to peer pressure (Spear 2000); clearly, these factors may contribute to a propensity to experiment with drugs during adolescence, a prerequisite for drug dependence.

The effects of drugs on behavior may also differ in adolescence. For example, preclinical studies have shown that adolescent rodents are less sensitive than adult animals to many of the negative effects of alcohol such as alcohol-induced sedation and motor impairment (Little et al. 1996), as well as alcohol withdrawal-induced social depression and anxiety (Varlinskaya and Spear 2004). Additionally, adolescent rodents are subject to greater reinforcing properties of alcohol (Pautassi et al. 2008) and have increased sensitivity to its social-facilitation effects compared to adult animals (Varlinskaya and Spear 2002). Similar studies have demonstrated that adolescent rodents show increased sensitivity to the rewarding effects of nicotine (Natividad et al. 2013). This 'enhanced' sensitivity may lead to prolonged periods of drug experimentation during adolescence and promote excessive consumption. Epidemiological studies indicate that, when drug use is initiated during adolescence (rather than during adulthood), there are higher lifetime rates of drug use and faster progression to dependency (Anthony and Petronis 1995; Grant and Dawson 1998; O'Brien and Anthony 2005). Together these data suggest that the adolescent brain may contribute to drug experimentation and excessive consumption and, at the same time, drug experimentation and excessive consumption at this age likely impact ongoing development of the brain.

Brain Development Throughout Adolescence and Its Susceptibility to the Effects of Drug Use

The structural and functional development of the brain is remarkably complex during infancy and childhood and continues on a dynamic trajectory throughout adolescence. Magnetic resonance imaging (MRI) studies indicate that white matter volume increases into the third decade of life whereas the volume of gray matter in the brain follows an inverted U-shaped trajectory, peaking in late childhood and decreasing through adolescence (Giedd et al. 1999; Giedd 2008). The increase in white matter volume may be associated with continued myelination of white matter tracts or increases in the caliber of axons (Paus et al. 2008). The decline in gray matter is thought to reflect the reorganization and refinement of synaptic connections through the process of synaptic pruning (Petanjek et al. 2011), or it may be

a result of the intracortical myelination and increased axonal caliber causing less tissue to be classified as gray matter in MRI scans (Blakemore 2012). Sensory and motor regions mature before frontal and temporal regions in terms of gray matter density (Gogtay et al. 2004) and cortical thickness (Shaw et al. 2008).

Does the continued development of the adolescent brain make it more vulnerable to environmental factors such as drug exposure? If so, then differences in the age of first encounter with psychoactive compounds may influence the subsequent trajectory of brain development, which may, in turn, affect patterns of drug-related behavior. For example, positive associations between hippocampal volumes and age of first alcohol use suggest that early adolescence may be a period of heightened risk for the neurotoxic effects of alcohol (De Bellis et al. 2000). Similarly, abnormal white matter microstructure in neocortical association pathways as well as in projection and thalamic pathways is found in adolescents with substance-use disorder (Baker et al. 2013). Finally, alcohol use disorder has been associated with increased volume of the PFC in adolescent males and decreased volume of the PFC in adolescent females compared to healthy controls (Medina et al. 2008).

Neural Substrates of Adolescent-Specific Drug-Related Behaviors

What is unique about the adolescent brain that promotes experimentation and excessive consumption of drugs? The differing developmental trajectories of two of the key regions implicated in drug dependence – the PFC and the striatum – are thought to contribute to the increased propensity to take drugs during adolescence (Casey et al. 2000). The PFC, known to be important for cognitive control, undergoes delayed maturation and continues to develop through the adolescent years. In contrast, the striatum, which is critical for detecting and learning about rewarding cues in the environment, develops sooner and is already mature in adolescence.

Post-mortem analyses (Bourgeois et al 1994; Huttenlocher 1979; Rakic et al. 1994) and in vivo MRI studies (Gogtay et al. 2004; Shaw et al. 2008) indicate that PFC structure continues to change throughout adolescence. At the molecular level, there are also developmental differences in the density of D1 and D2 dopamine receptors. In the striatum, D1/D2 density peaks early in adolescence, followed by a loss of these receptors by young adulthood in humans (Seeman et al. 1987) and in rats (Tarazi and Baldessarini 2000). In contrast, in the PFC, D1 and D2 receptor density does not peak until late adolescence and young adulthood in humans (Weickert et al. 2007) and in rats (Andersen et al. 2000). These differences in maturation may also be revealed at the functional level: when comparing adolescents to either children or adults, functional imaging studies demonstrate exaggerated ventral striatum activity, relative to PFC activity in response to reward manipulation (Galvan et al. 2006).

This imbalance in prefrontal and striatal systems in the brain has been proposed to underlie adolescent-specific behaviors that include (1) an increased propensity to take drugs and (2) an increased impact of drugs on behavior. Increased experimentation with drugs may result because mature striatal projections are sensitive to motivational stimuli and promote drug consumption; at the same time, drug consumption is left unchecked because delayed PFC maturation leads to poor top-down cognitive control and an impaired ability to manage drug consumption (Casey and Jones 2010). Drug-taking during the period of adolescence may exacerbate an already enhanced ventral striatum response, resulting in heightened reinforcing properties of the drug-impacting drug-induced behaviours.

Cocaine Use Is Associated with Structural Differences in the Brains of Adults as Detected by MRI

Repeated exposure to drugs of abuse can result in structural changes in the brain that can be probed in vivo with MRI. Advances in MRI technologies have allowed the detailed, noninvasive and safe assessment of human brain structure and function. Furthermore, the recent increase in availability of high-resolution clinical scanners allows for the collection of large samples of brain imaging data from drug-addicted individuals. These subjects have rich case histories that can be collected alongside the brain scans, which may allow for the identification of associations between these factors and imaging phenotypes. However because these subjects and their experiences are so distinct from one another, a large amount of heterogeneity is present in these studies.

Approximately 1 % (Bucello et al. 2010) of the 21 million users of cocaine worldwide (UNODC 2011) are considered to develop cocaine drug dependence. Cocaine is a psychostimulant and its mechanism of action is to directly increase dopamine in the reward centers of the brain by inhibiting dopamine reuptake. Though dependence on drugs of abuse other than cocaine is more common, other drugs typically modulate dopamine cell firing through their effects on other types of receptors. Thus cocaine is a good drug to study in terms of direct effects of long-term use on the reward systems of the brain.

There is a large collection of MRI studies that have investigated the effects of cocaine use on brain structure. Gray matter reductions in the frontal cortex (Franklin et al. 2002; Matochik et al. 2003) and temporal lobe (Bartzokis et al. 2000; Franklin et al. 2002) have been reported. Subcortical alterations include reduced amygdala volume (Makris et al. 2004) and increased (Ersche et al. 2011; Jacobsen et al. 2001) as well as decreased (Barros-Loscertales et al. 2011; Hanlon et al. 2011) caudate and putamen volumes in cocaine-dependent subjects. White matter alterations include lower fractional anisotropy – an index of white matter microstructure –in inferior frontal white matter (Lim et al. 2008), greater severity of white matter signal hyperintensities on MRI (Lyoo et al. 2004), less frontal white-matter volume

percentage than controls (Schlaepfer et al. 2006) and absence of age-related expansion in white matter volume in frontal and temporal lobe (Bartzokis et al. 2002) in cocaine-dependent subjects compared to controls.

Whether or not these morphological differences predated the onset of drug use or are a consequence of drug use is less clear. In the studies cited above, there were no associations between these imaging phenotypes and measures of drug use, suggesting that these morphological differences could be developmental in nature, potentially predisposing individuals to drug use. Consistent with this view, a recent study reported volumetric alterations in several gray matter regions, including the striatum and insular cortex in human cocaine addicts and their non-stimulant-dependent siblings, suggesting that certain brain abnormalities precede drug use and predispose these individuals to drug dependence (Ersche et al. 2012).

In contrast, some more recent studies have reported these types of associations between imaging phenotypes and measures of drug use, suggesting that these alterations may be a result of the drug use on the brain. For example, duration of cocaine use was negatively correlated with cerebellar gray matter volume (Sim et al. 2007), as well as with the volume of the bilateral middle frontal gyrus, left superior frontal gyrus, parahippocampus, posterior cingulate, amygdala, insula, and right middle temporal gyrus (Barros-Loscertales et al. 2011). Greater duration of cocaine dependence was correlated with greater gray matter volume reduction in orbitofrontal, cingulate and insular cortex (Ersche et al. 2011), and a thinner cortex was correlated with cocaine use in some regions (Makris et al. 2008). It seems as though morphological differences associated with cocaine dependence reflect both pre-existing abnormalities (that perhaps predispose individuals towards dependence) and the consequences of exposure to cocaine itself.

Limitations to Human Brain Imaging Studies That Investigate the Associations Between Drug Use and Brain Structure

What is clear from the human MRI literature is that cocaine dependence is associated with abnormalities in brain structure in humans. However, in addition to difficulties in teasing out which brain structural alterations predispose individuals to drug use and which are results of drug use, there are several inherent challenges associated with isolating the impact of cocaine on brain structure in these human imaging studies. First, alcohol, nicotine and caffeine consumption are characteristic of chronic drug users (Compton et al. 2007), so concurrent drug use or other lifestyle factors may contribute to structural variability in the brain. Second, comorbid diagnoses of mood, anxiety and disruptive behavior disorders are common in cocaine-dependent subjects (Swendsen et al. 2010), making it difficult to attribute morphological differences to cocaine use specifically. Third, typically there is variability in subjects' history of cocaine exposure (e.g., pattern and

duration of cocaine use, drug quality, route of administration, periods of abstinence and total lifetime intake) and in the accuracy of self-reports concerning these factors. Additionally, in human studies it is often challenging to isolate the effect of age of onset of drug use on brain structure while controlling for total amount of drug exposure in an individual. Given these limitations, there are some advantages in using experimental animals to address how drugs of abuse impact the developing and adult brain.

Describing Age Related Effects of Cocaine Exposure in the Mouse Brain with MRI

The impact of cocaine exposure on brain structure can be assessed under controlled conditions with experimental animal models, which allows for careful control of the dose and timing of drug exposure while keeping variability in genetic and environmental influences to a minimum. Previous studies have used this approach to investigate cocaine-related changes in receptor distribution (Wolf 2010) and brain structure at the microscopic level (Robinson and Kolb 2004). Using a similar approach to the human imaging studies, we scanned mouse brains with high resolution mouse MRI (Nieman et al. 2007) to assess cocaine-associated changes in brain structure at the macroscopic level (Wheeler et al. 2013). As drug use is often initiated during adolescence, which is a period of potentially increased vulnerability to drug-induced alterations in structure and function, we compared the impact of daily cocaine exposure during adolescence (27–46 days old) and young adulthood (60–79 days old). Following 30 days of abstinence, fixed brain T2 weighted MRIs were acquired on a 7T scanner at 32 μm isotropic voxel dimensions. Population differences in brain structure in cocaine- vs. saline-exposed mice were identified with three automated MRI analysis techniques: deformation-based morphometry, striatum shape analysis and cortical thickness assessment. Additionally, separate groups of mice underwent the same treatment regimen and were then assessed for sensitization to the locomotor stimulant effects of cocaine.

The imaging results indicated that exposure to cocaine-induced changes in brain structure, and these alterations were most pronounced in mice exposed to cocaine during adolescence. Many of these changes occurred in brain regions previously implicated in drug dependence and involved in mediating cocaine's acute and chronic effects associated with reward and sensitization (Table 1; Fig. 1). Components of the mesolimbic dopaminergic pathway – nucleus accumbens, ventral pallidum and medial forebrain bundle – were reduced in volume, consistent with cocaine's primary action on this system. Long-term alterations within this system likely contribute to locomotor sensitization (Vanderschuren and Pierce 2010). In addition, cocaine exposure caused a decrease in the volume of the cortical amygdaloid area and anterior cingulate cortex, regions where decreased metabolic activity has been associated with cocaine craving in human subjects (Childress

Table 1 Mouse brain regions where volumetric differences are observed following cocaine exposure during adolescence. Direction of changes refers to cocaine-exposed mice relative to saline-exposed mice. Changes were observed at the voxel level after correcting for multiple comparisons with 5 % FDR correction

Brain region	Direction	Hemisphere	Region implicated in cocaine dependence
Orbital frontal cortex	Increase	Right	Yes
Striatum (anterior)	Increase	Right	Yes
Striatum (posterior)	Decease	Bilateral	Yes
Nucleus accumbens	Decease	Bilateral	Yes
Anterior cingulate cortex	Decrease	Bilateral	Yes
Ventral pallidum	Decrease	Bilateral	Yes
Medial forebrain bundle	Decrease	Bilateral	Yes
Insular cortex	Decrease	Bilateral	Yes
Cortico amygdaloid area	Decrease	Bilateral	Yes
Substantia Nigra	Increase	Bilateral	Yes
Anterior olfactory area	Increase	Right	No
Corpus callosum	Increase	Bilateral	No
Rhinal cortex	Decrease	Bilateral	No
Piriform cortex	Decrease	Right	No
Medulla	Increase	Bilateral	No

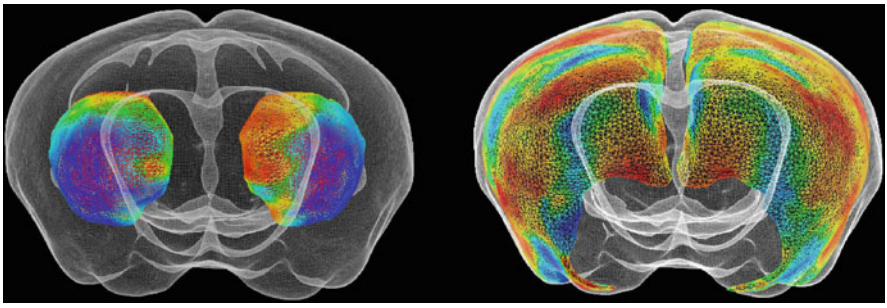

Fig. 1 Coronal view of morphological changes in the striatum and cortex induced by cocaine exposure during adolescence. Surface displacement of the striatum is color coded onto the surface of the 3D striatum and embedded in the mouse brain (*left*). Warm colors (*red*/*orange*) indicate inward displacement and cool colors (*blues*) indicate outward displacement of the striatum surface in animals treated with cocaine relative to animals treated with saline. Cortical thickness changes are color coded and superimposed onto the surface of the cortex and embedded in the mouse brain (*right*). Regions of the cortex that are thinner in adolescent animals treated with cocaine relative to saline-treated animals are indicated, with warm colors signifying bigger differences in thickness than cool colors

et al. 1999). The insular cortex also showed volume reduction, a region that has previously been associated with dependence on cocaine in human addicts (Ersche et al. 2011; Franklin et al. 2002). The substantia nigra, a dopamine-rich area of the midbrain showed an increase in volume associated with cocaine exposure, as did

the OFC. Previous imaging studies in humans and rodents implicate orbital frontal dysfunction in cocaine dependence through its impact on conditioned reinforcement and drug craving (Everitt et al. 2007).

There were two other major categories of morphological change that were most pronounced following adolescent cocaine exposure. First, we found volume as well as shape differences in the striatum following cocaine exposure in adolescent mice. Volume analysis showed that cocaine exposure led to portions of the anterior dorsal striatum increasing in size and the posterior striatum reducing in size, whereas shape analysis showed lateral outward displacement and medial inward displacement of the striatum bilaterally following cocaine exposure. Previous discrepant results from human imaging studies – with some reporting reduced (Barros-Loscertales et al. 2011; Hanlon et al. 2011) and others increased (Ersche et al. 2011; Jacobsen et al. 2001) striatal volume in cocaine-dependent subjects – may be accounted for by these bidirectional changes. Second, we found that adolescent but not adult cocaine exposure produced significant cortical thinning in regions of the right hemisphere. A comparison of mice treated with saline and cocaine during adolescence showed thinning of the cortex in the primary somatosensory, rhinal, piriform and insular cortices. Moreover, cocaine exposure caused locomotor sensitization, and these effects were again enhanced in mice exposed to cocaine during the adolescent period.

These mouse imaging results identify cocaine exposure as the cause of these morphological changes rather than being due to pre-existing anatomical differences, something that is difficult to tease apart in human imaging studies. Because not everyone who uses drugs goes onto develop a dependence, it is likely that these cocaine-induced effects may aggravate or interact with pre-existing abnormalities in brain structure, leading to behaviors related to drug dependence in humans. Additionally, these results suggest that altered brain structure following one month of abstinence may contribute to locomotor sensitization, a persistent drug-related behavior. Finally, the more pronounced alterations observed in adolescent animals suggest that adolescence is a sensitive period of brain development where brain structure morphology can be altered by drug exposure. This contributes to evidence that there is greater vulnerability of adolescents to experimentation with drugs of abuse and to subsequent drug dependence, supporting prevention of early exposure as an important strategy to curb drug dependence. Epidemiological studies show that the prevalence of drug use in adolescents has changed significantly over the past 30 years, and some of the decreases seem to be related to education about the risks of drugs (Volkow and Li 2004). Whole brain MRI for macroscopic analysis of brain structure in animal models is useful to complement human MRI results, due to the ability to maintain careful control of experimental conditions and reduce environmental and genetic influences on outcome measures.

References

Andersen SL, Thompson AT, Rutstein M, Hostetter JC, Teicher MH (2000) Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats. Synapse 37:167–169

Anthony JC, Petronis KR (1995) Early-onset drug use and risk of later drug problems. Drug Alcohol Depend 40:9–15

Baker ST, Yucel M, Fornito A, Allen NB, Lubman DI (2013) A systematic review of diffusion weighted MRI studies of white matter microstructure in adolescent substance users. Neurosci Biobehav Rev 37:1713–1723

Baler RD, Volkow ND (2006) Drug addiction: the neurobiology of disrupted self-control. Trends Mol Med 12:559–566

Barros-Loscertales A, Garavan H, Bustamante JC, Ventura-Campos N, Llopis JJ, Belloch V, Parcet MA, Avila C (2011) Reduced striatal volume in cocaine-dependent patients. Neuroimage 56:1021–1026

Bartzokis G, Beckson M, Lu PH, Edwards N, Rapoport R, Wiseman E, Bridge P (2000) Age-related brain volume reductions in amphetamine and cocaine addicts and normal controls: implications for addiction research. Psychiatry Res 98:93–102

Bartzokis G, Beckson M, Lu PH, Edwards N, Bridge P, Mintz J (2002) Brain maturation may be arrested in chronic cocaine addicts. Biol Psychiatry 51:605–611

Blakemore SJ (2012) Imaging brain development: the adolescent brain. Neuroimage 61:397–406

Bourgeois JP, Goldman-Rakic PS, Rakic P (1994) Synaptogenesis in the prefrontal cortex of rhesus monkeys. Cereb Cortex 4:78–96

Bucello C, Degenhardt L, Calabria B, Nelson P, Roberts A, Medina-Mora M (2010) NDARC technical report no. 30. National Drug and Alcohol Research Centre, University of NSW, Sydney

Casey BJ, Jones RM (2010) Neurobiology of the adolescent brain and behavior: implications for substance use disorders. J Am Acad Child Adolesc Psychiatry 49:1189–1201; quiz 1285

Casey BJ, Giedd JN, Thomas KM (2000) Structural and functional brain development and its relation to cognitive development. Biol Psychol 54:241–257

Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP (1999) Limbic activation during cue-induced cocaine craving. Am J Psychiatry 156:11–18

Compton WM, Thomas YF, Stinson FS, Grant BF (2007) Prevalence, correlates, disability, and comorbidity of DSM-IV drug abuse and dependence in the United States: results from the national epidemiologic survey on alcohol and related conditions. Arch Gen Psychiatry 64:566–576

De Bellis MD, Clark DB, Beers SR, Soloff PH, Boring AM, Hall J et al (2000) Hippocampal volume in adolescent-onset alcohol use disorders. Am J Psychiatry 157:737–744

Diana M (2011) The dopamine hypothesis of drug addiction and its potential therapeutic value. Front Psychiatry 2:64

Diana M, Muntoni AL, Pistis M, Melis M, Gessa GL (1999) Lasting reduction in mesolimbic dopamine neuronal activity after morphine withdrawal. Eur J Neurosci 11:1037–1041

Ersche KD, Barnes A, Jones PS, Morein-Zamir S, Robbins TW, Bullmore ET (2011) Abnormal structure of frontostriatal brain systems is associated with aspects of impulsivity and compulsivity in cocaine dependence. Brain 134(Pt 7):2013–2024

Ersche KD, Jones PS, Williams GB, Turton AJ, Robbins TW, Bullmore ET (2012) Abnormal brain structure implicated in stimulant drug addiction. Science 335:601–604

Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 8:1481–1489

Everitt BJ, Hutcheson DM, Ersche KD, Pelloux Y, Dalley JW, Robbins TW (2007) The orbital prefrontal cortex and drug addiction in laboratory animals and humans. Ann NY Acad Sci 1121:576–597

Franklin TR, Acton PD, Maldjian JA, Gray JD, Croft JR, Dackis CA, O'Brien CP, Childress AR (2002) Decreased gray matter concentration in the insular, orbitofrontal, cingulate, and temporal cortices of cocaine patients. Biol Psychiatry 51:134–142

Galvan A, Hare TA, Parra CE, Penn J, Voss H, Glover G, Casey BJ (2006) Earlier development of the accumbens relative to orbitofrontal cortex might underlie risk-taking behavior in adolescents. J Neurosci 26:6885–6892

Garavan H, Pankiewicz J, Bloom A, Cho JK, Sperry L, Ross TJ, Salmeron BJ, Risinger R, Kelley D, Stein EA (2000) Cue-induced cocaine craving: neuroanatomical specificity for drug users and drug stimuli. Am J Psychiatry 157:1789–1798

Giedd JN (2008) The teen brain: insights from neuroimaging. J Adolesc Health 42:335–343

Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, Paus T, Evans AC, Rapoport JL (1999) Brain development during childhood and adolescence: a longitudinal MRI study. Nat Neurosci 2:861–863

Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC et al (2004) Dynamic mapping of human cortical development during childhood through early adulthood. Proc Natl Acad Sci U S A 101:8174–8179

Goldstein RZ, Volkow ND (2002) Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. Am J Psychiatry 159:1642–1652

Grant BF, Dawson DA (1998) Age of onset of drug use and its association with DSM-IV drug abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. J Subst Abuse 10:163–173

Hanlon CA, Dufault DL, Wesley MJ, Porrino LJ (2011) Elevated gray and white matter densities in cocaine abstainers compared to current users. Psychopharmacology (Berl) 218:681–692

Hester R, Garavan H (2004) Executive dysfunction in cocaine addiction: evidence for discordant frontal, cingulate, and cerebellar activity. J Neurosci 24:11017–11022

Huttenlocher PR (1979) Synaptic density in human frontal cortex – developmental changes and effects of aging. Brain Res 163:195–205

Hyman SE (2005) Addiction: a disease of learning and memory. Am J Psychiatry 162:1414–1422

Jacobsen LK, Giedd JN, Gottschalk C, Kosten TR, Krystal JH (2001) Quantitative morphology of the caudate and putamen in patients with cocaine dependence. Am J Psychiatry 158:486–489

Kalivas PW, O'Brien C (2008) Drug addiction as a pathology of staged neuroplasticity. Neuropsychopharmacology 33:166–180

Lim KO, Wozniak JR, Mueller BA, Franc DT, Specker SM, Rodriguez CP, Silverman AB, Rotrosen JP (2008) Brain macrostructural and microstructural abnormalities in cocaine dependence. Drug Alcohol Depend 92:164–172

Little PJ, Kuhn CM, Wilson WA, Swartzwelder HS (1996) Differential effects of ethanol in adolescent and adult rats. Alcohol Clin Exp Res 20:1346–1351

Lyoo IK, Streeter CC, Ahn KH, Lee HK, Pollack MH, Silveri MM, Nassar L, Levin JM, Sarid-Segal O, Ciraulo DA, Renshaw PF, Kaufman MJ (2004) White matter hyperintensities in subjects with cocaine and opiate dependence and healthy comparison subjects. Psychiatry Res 131:135–145

Makris N, Gasic GP, Seidman LJ, Goldstein JM, Gastfriend DR, Elman I, Albaugh MD, Hodge SM, Ziegler DA, Sheahan FS, Caviness VS Jr, Tsuang MT, Kennedy DN, Hyman SE, Rosen BR, Breiter HC (2004) Decreased absolute amygdala volume in cocaine addicts. Neuron 44:729–740

Makris N, Gasic GP, Kennedy DN, Hodge SM, Kaiser JR, Lee MJ, Kim BW, Blood AJ, Evins AE, Seidman LJ, Iosifescu DV, Lee S, Baxter C, Perlis RH, Smoller JW, Fava M, Breiter HC (2008) Cortical thickness abnormalities in cocaine addiction–a reflection of both drug use and a pre-existing disposition to drug abuse? Neuron 60:174–188

Martinez D, Gil R, Slifstein M, Hwang DR, Huang Y, Perez A, Kegeles L, Talbot P, Evans S, Krystal J, Laruelle M, Abi-Dargham A (2005) Alcohol dependence is associated with blunted dopamine transmission in the ventral striatum. Biol Psychiatry 58:779–786

Matochik JA, London ED, Eldreth DA, Cadet JL, Bolla KI (2003) Frontal cortical tissue composition in abstinent cocaine abusers: a magnetic resonance imaging study. Neuroimage 19:1095–1102

Medina KL, McQueeny T, Nagel BJ, Hanson KL, Schweinsburg AD, Tapert SF (2008) Prefrontal cortex volumes in adolescents with alcohol use disorders: unique gender effects. Alcohol Clin Exp Res 32:386–394

Milton AL, Everitt BJ (2012) The persistence of maladaptive memory: addiction, drug memories and anti-relapse treatments. Neurosci Biobehav Rev 36:1119–1139

Natividad LA, Torres OV, Friedman TC, O'Dell LE (2013) Adolescence is a period of development characterized by short- and long-term vulnerability to the rewarding effects of nicotine and reduced sensitivity to the anorectic effects of this drug. Behav Brain Res 257:275–285

Nestler EJ (2005) Is there a common molecular pathway for addiction? Nat Neurosci 8:1445–1449

Nieman BJ, Bishop J, Dazai J, Bock NA, Lerch JP, Feintuch A, Chen XJ, Sled JG, Henkelman RM (2007) MR technology for biological studies in mice. NMR Biomed 20:291–303

O'Brien MS, Anthony JC (2005) Risk of becoming cocaine dependent: epidemiological estimates for the United States, 2000–2001. Neuropsychopharmacology 30:1006–1018

Paus T, Keshavan M, Giedd JN (2008) Why do many psychiatric disorders emerge during adolescence? Nat Rev Neurosci 9:947–957

Pautassi RM, Myers M, Spear LP, Molina JC, Spear NE (2008) Adolescent but not adult rats exhibit ethanol-mediated appetitive second-order conditioning. Alcohol Clin Exp Res 32:2016–2027

Petanjek Z, Judas M, Simic G, Rasin MR, Uylings HB, Rakic P, Kostovic I (2011) Extraordinary neoteny of synaptic spines in the human prefrontal cortex. Proc Natl Acad Sci U S A 108:13281–13286

Pierce RC, Kumaresan V (2006) The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? Neurosci Biobehav Rev 30:215–238

Porrino LJ, Daunais JB, Smith HR, Nader MA (2004) The expanding effects of cocaine: studies in a nonhuman primate model of cocaine self-administration. Neurosci Biobehav Rev 27:813–820

Rakic P, Bourgeois JP, Goldman-Rakic PS (1994) Synaptic development of the cerebral cortex: implications for learning, memory, and mental illness. Prog Brain Res 102:227–243

Robinson TE, Kolb B (2004) Structural plasticity associated with exposure to drugs of abuse. Neuropharmacology 47(Suppl 1):33–46

Rossetti ZL, Melis F, Carboni S, Diana M, Gessa GL (1992) Alcohol withdrawal in rats is associated with a marked fall in extraneuronal dopamine. Alcohol Clin Exp Res 16:529–532

Schlaepfer TE, Lancaster E, Heidbreder R, Strain EC, Kosel M, Fisch HU, Pearlson GD (2006) Decreased frontal white-matter volume in chronic substance abuse. Int J Neuropsychopharmacol 9:147–153

Seeman P, Bzowej NH, Guan HC, Bergeron C, Becker LE, Reynolds GP, Bird ED, Riederer P, Jellinger K, Tourtellotte WW (1987) Human brain dopamine receptors in children and aging adults. Synapse 1:399–404

Shaw P, Kabani NJ, Lerch JP, Eckstrand K, Lenroot R, Gogtay N, Greenstein D, Clasen L, Evans A, Rapoport JL, Giedd JN, Wise SP (2008) Neurodevelopmental trajectories of the human cerebral cortex. J Neurosci 28:3586–3594

Sim ME, Lyoo IK, Streeter CC, Covell J, Sarid-Segal O, Ciraulo DA, Kim MJ, Kaufman MJ, Yurgelun-Todd DA, Renshaw PF (2007) Cerebellar gray matter volume correlates with duration of cocaine use in cocaine-dependent subjects. Neuropsychopharmacology 32:2229–2237

Spear LP (2000) The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 24:417–463

Statistics Canada (2003) Canadian community health survey: mental health and well-being. Statistics Canada Catalogue no. 12-591-X. Ottawa, Ontario

Swendsen J, Conway KP, Degenhardt L, Glantz M, Jin R, Merikangas KR, Sampson N, Kessler RC (2010) Mental disorders as risk factors for substance use, abuse and dependence: results from the 10-year follow-up of the National Comorbidity Survey. Addiction 105:1117–1128

Tarazi FI, Baldessarini RJ (2000) Comparative postnatal development of dopamine D(1), D(2) and D(4) receptors in rat forebrain. Int J Dev Neurosci 18:29–37

Thomas KL, Arroyo M, Everitt BJ (2003) Induction of the learning and plasticity-associated gene Zif268 following exposure to a discrete cocaine-associated stimulus. Eur J Neurosci 17:1964–1972

Torregrossa MM, Corlett PR, Taylor JR (2011) Aberrant learning and memory in addiction. Neurobiol Learn Mem 96:609–623

Tremblay L, Schultz W (1999) Relative reward preference in primate orbitofrontal cortex. Nature 398:704–708

UNODC (2009) Principles of drug dependence treatment. United Nations Office on Drugs and Crime. Vienna, Austria

UNODC (2011) World drug report. United Nations Office on Drugs and Crime. Vienna, Austria

Vanderschuren LJ, Pierce RC (2010) Sensitization processes in drug addiction. Curr Top Behav Neurosci 3:179–195

Varlinskaya EI, Spear LP (2002) Acute effects of ethanol on social behavior of adolescent and adult rats: role of familiarity of the test situation. Alcohol Clin Exp Res 26:1502–1511

Varlinskaya EI, Spear LP (2004) Acute ethanol withdrawal (hangover) and social behavior in adolescent and adult male and female Sprague-Dawley rats. Alcohol Clin Exp Res 28:40–50

Volkow ND, Li TK (2004) Drug addiction: the neurobiology of behaviour gone awry. Nat Rev Neurosci 5:963–970

Volkow ND, Wang GJ, Fowler JS, Logan J, Hitzemann R, Ding YS, Ding YS, Pappas N, Shea C, Piscani K (1996) Decreases in dopamine receptors but not in dopamine transporters in alcoholics. Alcohol Clin Exp Res 20:1594–1598

Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Childress AR, Jayne M, Ma Y, Wong C (2006) Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. J Neurosci 26:6583–6588

Weickert CS, Webster MJ, Gondipalli P, Rothmond D, Fatula RJ, Herman MM, Kleinman JE, Akil M (2007) Postnatal alterations in dopaminergic markers in the human prefrontal cortex. Neuroscience 144:1109–1119

Wheeler AL, Lerch JP, Chakravarty MM, Friedel M, Sled JG, Fletcher PJ, Josselyn SA, Frankland PW (2013) Adolescent cocaine exposure causes enduring macroscale changes in mouse brain structure. J Neurosci 33:1797–1803

Wolf ME (2010) Regulation of AMPA receptor trafficking in the nucleus accumbens by dopamine and cocaine. Neurotox Res 18:393–409

Gonadal Hormones Organize the Adolescent Brain and Behavior

Cheryl L. Sisk

Abstract Sexual differentiation of the nervous system and behavior occurs through organizational effects of gonadal hormones acting during early neural development and again during puberty. In rodents, a transient elevation in testosterone around the time of birth masculinizes and defeminizes the male brain, creating structural sexual dimorphisms and programming sex-typical responses to gonadal hormones in adulthood. A second wave of sexual differentiation occurs when levels of gonadal hormones are elevated at the time of puberty. At this time, both testicular and ovarian hormones further masculinize and feminize the male and female brain, respectively, fine-tuning sex differences in adult behavior. To test the hypothesis that the peripubertal period is a sensitive period for hormone-dependent sexual differentiation that is separate and distinct from the perinatal period, exposure to testosterone was experimentally manipulated in male Syrian hamsters to occur either prepubertally, during puberty, or in young adulthood. This experiment revealed that the perinatal and peripubertal periods of masculinization of male sexual behavior are not two separate critical periods of sensitivity to organizing effects of testosterone. Instead, the two periods of masculinization are driven by the two naturally occurring elevations in gonadal hormones. To explore possible neural mechanisms of peripubertal organizational effects of gonadal hormones, cell birth-dating experiments in male and female rats revealed sex differences in the addition of new cells, including both neurons and glia, to sexually dimorphic cell groups in the hypothalamus and medial amygdala. These sex differences in cell addition were positively correlated with sex differences in the volume of these cell groups. Prepubertal gonadectomy abolished sex differences in the pubertal addition of new cells. These experiments provide evidence that gonadal hormone-dependent sex differences in pubertal cytogenesis contribute to the establishment or maintenance of sexual dimorphisms in the adult brain.

C.L. Sisk (✉)
Department of Psychology and Neuroscience Program, Michigan State University, 293 Farm Lane Giltner 108, East Lansing, MI 48824, USA
e-mail: sisk@msu.edu

J.-P. Bourguignon et al. (eds.), *Brain Crosstalk in Puberty and Adolescence*,
Research and Perspectives in Endocrine Interactions 13,
DOI 10.1007/978-3-319-09168-6_2

The transition from childhood to adulthood begins with the onset of puberty and the ensuing rise in sex steroid hormones, and it is completed by the end of adolescence. This period of development comprises extraordinary gain of function: individuals acquire the capacity to procreate, function independently within their social realm, and provide for themselves and their offspring. The metamorphosis of behavior is the product of a metamorphosis of underlying neural circuits, which necessarily occurs along different trajectories in females and males. In fact, adolescence can be regarded as a period of further sexual differentiation of brain and behavior, which is mediated in part by the actions of sex steroid hormones in the brain. This paper highlights research from my laboratory that has uncovered roles for gonadal hormones in shaping sex-specific behavioral and brain development during puberty and adolescence. I will first review experiments that establish that testicular hormones, acting during puberty, organize neural circuits underlying male social behaviors. Next I will present evidence that hormonal regulation of pubertal neuro- and gliogenesis is a potential mechanism for the establishment or maintenance of structural sex differences in the brain during adolescence.

Hormonal Organization of Male Social Behaviors During Puberty

The 1959 landmark paper by Phoenix et al. first posited the organizational-activational hypothesis of hormone-driven sex differences in brain and behavior. Within this framework, a transient rise in testosterone during prenatal or early postnatal development masculinizes and defeminizes neural circuits in males, whereas the absence of testosterone in females results in development of a feminine neural phenotype. Upon gonadal maturation during puberty, testicular and ovarian hormones act on previously sexually differentiated circuits to facilitate expression of sex-typical behaviors in particular social contexts. Research in the 1960–1970s identified a sensitive period for hormone-dependent sexual differentiation that occurs during late prenatal and early neonatal development (Baum 1979; Wallen and Baum 2002). Thus, the original conception was that gonadal steroid hormones organize brain structure during an early, developmentally sensitive period and activate behavior during puberty and into adulthood. Our work has refined the organizational-activational hypothesis to extend organizational effects of testicular hormones to the pubertal period. This second wave of brain organization builds on and refines the circuits that were sexually differentiated during early neural development.

Using the Syrian hamster as an animal model, we established that testicular hormones, acting during puberty and adolescence, organize male social behaviors. In these studies, we employed an experimental paradigm in which male hamsters were castrated at weaning, i.e., after the perinatal period of sexual differentiation and before the onset of puberty. In adulthood, testosterone was replaced and, 1–2 weeks later, social interactions with a receptive female or an intruder male were studied. The behavior of these hamsters was compared to that of other hamsters that were similarly treated, except that castration and hormone replacement occurred in

adulthood, several weeks after the pubertal elevation in testicular hormones. Thus, the two groups of hamsters differed in when they were deprived of testosterone, either during the normal time of puberty or in adulthood. This design allowed us to assess whether the presence of testicular hormones specifically during puberty and adolescence programmed behavioral responsiveness to testosterone in adulthood. In these experiments, we found that levels of sexual behavior, aggressive behavior, and flank-marking were all reduced in males in which testicular hormones were absent during adolescent brain development (i.e., males castrated before puberty), as compared to the behavior of males in which endogenous testicular hormones were present during adolescent brain development (i.e., males castrated after puberty; Schulz et al. 2004, 2006; Schulz and Sisk 2006). Even a prolonged period of testosterone replacement in adulthood failed to normalize behavior in males castrated before puberty. Thus, testicular hormones during puberty enhanced subsequent hormone activation of male social behaviors and masculinize behavioral responses in adulthood, outcomes similar to those of perinatal exposure to testosterone and indicative of organizational effects.

These findings prompted us to propose a two-stage model of behavioral development in which the perinatal period of steroid-dependent sexual differentiation is followed by a second wave of steroid-dependent neural organization during puberty and adolescence (Sisk et al. 2003; Sisk and Zehr 2005; Schulz and Sisk 2006; Fig. 1). During the second wave, pubertal hormones first organize neural circuits in the developing adolescent brain and then facilitate the expression of sex-typical adult behaviors in specific social contexts by activating those circuits. We view

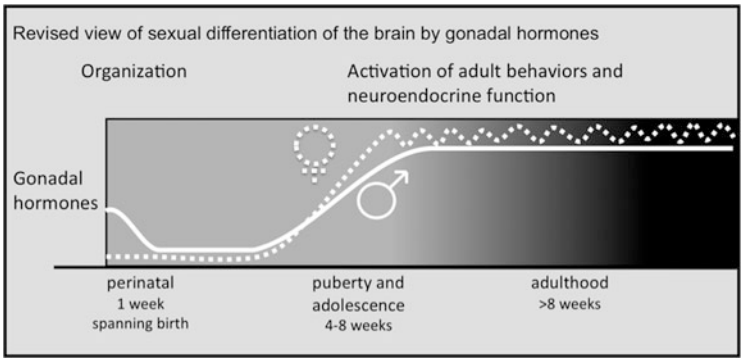

Fig. 1 Contemporary view of the sexual differentiation of brain and behavior by gonadal hormones in rodents. Two phases of sexual differentiation occur during natural developmental elevations in gonadal hormone secretion. The perinatal phase involves the masculinization and defeminization of the brain by a transient rise in testosterone secretion in males. The ovary is quiescent at this time. The pubertal phase involves the masculinization and feminization of the brain by the pubertal rise in gonadal hormones. Testicular and ovarian hormones both play an active role during this phase of sexual differentiation, which builds on the sex differences that were created during perinatal development. The transition from *gray* to *black* shading depicts the decreasing ability for gonadal hormones to exert organizational influences on brain and behavior (Reprinted from Juraska et al. 2013)

hormone-driven adolescent organization as a refinement of the sexual differentiation that occurred during perinatal neural development. That is, what occurs during perinatal brain organization determines the substrate upon which pubertal hormones act during adolescent organization. During the adolescent phase of organization, steroid-dependent refinement of neural circuits results in long-lasting structural changes that further program adult behavioral responses to hormones and socially relevant sensory stimuli.

We next asked whether the perinatal and peripubertal periods of hormone-dependent organization involve two discrete periods of enhanced sensitivity to hormones. We know that a window of sensitivity opens in rodents during late embryonic development to permit the initial sexual differentiation of the brain organized by testicular hormones (Wallen and Baum 2002). We also know that a window of developmental sensitivity to hormone-dependent organization closes (or nearly so) by the end of adolescence, because a prolonged duration of testosterone replacement does not reverse or ameliorate the adverse consequences of the absence of testosterone during puberty on adult social behaviors (Schulz et al. 2004). What was not clear is the extent to which the perinatal and peripubertal windows overlap: does the perinatal window close before the peripubertal window opens, or do the perinatal and peripubertal windows overlap enough to be considered a single period of sensitivity to hormone-dependent organization of the nervous system?

To address these questions, we manipulated the time during postnatal development that male hamsters were exposed to testosterone. If puberty marks the opening of a unique sensitive period for testosterone-dependent behavioral organization, separate from the perinatal sensitive period, then males exposed to testosterone during the normal time of puberty and adolescence, but not males exposed to testosterone before or after, should display the full complement of adult-typical mating behaviors. Male hamsters were castrated after the perinatal window of sensitivity to organizing influences of testosterone on postnatal day (P; P0 = day of birth) 10 and were implanted with testosterone-filled or empty Silastic capsules on P10, P29, or P63. Capsules remained in place for 19 days and then were removed on P29, P48, or P82, respectively. Thus, the groups receiving testosterone-filled capsules were exposed to testosterone either before (P10–29), during (P29–48), or after (P63–82) the normal time of puberty and adolescence (roughly P30–P50). Twenty-eight days after the removal of testosterone or blank pellets, when hamsters in all groups were in young adulthood, all males were implanted with testosterone pellets to activate mating behavior and were tested with a sexually receptive female 7 days later.

We found that testosterone treatment before and during adolescence, but not after, facilitated mating behavior in adulthood (Schulz et al. 2009; Fig. 2). In addition, prepubertal testosterone treatment more effectively facilitated adult mating behavior than at any other age. These findings extend our knowledge of neurobehavioral development by demonstrating (1) adolescence is not a sensitive period for testosterone-dependent behavioral organization distinct or separate from the neonatal period but rather adolescence is part of a protracted sensitive period that likely begins perinatally and ends in late adolescence; and (2) the

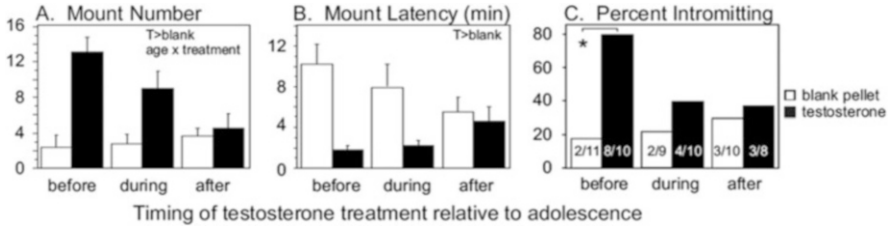

Fig. 2 Effects of periadolescent testosterone exposure on adult reproductive behaviors. Testosterone treatments were designed to simulate early, on-time, and late pubertal development, and all behavior testing occurred in adulthood after a 1-week treatment with testosterone-filled or blank subcutaneous pellets. Only before- and during-adolescent testosterone treatments facilitated mounting behavior in response to testosterone in adulthood. The percentage of male hamsters that showed intromissions was increased only by before-adolescent testosterone treatments. These data suggest that early testosterone treatments enhance behavioral responsiveness to testosterone in adulthood. *Asterisk* indicates a significant difference ($p < 0.05$) between groups (Reprinted from Schulz et al. 2009)

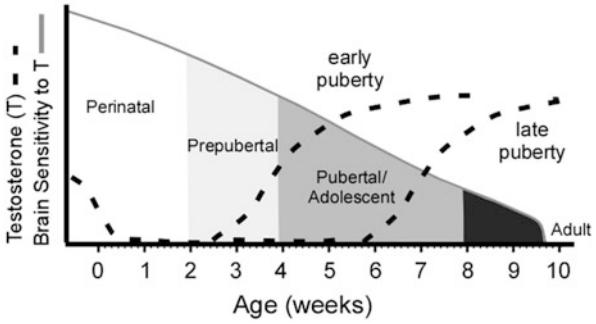

Fig. 3 Illustration depicting the results of our study investigating the effects of early, on-time, and late adolescent testosterone treatments on adult mating behavior. On the y-axis, the *dashed line* approximates testosterone secretion across development, whereas the *solid line* depicts decreasing sensitivity to the organizing actions of testosterone across development. Shading approximates the timing of perinatal, prepubertal, adolescent, and adult periods in the Syrian hamster. Given that early adolescent testosterone treatment was initiated immediately following the period of sexual differentiation (postnatal day 10), our data suggest that adolescence is part of a protracted sensitive period for the organizing actions of testosterone (area under the *solid gray curve*). In addition, because early adolescent treatments most effectively organized adult mating behavior, we propose that sensitivity to the organizing actions of testosterone decreases across postnatal development. If sensitivity to the organizing actions of testosterone decreases with time, then differences in the timing of pubertal onset may result in differences in brain development and adult behavior (Reprinted from Schulz et al. 2009)

timing of testosterone secretion within this postnatal window programs expression of adult mating behavior, with the earlier exposure to testosterone, the greater impact on behavior. Thus, these data have prompted us to revise the two-stage model to include a large postnatal window of decreasing sensitivity to the organizing actions of testosterone (Fig. 3). Importantly, the original two-stage model of

hormone-dependent organization is still relevant, but the stages are not defined by distinct windows of sensitivity to steroid hormones but instead by the two periods of elevated hormone secretion within a prolonged postnatal window of sensitivity to organizational effects of gonadal steroid hormones.

Sexual Differentiation of Brain Structure During Adolescence

In addition to proposing that sexually differentiated adult behaviors are organized by testosterone during early development, Phoenix et al. (1959) also correctly surmised that these early developmental effects of testicular hormones must involve organizational effects on the structure of the developing nervous system. Over the three decades following this seminal paper, an enormous literature emerged describing numerous sexual dimorphisms in the adult nervous system and uncovering the developmental processes by which these sex differences come to be. Structural sexual dimorphisms are typically manifested as sex differences in cell group volume, neuron or glial cell number, soma size, dendritic arborizations, dendritic spine density, degree of myelination, or some combination of these features. One principle derived from early studies of developmental mechanisms of sexual dimorphisms was that testicular hormones drive the perinatal creation of sexual dimorphisms in nervous system structure. This principle was based in large part on experiments showing that either pharmacological blockade or surgical removal of testicular hormone influences during the perinatal period was sufficient to prevent both masculinization and defeminization of sexually dimorphic hypothalamic regions (reviewed in Cooke et al. 1998). In contrast, removal of the ovaries immediately after birth did not have robust consequences on sexual differentiation, which is not surprising given that ovarian hormones are not secreted at this time. These studies led to the idea that, in the absence of testicular hormones, the default phenotype is essentially female and ovarian hormones are not actively involved in early sexual differentiation of the nervous system. Another principle was that the period of sexual differentiation of the nervous system structure is confined to a sensitive period during prenatal and early postnatal life, which in rodents constitutes a week bracketing the day of birth. This principle rested on studies in rodents showing that manipulation of testicular hormones after P7 failed to alter the adult phenotype of sexually dimorphic hypothalamic regions (reviewed in Cooke et al. 1998).

Each of these tenets began to be challenged as scientific interest in adolescent brain development gathered speed in the late twentieth century. Evidence began accumulating in the 1990s that testicular hormones, acting during puberty, masculinize male social behaviors, including male–female interactions, male-male

interactions, and scent-marking (Primus and Kellogg 1990; Bloch et al. 1995; Romeo et al. 2002; Schulz and Sisk 2006). Then came reports that ovarian hormones, acting during puberty, feminize food-guarding behavior and behavioral responses to metabolic challenge (Field et al. 2004; Swithers et al. 2008). These behavioral data encouraged investigations of the potential organizational effects of gonadal hormones on the masculinization and feminization of behavioral circuits during puberty and adolescence and a re-examination of the role of pubertal ovarian hormones in sexual dimorphisms in brain structure (reviewed in Juraska et al. 2013). Here I will discuss experiments from my laboratory that provide evidence that (1) ovarian hormones contribute to sexual differentiation of the brain during puberty, and (2) sex differences in cytogenesis are a potential mechanism for establishment or maintenance of structural sexual dimorphisms in the adolescent brain.

Sexual Differentiation of Hypothalamic Regions During Adolescence: The Anteroventral Periventricular Nucleus (AVPV) and Sexually Dimorphic Nucleus of the Preoptic Area (SDN)

The AVPV and SDN of adult rats are sexually dimorphic, with the AVPV being larger in females than in males (female-biased) and the SDN being larger in males (male-biased). The AVPV is essential for the generation of the preovulatory surge of gonadotropins (Wiegand and Terasawa 1982; Petersen and Barraclough 1989), a neuroendocrine event displayed by female rats only after puberty and never displayed by male rats (Hoffman et al. 2005). SDN function is linked to male sexual behavior, particularly partner preference (Kondo et al. 1990; Baum et al. 1996). Sex differences in the AVPV and SDN were among the first forebrain cell groups to be studied with respect to the mechanisms of sexual differentiation. It was established that the perinatal surge of testosterone secretion in males promotes survival of SDN cells, while simultaneously promoting death of AVPV cells during early postnatal development. Conversely, the absence of testosterone in females favors cell death in the SDN and cell survival in the AVPV (Dohler et al. 1982, 1984; Jacobson et al. 1985; Murakami and Arai 1989; Arai et al. 1994, 1996; Davis et al. 1996a, b). For both AVPV and SDN, then, adult sexual dimorphisms are hormonally programmed during perinatal brain development, with testosterone (or a metabolite) playing the primary role in masculinization and defeminization of these cell groups.

In spite of the evidence that neonatal testosterone promotes cell death in the AVPV during the perinatal period, an early investigation of sex differences in AVPV volume indicated that the female AVPV does not become larger than the male AVPV until puberty; between P30 and P90, AVPV volume grows in females but remains stable in males (Davis et al. 1996a). This finding suggests that the

perinatal programming of sex differences in AVPV volume is not immediately manifested and that pubertal processes contribute to the sexual dimorphism present in adulthood. To determine whether the pubertal increase in AVPV volume is due to the addition of new cells, we used the cell birthdate marker bromo-deoxyuridine (BrdU) to label cells born during puberty in male and female rats (Ahmed et al. 2008). BrdU was administered to rats that were at a prepubertal (P20), early-pubertal (P30), or mid-pubertal (P40) stage, and BrdU-labeled cells were quantified 3 weeks later, allowing time for the new cells to mature and potentially differentiate. Across all ages at which BrdU was administered, more BrdU cells were found in the female than in the male AVPV. Phenotyping studies revealed that some of these AVPV cells that had been born during puberty had differentiated 3 weeks later into mature neurons (Fig. 4). BrdU-labeled cells were also found in the SDN, with greater numbers of pubertally born cells in males than in females. However, in the SDN, there was no evidence for co-labeling of BrdU with either mature neuron or astrocyte markers, so the phenotype of pubertally added SDN cells remains unknown. It is possible that 3–4 weeks is insufficient time for these cells to fully differentiate as mature neurons or glial cells, as reported for cortical interneurons (Cameron and Dayer 2008). The results of these studies offer a novel mechanism for establishing and/or maintaining sexual dimorphisms in the hypothalamus during puberty, when new, sex-specific physiological functions and behaviors are acquired as the transition to adulthood proceeds.

A role for gonadal hormones in the addition of new cells during puberty is evident from additional experiments showing that prepubertal gonadectomy of male and female rats completely abolished the sex differences in the pubertal

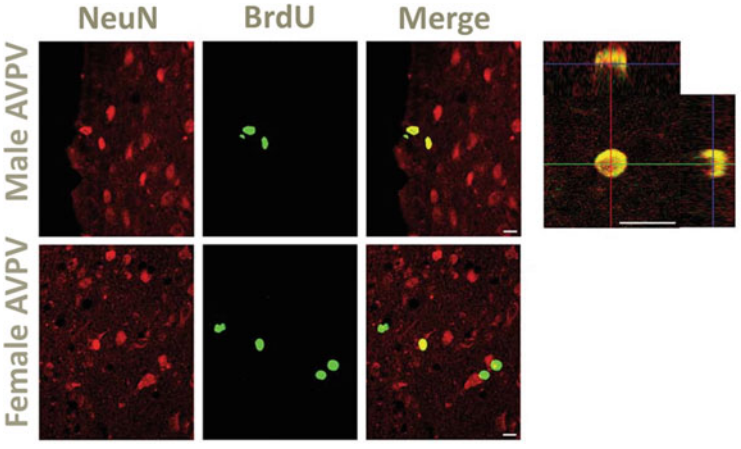

Fig. 4 Confocal images of cells in male and female rat AVPV. Sections were processed for double-label BrdU (*green*) and the mature neuron marker NeuN (*red*); colocalization is *yellow*. Orthogonal views (*right*) of confocal images verify colocalization of BrdU and NeuN in the female AVPV of a neuron that was born on P30–32. Scale bars: 10 μm (Reprinted from Ahmed et al. 2008)

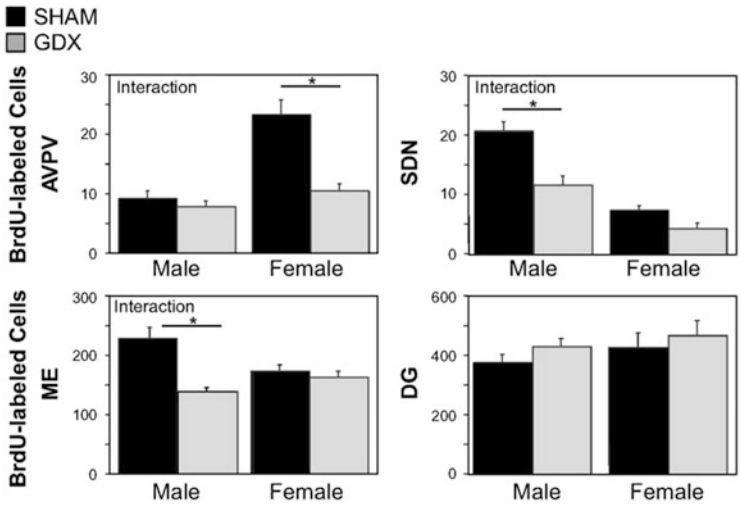

Fig. 5 The effect of prepubertal gonadectomy (GDX) on the number of BrdU-labeled cells depends on sex and brain region. Male and female rats were gonadectomized or sham gonadectomized at 20 days of age ($n = 8$/sex and treatment). A daily injection of BrdU was given on 30–32 days of age and brain tissue was collected at 50 days of age. Prepubertal GDX significantly decreased the number of BrdU-labeled cells in female but not male AVPV (interaction between sex and treatment). Prepubertal GDX decreased the number of BrdU-labeled cells in male but not female SDN and ME (interaction between sex and treatment). Prepubertal GDX did not affect BrdU-labeled cells in the dentate gyrus (*DG*) of either males or females. Data are presented as means ± SEM. *Asterisks* indicate $p < 0.05$ (post hoc Fisher test between groups) (Reprinted from Ahmed et al. 2008)

addition of new cells to the AVPV and SDN (Ahmed et al. 2008; Fig. 5). Prepubertal ovariectomy reduced the volume of the AVPV of females to a volume similar to males, and this reduction in volume was accompanied by a significant reduction in stereological estimates of total neuron number to a number similar to that of males. In contrast, prepubertal gonadectomy had no effect on the volume or pubertal addition of new cells to the AVPV of males. The effects of prepubertal castration on cell addition to the SDN paralleled those for the AVPV, but in a reverse direction. That is, prepubertal gonadectomy of male rats eliminated the sex difference in the number of BrdU-labeled cells in the SDN, reducing the number of pubertally added cells in males to the number observed in females. In contrast, prepubertal ovariectomy did not affect the number of BrdU-labeled cells added to the female SDN. Thus, ovarian hormones during puberty (or just before) feminize the AVPV but do not appear to actively feminize the SDN at this time. And, conversely, testicular hormones further masculinize the SDN during puberty but do not appear to actively masculinize the AVPV.

Sexual Differentiation of the Posterodorsal Medial Amygdala (MePD) During Adolescence

The rodent MePD evaluates chemosensory stimuli from conspecifics and integrates this information with the internal hormonal milieu, thereby coordinating the external and internal signals that regulate social behaviors. MePD volume is larger in adult male rats than in female rats, with this sex difference being more pronounced in the right hemisphere than in the left (Cooke and Woolley 2005; Cooke et al. 2007; Morris et al. 2008). This adult sexual dimorphism is the product of two successive phases of masculinization, one during early postnatal life and one during puberty. At P25, still before the onset of puberty, the rat MePD is already larger in males than in females (Cooke et al. 2007; Johnson et al. 2008, 2012a). Between the time of puberty and young adulthood, the sex difference in the MePD volume becomes even more exaggerated (Johnson et al. 2008, 2012a). BrdU cell birth dating studies corroborate these findings by showing that, during puberty, more cells are added to the male than to the female MePD (Ahmed et al. 2008). Some of these pubertally added cells are neurons and some are astrocytes (Ahmed et al. 2008), but it is not known at this time whether there are either sex or hemispheric differences in the pubertal addition of neurons vs astrocytes to the MePD. It does appear that pubertally born cells in the MePD become functionally integrated into behavioral circuits, as some pubertally born cells in the male hamster MePD express the immediate-early gene product fos after a sociosexual interaction with a receptive female (Mohr and Sisk 2013).

The pubertal phase of sexual differentiation of the MePD appears to involve, at least in part, testicular hormones acting via androgen receptors. The evidence comes from comparing the pubertal development of MePD in wildtype (wt) male and female rats with that of male rats carrying the testicular feminization mutation (tfm) of the androgen receptor, which confers androgen insensitivity. These studies show that the pubertal increase in MePD astrocyte number and astrocytic branching seen in wt male rats does not occur in male tfm rats, indicating that an androgen receptor-dependent mechanism is normally involved in these pubertal processes (Johnson et al. 2012a, b). Furthermore, the sex difference in the pubertal addition of new cells to the MePD is abolished by prepubertal gonadectomy of male rats, indicating that testicular hormones normally promote proliferation and/or survival of pubertally added cells in the male MePD (Ahmed et al. 2008). In contrast, prepubertal ovariectomy does not alter the number of pubertally added cells to the female MePD (Ahmed et al. 2008). Thus, the MePD provides a solid example of a region in which sex differences arise during early development but are magnified during puberty, and in the MePD the sexual differentiation that occurs during puberty involves a testicular hormone-, androgen receptor-dependent mechanism that promotes the addition of new neurons and astrocytes and astrocyte branching in males.

Future Directions

Our work demonstrates that sexually differentiated social behaviors and their underlying neural circuits are further masculinized and feminized during puberty and adolescence by gonadal hormones. Our future work will investigate the mechanisms by which the pubertal elevation in testosterone organizes male social behaviors. For example, are social information processing, social learning, and/or executive regulation of behavior under the organizational influences of testosterone during puberty, and if so, what neural circuits and neurotransmitters are involved and how are they permanently changed by testosterone? What are the roles of pubertally born neurons and glial cells in the adolescent maturation of sexually differentiated behaviors? We hope that elucidation of the mechanisms underlying typical sexual differentiation of the brain and behavior during puberty and adolescence will inform our understanding of the etiology of sex-biased psychopathologies that emerge during this period of development.

References

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

Arai Y, Murakami S, Nishizuka M (1994) Androgen enhances neuronal degeneration in the developing preoptic area: apoptosis in the anteroventral periventricular nucleus (AVPvN-POA). Horm Behav 28:313–319

Arai Y, Sekine Y, Murakami S (1996) Estrogen and apoptosis in the developing sexually dimorphic preoptic area in female rats. Neurosci Res 25:403–407

Baum MJ (1979) Differentiation of coital behavior in mammals: a comparative analysis. Neurosci Biobehav Rev 3:265–284

Baum MJ, Tobet SA, Cherry JA, Paredes RG (1996) Estrogenic control of preoptic area development in a carnivore, the ferret. Cell Mol Neurobiol 16:117–128

Bloch G, Mills R, Gale S (1995) Prepubertal testosterone treatment of female rats: defeminization of behavioral and endocrine function in adulthood. Neurosci Biobehav Rev 19:177–186

Cameron HA, Dayer AG (2008) New interneurons in the adult neocortex: small, sparse, but significant? Biol Psychiatry 63:650–655

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

Cooke B, Hegstrom CD, Villeneuve LS, Breedlove SM (1998) Sexual differentiation of the vertebrate brain: principles and mechanisms. Front Neuroendocrinol 19:323–362

Cooke BM, Stokas MR, Woolley CS (2007) Morphological sex differences and laterality in the prepubertal medial amygdala. J Comp Neurol 501:904–915

Davis EC, Shryne JE, Gorski RA (1996a) Structural sexual dimorphisms in the anteroventral periventricular nucleus of the rat hypothalamus are sensitive to gonadal steroids perinatally, but develop peripubertally. Neuroendocrinology 63:142–148

Davis EC, Popper P, Gorski RA (1996b) The role of apoptosis in sexual differentiation of the rat sexually dimorphic nucleus of the preoptic area. Brain Res 734:10–18

Dohler KD, Coquelin A, Davis F, Hines M, Shryne JE, Gorski RA (1982) Differentiation of the sexually dimorphic nucleus in the preoptic area of the rat brain is determined by the perinatal hormone environment. Neurosci Lett 33:295–298

Dohler KD, Coquelin A, Davis F, Hines M, Shryne JE, Gorski RA (1984) Pre- and postnatal influence of testosterone propionate and diethylstilbestrol on differentiation of the sexually dimorphic nucleus of the preoptic area in male and female rats. Brain Res 302:291–295

Field EF, Whishaw IQ, Forgie ML, Pellis SM (2004) Neonatal and pubertal, but not adult, ovarian steroids are necessary for the development of female-typical patterns of dodging to protect a food item. Behav Neurosci 118:1293–1304

Hoffman GE, Le WW, Schulterbrandt T, Legan SJ (2005) Estrogen and progesterone do not activate Fos in AVPV or LHRH neurons in male rats. Brain Res 1054:116–124

Jacobson CD, Davis FC, Gorski RA (1985) Formation of the sexually dimorphic nucleus of the preoptic area: neuronal growth, migration and changes in cell number. Brain Res 353:7–18

Johnson RT, Breedlove SM, Jordan CL (2008) Sex differences and laterality in astrocyte number and complexity in the adult rat medial amygdala. J Comp Neurol 511:599–609

Johnson RT, Breedlove SM, Jordan CL (2012a) Androgen receptors mediate masculinization of astrocytes in the rat posterodorsal medial amygdala during puberty. J Comp Neurol 521:2298–2309

Johnson RT, Schneider A, DonCarlos LL, Breedlove SM, Jordan CL (2012b) Astrocytes in the rat medial amygdala are responsive to adult androgens. J Comp Neurol 520:2531–2544

Juraska JM, Sisk CL, DonCarlos LL (2013) Sexual differentiation of the adolescent rodent brain: hormonal influences and developmental mechanisms. Horm Behav 64:203–210

Kondo Y, Shinoda A, Yamanouchi K, Arai Y (1990) Role of septum and preoptic area in regulating masculine and feminine sexual behavior in male rats. Horm Behav 24:421–434

Mohr MA, Sisk CL (2013) Pubertally born neurons and glia are functionally integrated into limbic and hypothalamic circuits of the male Syrian hamster. Proc Natl Acad Sci U S A 110:4792–4797

Morris JA, Jordan CL, Breedlove SM (2008) Sexual dimorphism in neuronal number of the posterodorsal medial amygdala is independent of circulating androgens and regional volume in adult rats. J Comp Neurol 506:851–859

Murakami S, Arai Y (1989) Neuronal death in the developing sexually dimorphic periventricular nucleus of the preoptic area in the female rat: effect of neonatal androgen treatment. Neurosci Lett 102:185–190

Petersen L, Barraclough C (1989) Suppression of spontaneous LH surges in estrogen-treated ovariectomized rats by microimplants of antiestrogens into the preoptic brain. Brain Res 484:279–289

Phoenix C, Goy R, Gerall A, Young W (1959) Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. Endocrinology 65:369–382

Primus R, Kellogg C (1990) Gonadal hormones during puberty organize environment-related social interaction in the male rat. Horm Behav 24:311–323

Romeo RD, Richardson HN, Sisk CL (2002) Puberty and the maturation of the male brain and sexual behavior: recasting a behavioral potential. Neurosci Biobehav Rev 26:381–391

Schulz KM, Sisk CL (2006) Pubertal hormones, the adolescent brain, and the maturation of social behaviors: lessons from the Syrian hamster. Mol Cell Endocrinol 254–255:120–126

Schulz KM, Richardson HM, Zehr JL, Osetek AJ, Menard TA, Sisk CL (2004) Gonadal hormones masculinize and defeminize reproductive behaviors during puberty in the male Syrian hamster. Horm Behav 45:242–249

Schulz KM, Menard TA, Smith DA, Albers HE, Sisk CL (2006) Testicular hormone exposure during adolescence organizes flank-marking behavior and vasopressin receptor binding in the lateral septum. Horm Behav 50:477–483

Schulz KM, Zehr JL, Salas-Ramirez KY, Sisk CL (2009) Testosterone programs adult social behavior before and during, but not after, adolescence. Endocrinology 150:3690–3698

Sisk CL, Zehr JL (2005) Pubertal hormones organize the adolescent brain and behavior. Front Neuroendocrinol 26:163–174

Sisk CL, Schulz KM, Zehr JL (2003) Puberty: a finishing school for male social behavior. Ann N Y Acad Sci 1007:189–198

Swithers SE, McCurley M, Hamilton E, Doerflinger A (2008) Influence of ovarian hormones on development of ingestive responding to alterations in fatty acid oxidation in female rats. Horm Behav 54:471–477

Wallen K, Baum MJ (2002) Masculinization and defeminization in altricial and precocial mammals: comparative aspects of steroid hormone action. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, Rubin RT (eds) Hormones, brain and behavior. Elsevier, San Diego, pp 385–423

Wiegand SJ, Terasawa E (1982) Discrete lesions reveal functional heterogeneity of suprachiasmatic structures in regulation of gonadotropin secretion in the female rat. Neuroendocrinology 34:395–404

The Role of Pubertal Hormones in the Development of Gender Identity: fMRI Studies

S.M. Burke and J. Bakker

Abstract The present study is the first to demonstrate that sex differences in hypothalamic activation upon smelling the chemo-signal androstadienone are not acquired during sexual maturation, under the influence of gonadal hormones during puberty, but might be considered hard-wired responses, which already can be observed in prepubertal children. Moreover, the current study is the first to explore sex-atypical hypothalamic responses to androstadienone in male and female individuals with GD at two different developmental stages. Our results suggest that individuals with GD possess certain functional brain characteristics of their experienced gender and might have undergone atypical neuronal sexual differentiation, most likely during early brain development and not during puberty. Additional analyses in other functional (e.g., mental rotation, emotional face matching, resting state data) and structural (e.g., gray and white matter volumes or diffusion tensor imaging data) MRI measures, which are in preparation, should corroborate the present preliminary findings.

S.M. Burke (✉)
Center of Expertise on Gender Dysphoria, Department of Medical Psychology, Neuroscience Campus Amsterdam, VU University Medical Center, De Boelelaan 1131, 1081 HX Amsterdam, The Netherlands

Netherlands Institute for Neuroscience, Meibergdreef 47, 1105 BA Amsterdam, The Netherlands
e-mail: s.burke@vumc.nl

J. Bakker
Center of Expertise on Gender Dysphoria, Department of Medical Psychology, Neuroscience Campus Amsterdam, VU University Medical Center, De Boelelaan 1131, 1081 HX Amsterdam, The Netherlands

Netherlands Institute for Neuroscience, Meibergdreef 47, 1105 BA Amsterdam, The Netherlands

GIGA Neuroscience, University of Liege, Avenue de l'Hôpital 1, 4000 Liege, Belgium
e-mail: jbakker@ulg.ac.be

J.-P. Bourguignon et al. (eds.), *Brain Crosstalk in Puberty and Adolescence*,
Research and Perspectives in Endocrine Interactions 13,
DOI 10.1007/978-3-319-09168-6_3

Introduction

Definition

Gender dysphoria (GD) is a condition in which people suffer from an incongruence between their natal sex and their gender identity, i.e., their experienced gender (DSM-5; American Psychiatric Association 2013). GD is also referred to as transsexualism (ICD-10; World Health Organization 1992). Although no formal epidemiological studies exist on the prevalence of GD in children, adolescents, or adults, severe and persisting GD is relatively rare. Based on studies conducted in clinical samples (Zucker and Lawrence 2009), the prevalence of GD in adults was estimated to range from 1:7,400 to 1:100,000 in natal males and from 1:30,400 to 1:400,000 in natal females. However, these estimates are based on adults turning to health services and thus seeking medical treatment. Since individuals might be hesitant to seek medical care related to GD, these studies probably underestimate the prevalence of GD. Indeed, a recent Dutch study (Kuyper and Wijsen 2014) based on self-report showed that 0.6 % of men and 0.2 % of women had ambivalent and incongruent gender identities, combined with a dislike of their body and a wish to obtain hormones/surgery.

Etiology

The etiology of GD is still largely unknown, although various hypotheses have been formulated. It has been hypothesized that psychosocial (vulnerability) factors (Coates and Person 1985; Coates 1990; Zucker and Bradley 1995), stressful events during pregnancy (Ward et al. 2002; Seckl and Holmes 2007), or hormonal alterations during critical periods of development (Cohen-Kettenis et al. 1998; Schagen et al. 2012) might cause GD.

Most of our knowledge of how sex differences emerge in the brain and behavior is derived from studies in animal models. These studies have clearly shown that testosterone, secreted by the testes, induces male-typical neural and behavioral characteristics during perinatal development, whereas female-typical neural and behavioral characteristics are supposed to develop in the absence of testosterone ("by default"). A default organization of the female brain and behavior has been recently challenged by the observation that estradiol feminizes the brain in female mice over a specific prepubertal period (Brock et al. 2011). Thus, the current view of the sexual differentiation of the brain is that different critical windows exist for the development of the male versus the female brain, with the male brain developing during pre- and early postnatal development under the influence of testosterone whereas the female brain develops during prepubertal development under the influence of estradiol (Bakker and Brock 2010). These effects of sex hormones on the developing brain are also known as "organizational" effects, since they are

permanent and irreversible. Furthermore, it is important to note that sex hormones also have activational effects on the brain and behavior. For instance, male sexual behavior is highly dependent on testosterone. In all mammalian species studied, the display of mating behavior in adult males is stimulated by testosterone. The increase in testosterone production at puberty underlies the increase in sexual activity in maturing males, and if the source of testosterone, the testes, is removed surgically, sexual activity typically declines. Likewise, in most female species (with the exception of some primate species and humans), sexual behavior coincides with ovulation and thus with high levels of the ovarian hormones, estradiol and progesterone. When the ovaries are removed surgically, the female will no longer be sexually receptive. These effects are thus not permanent and reversible and are therefore referred to as "activational".

Similar to what is seen in non-human animals, sex hormones have been proposed to affect the sexual differentiation of the human brain. Clinical observations have suggested that testosterone indeed induces male-typical psychosexual differentiation in men prenatally (Baum 2006; Swaab 2007). The strongest clinical evidence derives from a syndrome in genetic males (46, XY) with complete androgen insensitivity (CAIS). These individuals carry a mutation in the androgen receptor; as a consequence, all tissues, including the brain, are insensitive to testosterone. They are born with external female genitalia, since the development of the external genitalia entirely depends on testosterone action, but they have testes and internal male genitalia, because these develop independently of testosterone action during embryonic development (genetic factors and anti-müllerian hormone). CAIS is often discovered at puberty because of amenorrhea. Most remarkably, individuals with CAIS have a female gender identity and gender role and they are sexually attracted to men, thereby providing strong evidence that, in the absence of testosterone, a female-typical gender identity develops. Additional evidence for a role of testosterone in the development of male-typical characteristics comes from genetic women (46, XX) with congenital adrenal hyperplasia (CAH). These women have been exposed prenatally to excessive amounts of androgen due to a mutation in the gene encoding 21-hydroxylase, which is responsible for the conversion of progesterone into deoxycorticosterone and that of 17-dehydroxyprogesterone into 11, deoxycortisol in the adrenal glands. Since the biosynthetic pathway to aldosterone and cortisol is blocked, all progesterone will be converted into androgens by the adrenal glands. Furthermore, the absence of negative feedback by cortisol on the pituitary will lead to increased production and release of ACTH, which will stimulate the adrenal glands to produce even more androgens. These women are often born with clearly virilized external genitalia (to various degrees, depending on the amount of androgen exposure). Furthermore, they show a masculinization in certain behaviors, such as toy preferences (Hines 2010), as well as an increased incidence of bisexual orientation and GD (Meyer-Bahlburg et al. 2008). However, the majority of these CAH women do not suffer from GD, indicating that there is no direct relationship between testosterone exposure and developing a male-typical gender identity.

At present, there is only evidence for a role of testosterone in the sexual differentiation of the human brain and no clear evidence for a feminizing role of

estradiol, as has been suggested by recent studies using mouse models (Bakker and Brock 2010). However, several studies (Downey et al. 1989; Rolstad et al. 2007; Shaeffer et al. 2008) have shown that women with Turner Syndrome (TS), a disorder in which all or part of the X chromosome is missing and with ovarian dysgenesis as one of several morphological consequences, reported significantly lower heterosexual function (e.g., ever engaging in genital petting or sexual intercourse, ever having a boyfriend) than control women. Furthermore, Ross et al. (1998) reported some beneficial effects of prepubertal low-dose estrogen treatment on cognitive function. Twenty-four TS girls exhibited a significant improvement in their motor function and nonverbal processing speed after estradiol treatment when compared to their TS peers who received a placebo treatment. Interestingly, CAIS individuals who are insensitive to testosterone are not insensitive to estradiol (testosterone is converted into estradiol by the enzyme aromatase); thus it might be postulated that estrogens induce a female-typical gender identity in these individuals. Clearly more research is needed to determine whether estradiol feminizes the brain in humans.

Based on the important role of testosterone in the development of gender identity, i.e., inducing a male-typical gender identity, the leading hypothesis on the etiology of GD involves a sex-atypical cerebral programming that diverges from the sexual differentiation of the rest of the body (Cohen-Kettenis and Gooren 1999; van Goozen et al. 2002; Gooren 2006; Hines 2010; Savic et al. 2010; Bao and Swaab 2011). It should be noted that the primary sexual differentiation of the genitals and the brain take place during different periods of fetal development, thereby allowing a time window for a potential divergence of the in utero endocrine climate driving the sexual differentiation of these organs. Some twin studies (Coolidge et al. 2002; Heylens et al. 2012) suggested a role for genetic factors in the development of GD, potentially involving polymorphisms in genes encoding elements of the sex steroid signaling or metabolic pathways, such as the androgen receptor (Hare et al. 2009), the estrogen receptor (Henningsson et al. 2005; Fernández et al. 2013), and aromatase (Bentz et al. 2008). However, these latter studies still await replication.

Neural Correlates

Postmortem studies investigating the brain of individuals with GD have observed a reduced volume and number of neurons in the central subdivision of the sexually dimorphic bed nucleus of the stria terminalis and the third interstitial nucleus of the anterior hypothalamus (INAH3) in males-to-females (MFs) in comparison to their natal sex controls (Zhou et al. 1995; Kruijver et al. 2000; Garcia-Falgueras and Swaab 2008). A female-typical expression of neurokinin B, an important regulator of GnRH release and thus reproductive functioning, has also been detected in the hypothalamus of MFs (Taziaux et al. 2012). In a female-to-male (FM) individual,

on the other hand, an INAH3 neuronal number in the male range was observed (Garcia-Falgueras and Swaab 2008).

More recently, the introduction of neuroimaging techniques has allowed in vivo assessment of the brain, thus enabling larger-scale projects involving more substantial subject samples and a more flexible study design. Several imaging studies have investigated the brains of individuals with GD. Studies using functional magnetic resonance imaging (fMRI) examining brain activation during a task of mental spatial rotation in MFs showed a divergence from the natal sex in the recruitment of parietal regions subserving this function (Carrillo et al. 2010; Schöning et al. 2010). Accordingly, a female-like pattern of brain activation was observed in MFs while viewing erotic stimuli (Gizewski et al. 2009). Exposure to 4,16-androstadien-3-one (AND), a steroid compound with postulated pheromonal properties, also elicited a female-like hypothalamic response in MF adults (Berglund et al. 2008). However, unlike in women, the hypothalamus of MFs also responded to estra-1,3,5(10),16-tetraen-3-ol (EST), a putative female chemosignal (Berglund et al. 2008).

Several imaging studies have also examined the structure of the brain in individuals with GD. MRI studies examining white matter tracts using diffusion tensor imaging have shown deviations from the natal sex in white matter microstructure in both FMs and MFs (Rametti et al. 2011a, b) . To date, five neuroimaging studies have investigated gray matter (GM) in individuals with GD, observing brain volumes largely concordant with the natal sex, although subtle signs of a sex-atypical sexual differentiation of the brain were also detected (Luders et al. 2009; Savic and Arver 2011; Zubiaurre-Elorza et al. 2013, 2014). A recent study by Zubiaurre-Elorza et al. (2013) was the first to examine GM in FMs. Interestingly, they observed signs of subcortical masculinization in FM adults and of cortical feminization in MF adults, providing the first insights into the developmental processes underlying GD in both sexes. Taken together, these findings provide a strong suggestion of an atypical sexual differentiation of the brain in individuals suffering from GD. However, all the above-mentioned studies examined individuals in adulthood, when both organizational and activational steroid hormone effects have already sculpted the brain into a sex-specific configuration.

Objectives

GD often shows up at early pre-pubertal ages. In general, sex reassignment for individuals with GD was met with a great deal of skepticism, and this is even more evident when it comes to the treatment of young people. Thus, no irreversible interventions such as hormonal and surgical treatments were allowed before adulthood. However, this policy has changed over the years, particularly since early hormonal treatment of GD individuals has led to positive results (Gooren and Delemarre-van de Waal 1996; Cohen-Kettenis and van Goozen 1998). According to the new Dutch protocol established by Cohen-Kettenis and Delemarre-van de

Waal, adolescents who have experienced severe GD since early childhood are allowed, after a careful psychological diagnostic procedure, to start using GnRH agonists to suppress pubertal maturation (Kreukels and Cohen-Kettenis 2011; de Vries and Cohen-Kettenis 2012). A minimum age of 12 years and at least Tanner Stage 2/3 should be reached before any medical treatment is started. Several advantages of this treatment are that further development of the secondary sex characteristics is suppressed, the psychological well-being of adolescents improves (stress relief), the adolescent can take his/her time to weigh the possibilities of an actual sex reassignment procedure and, finally, arresting the development of the secondary sex characteristics positively influences later physical appearance.

From the age of 16 years on, as a first step in the actual sex reassignment, adolescents with persisting GD may receive cross-sex hormones (testosterone for natal girls and estradiol for natal boys), in addition to their treatment with GnRH agonists, in order to develop secondary sex characteristics of their experienced gender (Delemarre-van de Waal and Cohen-Kettenis 2006; Hembree et al. 2009). However, empirical evidence for the favorable effects of this protocol is needed to counter critics who raise ethical considerations about medical interventions in minors. It has been argued that experiencing all stages of (physical) puberty and having age-appropriate (socio-) sexual experiences are crucial for psychological (cognitive and emotional) maturation (Meyenburg 1999; Spriggs 2004; Korte et al. 2008). Others have pointed out that inhibition of endogenous sex hormone functioning might have detrimental effects on bone maturation and growth (Viner et al. 2005; Houk and Lee 2006). Finally, several studies, although only conducted in animal species so far, have suggested that puberty might reflect a second organizational period in brain development (Sisk et al. 2003).

Therefore, the first aim of our studies was to determine whether sex-related brain functions and sex differences in brain structures are already established during early development and thus would be present in prepubertal children, or whether sex-specific characteristics of the brain emerge during puberty, as a second organizational period in the sexual differentiation of the brain. The second aim was to determine whether individuals with GD might have undergone a sex-atypical sexual differentiation, and thus whether their neurobiological characteristics reflect and match their experienced gender rather than their natal sex. In this review, we focus on one small part of these rather extensive studies, i.e., the study in which prepubertal children and adolescents, both GD and controls, were exposed to the putative male chemo-signal 4,16-androstadien-3-one (AND). As mentioned above, studies by the group of Savic et al. (2001) using positron emission topography (PET) have shown sex differences in hypothalamic activation upon exposure to this odorous steroid, with a significant activation in the hypothalamus of heterosexual women but not in heterosexual men. We recently replicated this sex difference in groups of adult men and women by using functional MRI, since PET is considered to be too invasive in pediatric populations (Burke et al. 2012).

Methods

Subjects

All subjects diagnosed with GD were recruited via the Center of Expertise on Gender Dysphoria at the VU University Medical Center in Amsterdam. The control participants were recruited via several primary and secondary schools in The Netherlands and by inviting friends and relatives of the participants with GD.

The children's sample consisted of 19 control girls [mean (±SD) years of age = 9.7 ± 0.9], 20 control boys (9.5 ± 1.1), 17 girls with GD (9.6 ± 1.1), and 19 boys with GD (10.4 ± 0.9). All children underwent a short physical examination by a pediatric endocrinologist to ascertain their prepubertal status (Tanner stage 1; Marshall and Tanner 1969, 1970).

The adolescent groups consisted of 21 control girls (16.3 ± 0.9), 20 control boys (15.0 ± 0.6), 21 girls with GD (16.1 ± 0.8), and 17 boys with GD (15.3 ± 1.2). The adolescent participants diagnosed with GD had been treated monthly with 3.75 mg of Triptorelin (Decapeptyl-CR®, Ferring, Hoofddorp, The Netherlands), a GnRH agonist, by injection for on average 24 months (range 2–48 months), resulting in complete suppression of gonadal hormone production. Female adolescent controls were tested randomly according to their menstrual cycle, and 11 of 21 control girls reported using hormonal contraception. All subjects and their legal guardians gave their informed consent according to the Declaration of Helsinki, and the study was approved by the Ethics Committee of the VU University Medical Center Amsterdam (application number NL31283.029.10).

Olfactory Function and Stimulation

First, normal olfactory function was ascertained by means of an extended version of the "Sniffin' Sticks" test battery (32-item odor identification test and olfactory threshold measurement; Kobal et al. 1996, 2000; Hummel et al. 2007). Then participants were asked to report the perceived intensity of a 10 mM androstadienone solution (on a scale from 0 to 10). For the olfactory stimulation in the MRI, androstadienone (Steraloids Inc., Newport, RI 02840, US) was diluted in propylene glycol (Sigma) to a concentration of 10 mM, according to the "high" concentration used in our previous study (Burke et al. 2012). The volume of the solution used during the fMRI experiments was 20 ml. Olfactory stimuli were delivered through a tubing system to the subjects' nostrils by means of a custom-built air-dilution olfactometer (for details of the olfactometer set-up and procedure see Burke et al. 2012). With a total air flow of about 1 l per minute, during "ON" periods the odor was delivered every 2 s for 1 s, whereas during "OFF" periods subjects received odorless air.

Image Acquisition and Processing

Scans were performed on a 3.0 T GE Signa HDxt scanner (General Electric, Milwaukee, WI, USA). A gradient echo echoplanar imaging sequence was used for functional imaging (19.2 cm^2 field of view, TR of 1950 ms, TE of 25 ms, an 80° flip angle, isotropic voxels of 3 mm, and 36 slices). Before each imaging session, a local high-order shimming technique was used to reduce susceptibility artefacts. A scanning session consisted of six alternating ON-OFF cycles over 108 volumes in a classical block design (one block consisted of nine volumes), lasting 3.6 min. For co-registration with the functional images, a T1-weighted scan was obtained (3D FSPGR sequence, 25 cm^2 field of view, TR of 7.8 ms, TE of 3.0 ms; slice thickness of 1 mm, and 176 slices).

Data analysis was performed with SPM8 software (Statistical Parametric Mapping; Wellcome Department of Imaging Neuroscience, Institute of Neurology at the University College London, UK) implemented in Matlab R2009b (Math Works Inc., Natick, MA, USA). Functional images were slice-timed and realigned to the mean image, followed by unwarp. Applying the 'New Segment' and 'Create Template' options of the DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra) toolbox, structural images were segmented. Then, gray matter and white matter images were used for creating age group-specific templates (one each for the children and the adolescents samples), registered in Montreal Neurological Institute (MNI) space. Functional images were spatially normalized to their respective group-template, applying each individual's DARTEL flow field and, finally, images were smoothed by means of a 5 mm full width half maximum (FWHM) isotropic Gaussian kernel.

Individual image data were analyzed using boxcar regressors convolved with a synthetic hemodynamic response function and a first-order time modulation (TM) regressor to test for possible effects of adaptation/sensitization to androstadienone. To account for assumed late "wash-out" effects during OFF blocks and an early peak response to the odor stimulation during ON blocks, first-level contrast images were built by subtracting the second half (b) of the OFF blocks (four volumes) from the first part (a) of the ON blocks (four volumes). Accordingly, this was done with the associated TM regressor blocks. Further, based on the image realignment process, individual head jerks were identified (>1 mm displacement; Lemieux et al. 2007). Together with the six motion parameters, these so-called scan nulling regressors were included in every first-level design matrix to account for the effects of excessive head motion.

Statistical Analyses

First, to test whether the sex difference in response to androstadienone was present in the control groups (i.e., prepubertal and adolescent), and whether a potential sex

difference in responsiveness varied as a function of adaptation/sensitization to the odor, we conducted a *sex* (control boys, control girls) by *odor stimulation* ANOVA for both the prepubertal and the adolescent control groups. The factor *odor stimulation* consisted of two levels, a regressor modeling the condition ON_a – OFF_b effect, i.e., the hypothesized hypothalamic response to the odor, and a first order parametric modulation TM ON_a–TM OFF_b regressor, which indicates how well the hypothalamic response correlates with changes over time, thus modeling possible effects of adaptation or sensitization.

Second, by means of four *gender* by *odor stimulation* ANOVAs separately for the prepubertal and the adolescent groups, we tested whether boys and girls diagnosed with GD differed significantly in response to the steroid odor in comparison to their respective natal sex or their experienced gender control group.

Analyses were restricted to the hypothalamus area as region of interest (ROI), defined (with Marsbar; Brett et al. 2002) as a sphere (centered at MNI coordinates $x = 0$, $y = -10$, $z = -7$; with a 7 mm radius), and based on anatomical demarcations following Makris et al. (2013) and Baroncini et al. (2012). The threshold for statistical significance was set at $p < 0.05$ family-wise error (FWE)-corrected for the extent of the hypothalamus ROI.

Results

Sex Difference in Control Groups

Prepubertal girls and boys did not differ in terms of general hypothalamic responsiveness (condition effect ON > OFF) to androstadienone, but directional *t*-tests showed that the sex difference (girls > boys) was significantly modulated by the effect of the TM regressor ($t = 3.33$; $p = 0.033$), indicating that hypothalamic activation in boys differed from that of the girls during the course of the scanning session and thus suggesting differences in adaptation and/or sensitization to the odor between groups (Fig. 1). Accordingly, similar comparisons were done in the adolescent groups. Again, the sex difference in hypothalamic activation (girls > boys) was significantly dependent on the factor time ($t = 3.52$; $p = 0.019$; Fig. 1). Visual inspection of the data revealed that activation increased in girls with repeated exposure to androstadienone (sensitization), particularly towards the end of the session, whereas the responsiveness to the steroid odor seemed relatively stable in boys, showing a slight decrease in activation over the course of stimulation.

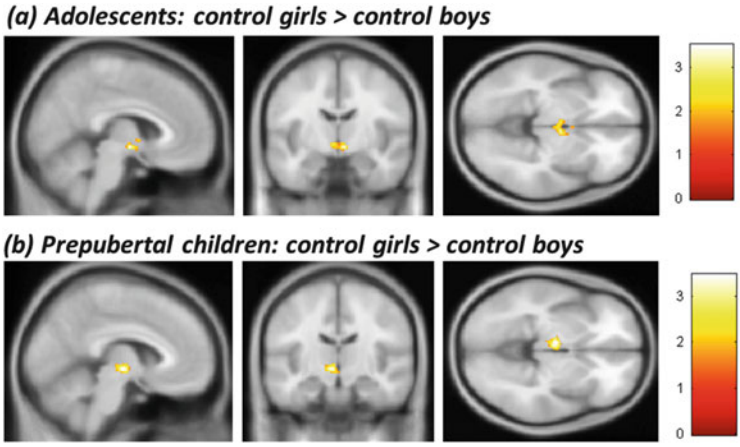

Fig. 1 Sex differences in hypothalamic activation in both the (**a**) adolescent and the (**b**) prepubertal control groups. Irrespective of their pubertal status, girls compared with boys (directional t-contrast) showed a stronger hypothalamic activation in response to smelling the chemo-signal androstadienone. The *color bar* indicates the t statistic

Responses in Girls Diagnosed with GD

Prepubertal girls with GD differed significantly from neither their experienced (control boys) nor from their natal sex (control girls) in terms of hypothalamic activation when smelling androstadienone. None of the comparisons between prepubertal control girls and prepubertal girls with GD revealed any differences in hypothalamic activation upon smelling androstadienone. However, no sex-typical effects (i.e., a female-typical hypothalamus response), when compared to control boys, could be confirmed. In contrast, the activation in adolescent girls with GD was very similar to that in adolescent control boys, i.e., remaining stable over the course of stimulation, whereas control girls showed a significantly stronger hypothalamic response to androstadienone over time as described earlier (Fig. 2). Thus the comparison of adolescent control girls to girls diagnosed with GD revealed a significant effect of *gender* (control girls > girls with GD), which was mainly explained by the effect of the TM regressor ($t = 4.3$; $p = 0.002$).

Responses in Boys Diagnosed with GD

Prepubertal boys with GD showed a pattern of hypothalamic activation that was similar to that of the prepubertal control boys. However, when adolescent boys with GD were compared to adolescent control boys, a significant effect of condition (ON > OFF) was observed (Fig. 2). Thus, adolescent boys with GD showed a significantly stronger, thus sex-atypical response, to androstadienone compared to

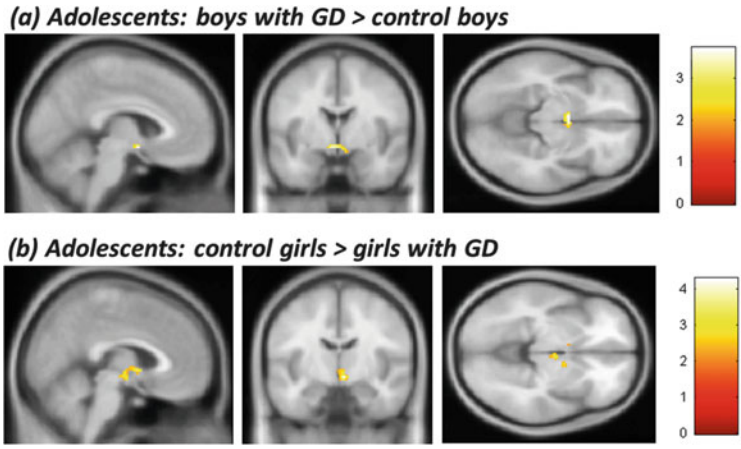

Fig. 2 Sex-atypical hypothalamic responses in the (**a**) natal boys with Gender Dysphoria (GD) and (**b**) natal girls with GD. Adolescent natal boys showed a similar hypothalamic activation as control girls when smelling androstadienone, whereas adolescent natal girls with GD showed a male-typical response pattern. The *color bar* indicates the t statistic

control boys, irrespective of the factor time, which indicates that adolescent boys with GD showed female-typical hypothalamic responses upon smelling androstadienone without, however, any effects due to sensitization.

Discussion

Sex differences in hypothalamic activation upon smelling androstadienone are already present before puberty: both prepubertal and adolescent control girls showed a stronger hypothalamic activation compared to boys. This sex difference seemed to be crucially modulated by effects of sensitization to androstadienone. So, in answer to the first aim, sex differences in hypothalamic responses to androstadienone seemed to have developed during early brain development, at least before puberty. Furthermore, adolescents with GD, both natal boys and girls, showed hypothalamic responses that were in line with their experienced gender, suggesting that they might indeed have undergone an atypical sexual differentiation of the brain. However, the hypothalamic response was not observed in the prepubertal subjects with GD, although girls with GD showed neither a male- nor a female-typical hypothalamic response. By contrast, prepubertal boys with GD showed similar responses as control boys.

The absence of a clear effect in the prepubertal sample of GD suggests that pubertal hormones might somehow be involved in the development of GD. However, it should be noted that the prepubertal groups with GD constitute rather heterogeneous groups with respect to future-persisting GD. It has been shown

that only about 20 % of the childhood GD cases will eventually lead to adult GD (Steensma et al. 2011), although overall women present early-onset cases more often (Nieder et al. 2011) and girls show higher persistency rates of GD into adolescence compared to boys (Steensma et al. 2013). Thus, our finding of prepubertal girls with GD showing neither a clear-cut female- nor male-typical response to androstadienone awaits confirmation in the future, when it will be known who of our participants showed persisting GD into adolescence and adulthood. Regarding the prepubertal boys with GD, who were similar to control boys in their hypothalamic responses, the same argument probably holds, i.e., they present a heterogeneous group with respect to future-persisting GD and further confirmation is needed, especially since the adolescent boys with GD showed female-typical hypothalamic activations.

Acknowledgments This study was funded by a VICI grant (453-08-003) from the Dutch Science Foundation (Nederlandse Organisatie voor Wetenschappelijk Onderzoek) to Dr. J. Bakker. Dr. J. Bakker is a senior research associate of the Belgian Fonds National de la Recherche Scientifique.

References

American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders. DSM-5, 5th edn. American Psychiatric Association, Arlington

Bakker J, Brock O (2010) Early oestrogens in shaping reproductive networks: evidence for a potential organisational role of oestradiol in female brain development. J Neuroendocrinol 22:728–732

Bao A-M, Swaab DF (2011) Sexual differentiation of the human brain: relation to gender identity, sexual orientation and neuropsychiatric disorders. Front Neuroendocrinol 32:214–226

Baroncini M, Jissendi P, Balland E, Besson P, Pruvo J-P, Francke J-P, Dewailly D, Blond S, Prevot V (2012) MRI atlas of the human hypothalamus. Neuroimage 59:168–180

Baum MJ (2006) Mammalian animal models of psychosexual differentiation: when is "translation" to the human situation possible? Horm Behav 50:579–588

Bentz E-K, Hefler LA, Kaufmann U, Huber JC, Kolbus A, Tempfer CB (2008) A polymorphism of the CYP17 gene related to sex steroid metabolism is associated with female-to-male but not male-to-female transsexualism. Fertil Steril 90:56–59

Berglund H, Lindström P, Dhejne-Helmy C, Savic I (2008) Male-to-female transsexuals show sex-atypical hypothalamus activation when smelling odorous steroids. Cereb Cortex 18:1900–1908

Brett M, Anton J, Valabregue R, Poline J-B (2002) Region of interest analysis using an SPM toolbox (abstract). In: Presented at the 8th international conference on functional mapping of the human brain. Sendai (available on CD–RO NeuroImage, Vol 16, No 2)

Brock O, Baum MJ, Bakker J (2011) The development of female sexual behavior requires prepubertal estradiol. J Neurosci 31:5574–5578

Burke SM, Veltman DJ, Gerber J, Hummel T, Bakker J (2012) Heterosexual men and women both show a hypothalamic response to the chemo-signal androstadienone. PLoS One 7:e40993

Carrillo B, Gómez-Gil E, Rametti G, Junque C, Gomez A, Karadi K, Segovia S, Guillamon A (2010) Cortical activation during mental rotation in male-to-female and female-to-male transsexuals under hormonal treatment. Psychoneuroendocrinology 35:1213–1222

Coates S (1990) Ontogenesis of boyhood gender identity disorder. J Am Acad Psychoanal 18:414–438
Coates S, Person ES (1985) Extreme boyhood femininity: isolated behavior or pervasive disorder? J Am Acad Child Psychiatry 24:702–709
Cohen-Kettenis PT, Gooren LJ (1999) Transsexualism: a review of etiology, diagnosis and treatment. J Psychosom Res 46:315–333
Cohen-Kettenis PT, van Goozen SH (1998) Pubertal delay as an aid in diagnosis and treatment of a transsexual adolescent. Eur Child Adolesc Psychiatry 7:246–248
Cohen-Kettenis PT, van Goozen SH, Doorn CD, Gooren LJ (1998) Cognitive ability and cerebral lateralisation in transsexuals. Psychoneuroendocrinology 23:631–641
Coolidge FL, Thede LL, Young SE (2002) The heritability of gender identity disorder in a child and adolescent twin sample. Behav Genet 32:251–257
De Vries ALC, Cohen-Kettenis PT (2012) Clinical management of gender dysphoria in children and adolescents: the Dutch approach. J Homosex 59:301–320
Delemarre-van de Waal HA, Cohen-Kettenis PT (2006) Clinical management of gender identity disorder in adolescents: a protocol on psychological and paediatric endocrinology aspects. Eur J Endocrinol 155:S131–S137
Downey J, Ehrhardt AA, Gruen R, Bell JJ, Morishima A (1989) Psychopathology and social functioning in women with Turner syndrome. J Nerv Ment Dis 177:191–201
Fernández R, Esteva I, Gómez-Gil E, Rumbo T, Almaraz MC, Roda E, Haro-Mora J-J, Guillamón A, Pásaro E (2013) The (CA)n polymorphism of ERβ gene is associated with FtM transsexualism. J Sex Med 11:720–728
Garcia-Falgueras A, Swaab DF (2008) A sex difference in the hypothalamic uncinate nucleus: relationship to gender identity. Brain 131:3132–3146
Gizewski ER, Krause E, Schlamann M, Happich F, Ladd ME, Forsting M, Senf W (2009) Specific cerebral activation due to visual erotic stimuli in male-to-female transsexuals compared with male and female controls: an fMRI study. J Sex Med 6:440–448
Gooren L (2006) The biology of human psychosexual differentiation. Horm Behav 50:589–601
Gooren L, Delemarre-van de Waal H (1996) The feasibility of endocrine interventions in juvenile transsexuals. J Psychol Hum Sex 8:69–74
Hare L, Bernard P, Sánchez FJ, Baird PN, Vilain E, Kennedy T, Harley VR (2009) Androgen receptor repeat length polymorphism associated with male-to-female transsexualism. Biol Psychiatry 65:93–96
Hembree WC, Cohen-Kettenis P, Delemarre-van de Waal HA, Gooren LJ, Meyer WJ, Spack NP, Tangpricha V, Montori VM (2009) Endocrine treatment of transsexual persons: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 94:3132–3154
Henningsson S, Westberg L, Nilsson S, Lundström B, Ekselius L, Bodlund O, Lindström E, Hellstrand M, Rosmond R, Eriksson E, Landén M (2005) Sex steroid-related genes and male-to-female transsexualism. Psychoneuroendocrinology 30:657–664
Heylens G, De Cuypere G, Zucker KJ, Schelfaut C, Elaut E, Vanden Bossche H, De Baere E, T'Sjoen G (2012) Gender identity disorder in twins: a review of the case report literature. J Sex Med 9:751–757
Hines M (2010) Sex-related variation in human behavior and the brain. Trends Cogn Sci 14:448–456
Houk CP, Lee PA (2006) The diagnosis and care of transsexual children and adolescents: a pediatric endocrinologists' perspective. J Pediatr Endocrinol Metab 19:103–109
Hummel T, Kobal G, Gudziol H, Mackay-Sim A (2007) Normative data for the "Sniffin' Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. Eur Arch Oto-Rhino-Laryngol 264:237–243
Kobal G, Hummel T, Sekinger B, Barz S, Roscher S, Wolf S (1996) "Sniffin' sticks": screening of olfactory performance. Rhinology 34:222–226
Kobal G, Klimek L, Wolfensberger M, Gudziol H, Temmel A, Owen CM, Seeber H, Pauli E, Hummel T (2000) Multicenter investigation of 1,036 subjects using a standardized method for

the assessment of olfactory function combining tests of odor identification, odor discrimination, and olfactory thresholds. Eur Arch Otorhinolaryngol 257:205–211
Korte A, Lehmkuhl U, Goecker D, Beier KM, Krude H, Grüters-Kieslich A (2008) Gender identity disorders in childhood and adolescence: currently debated concepts and treatment strategies. Dtsch Arztebl Int 105:834–841
Kreukels BPC, Cohen-Kettenis PT (2011) Puberty suppression in gender identity disorder: the Amsterdam experience. Nat Rev Endocrinol 7:466–472
Kruijver FPM, Zhou JN, Pool CW, Hofman MA, Gooren LJ, Swaab DF (2000) Male-to-female transsexuals have female neuron numbers in a limbic nucleus. J Clin Endocrinol Metab 85:2034–2041
Kuyper E, Wijsen C (2014) Gender identities and gender dysphoria in the Netherlands. Arch Sex Behav 43:377–385
Lemieux L, Salek-Haddadi A, Lund TE, Laufs H, Carmichael D (2007) Modelling large motion events in fMRI studies of patients with epilepsy. Magn Reson Imaging 25:894–901
Luders E, Sánchez FJ, Gaser C, Toga AW, Narr KL, Hamilton LS, Vilain E (2009) Regional gray matter variation in male-to-female transsexualism. Neuroimage 46:904–907
Makris N, Swaab DF, van der Kouwe A, Abbs B, Boriel D, Handa RJ, Tobet S, Goldstein JM (2013) Volumetric parcellation methodology of the human hypothalamus in neuroimaging: normative data and sex differences. Neuroimage 69:1–10
Marshall WA, Tanner JM (1969) Variations in pattern of pubertal changes in girls. Arch Dis Child 44:291–303
Marshall WA, Tanner JM (1970) Variations in the pattern of pubertal changes in boys. Arch Dis Child 45:13–23
Meyenburg B (1999) Gender identity disorder in adolescence: outcomes of psychotherapy. Adolescence 34:305–313
Meyer-Bahlburg HFL, Dolezal C, Baker SW, New MI (2008) Sexual orientation in women with classical or non-classical congenital adrenal hyperplasia as a function of degree of prenatal androgen excess. Arch Sex Behav 37:85–99
Nieder TO, Herff M, Cerwenka S, Preuss WF, Cohen-Kettenis PT, De Cuypere G, Haraldsen IRH, Richter-Appelt H (2011) Age of onset and sexual orientation in transsexual males and females. J Sex Med 8:783–791
Rametti G, Carrillo B, Gomez-Gil E, Junque C, Segovia S, Gomez A, Guillamon A (2011a) White matter microstructure in female to male transsexuals before cross-sex hormonal treatment. A diffusion tensor imaging study. J Psychiatr Res 45:199–204
Rametti G, Carrillo B, Gomez-Gil E, Junque C, Zubiarre-Elorza L, Segovia S, Gomez A, Guillamon A (2011b) The microstructure of white matter in male to female transsexuals before cross-sex hormonal treatment. A DTI study. J Psychiatr Res 45:949–954
Rolstad SG, Moller A, Byrman I, Boman UW (2007) Sexual functioning and partner relationships in women with turner syndrome: some empirical data and theoretical considerations regarding sexual desire. J Sex Marital Ther 33:231–247
Ross JL, Roeltgen D, Feuillan P, Kushner H, Cutler GB (1998) Effects of estrogen on nonverbal processing speed and motor function in girls with Turner's syndrome. J Clin Endocrinol Metab 83:3198–3204
Savic I, Arver S (2011) Sex dimorphism of the brain in male-to-female transsexuals. Cereb Cortex 21:2525–2533
Savic I, Berglund H, Gulyas B, Roland P (2001) Smelling of odorous sex hormone-like compounds causes sex-differentiated hypothalamic activations in humans. Neuron 31:661–668
Savic I, Garcia-Falgueras A, Swaab DF (2010) Sexual differentiation of the human brain in relation to gender identity and sexual orientation. Prog Brain Res 186:41–62
Schagen SEE, Delemarre-van de Waal HA, Blanchard R, Cohen-Kettenis PT (2012) Sibling sex ratio and birth order in early-onset gender dysphoric adolescents. Arch Sex Behav 41:541–549
Schöning S, Engelien A, Bauer C, Kugel H, Kersting A, Roestel C, Zwitserlood P, Pyka M, Dannlowski U, Lehmann W, Heindel W, Arolt V, Konrad C (2010) Neuroimaging differences

in spatial cognition between men and male-to-female transsexuals before and during hormone therapy. J Sex Med 7:1858–1867
Seckl JR, Holmes MC (2007) Mechanisms of disease: glucocorticoids, their placental metabolism and fetal "programming" of adult pathophysiology. Nat Clin Pract Endocrinol Metab 3:479–488
Shaeffer AT, Lange E, Bondy CA (2008) Sexual function in women with Turner syndrome. J Women's Health 17:27–33
Sisk CL, Schulz KM, Zehr J (2003) Puberty: a finishing school for male social behavior. Ann N Y Acad Sci 1007:189–198
Spriggs MP (2004) Ethics and the proposed treatment for a 13-year-old with atypical gender identity. Med J Aust 181:319–321
Steensma TD, Biemond R, de Boer F, Cohen-Kettenis PT (2011) Desisting and persisting gender dysphoria after childhood: a qualitative follow-up study. Clin Child Psychol Psychiatry 16:499–516
Steensma TD, McGuire JK, Kreukels BPC, Beekman AJ, Cohen-Kettenis PT (2013) Factors associated with desistence and persistence of childhood gender dysphoria: a quantitative follow-up study. J Am Acad Child Adolesc Psychiatry 52:582–590
Swaab DF (2007) Sexual differentiation of the brain and behavior. Best Pract Res Clin Endocrinol Metab 21:431–444
Taziaux M, Swaab DF, Bakker J (2012) Sex differences in the neurokinin B system in the human infundibular nucleus. J Clin Endocrinol Metab 97:E2210–E2220
Van Goozen SHM, Gooren LJG, Sanders G, Cohen-Kettenis PT, Slabbekoorn D (2002) Organizing and activating effects of sex hormones in homosexual transsexuals. Behav Neurosci 116:982–988
Viner RM, Brain C, Carmichael P, Di Ceglie D (2005) Sex on the brain: dilemmas in the endocrine management of children and adolescents with gender identity disorder [abstract G200]. Arch Dis Child 90:A77–A81
Ward OB, Ward IL, Denning JH, Hendricks SE, French JA (2002) Hormonal mechanisms underlying aberrant sexual differentiation in male rats prenatally exposed to alcohol, stress, or both. Arch Sex Behav 31:9–16
World Health Organization (1992) International statistical classification of diseases and related health problems. 10th Revision (ICD-10). Chapter V. Mental and behavioural disorders, Geneva, pp F00–F9
Zhou J-NN, Hofman MA, Gooren LJG, Swaab DF (1995) A sex difference in the human brain and its relation to transsexuality. Nature 378:68–70
Zubiaurre-Elorza L, Junque C, Gómez-Gil E, Segovia S, Carrillo B, Rametti G, Guillamon A (2013) Cortical thickness in untreated transsexuals. Cereb Cortex 23:2855–2862
Zubiaurre-Elorza L, Junque C, Gómez-Gil E, Guillamon A (2014) Effects of cross-sex hormone treatment on cortical thickness in transsexual individuals. J Sex Med 11:1248–1261
Zucker KJ, Bradley SJ (1995) Gender identity disorder and psychosexual problems in children and adolescents. Guilford Press, New York
Zucker KJ, Lawrence AA (2009) Epidemiology of gender identity disorder. Int J Transgenderism 11:8–18

Pubertal Timing, Exploratory Behavior and Mental Health: A View from a Clinician and Public Health Practitioner

P.-A. Michaud

Abstract The potential impact of early versus late pubertal timing on adolescent psychological and behavioral development and adjustment has led to interesting but conflicting results. Early- and late-maturing adolescents engage in exploratory/risk behavior differently, as compared with their "on time" peers. This impact is not the same among males and females and probably varies within different environmental contexts. Moreover, we do not clearly understand the origin of such a phenomenon: recent publications tend to indicate that one's self image representation may have an effect on the behavior of early-maturing females, whereas others suggest that poor psychosocial adjustment may antedate the appearance of puberty among early-maturing adolescents of both sexes. The meaning of these pieces of research should be integrated into the everyday care of adolescents and in the public health area.

Introduction

Human puberty is a very puzzling and sophisticated process. According to Bogin (1994) and Gluckman (2005; (Gluckman and Hanson 2006), humans are the only mammalian species that undergo a second growth spurt after a first period of rapid growth following birth. In an ontogenic perspective, this may be seen as a way to allow for a more important growth of the brain as compared with other species, such as apes. This is all the more understandable when we take into account what we know from recent research, that the development of the adolescent brain lasts until late adolescence or early adulthood (Giedd 2008), as is discussed in other chapters of this volume. Another rather interesting phenomenon in the human species is the secular trend in the reduction of the age at which puberty starts, a phenomenon that is not totally understood but that, at least partly, relates to secular trends in the nutritional state of pregnant women as well as the presence in the environment of

P.-A. Michaud (✉)
Multidisciplinary Unit for Adolescent Health, Lausanne University Hospital – CHUV, Lausanne, Switzerland
e-mail: Pierre-andre.michaud@chuv.ch

J.-P. Bourguignon et al. (eds.), *Brain Crosstalk in Puberty and Adolescence*, Research and Perspectives in Endocrine Interactions 13,
DOI 10.1007/978-3-319-09168-6_4

endocrine disruptors (Parent et al. 2003; Bellis et al. 2006; Gluckman and Hanson 2006; Bourguignon et al. 2010, 2013). A major consequence of an earlier puberty in the human species is that the period separating the attainment of biologic maturity and social maturity is enlarging. This kind of mismatch, or so-called maturational gap (Moffitt 1993; Waylen and Wolke 2004; Gluckman and Hanson 2006), has a profound developmental impact on individuals who are neither children nor adults for several years. A third singularity of human puberty is the wide span between those individuals who mature earlier and those who mature later. Since the seminal work of Tanner (1962), we know that a period of around five years separates early- and late-maturing adolescents, with a mean difference of one year between girls and boys, the former being the ones who enter puberty first.

It is important to stress the difference between premature/precocious puberty and early puberty, as well as between late and delayed puberty: both early- and late-maturing individuals are in the normal range of pubertal timing, whereas precocious and delayed puberty usually command clinical and laboratory investigations. Although, once the process of puberty begins, it tends to follow a fairly strict sequence of events (Tanner 1962), the question arises as to whether young people who mature earlier or later exhibit differences in their health status or their psychosocial development. There is an extensive literature available on this issue, which stresses both the impact of early or late maturation among girls as well as boys (Graber et al. 1997, 2004; Kaltiala-Heino et al. 2003a, b; Ostovich and Sabini 2005; Patton and Viner 2007).

Before discussing in detail the impact of pubertal timing on the health and development of young people, and especially on behavior, a few general comments are needed.

Conceptual Comments

The relationship between pubertal timing and the mental health and behavioral conduct of adolescents has often been based on cross-sectional studies, whereas more recent longitudinal publications have drawn attention to the fact that, on one hand, some factors may precede the occurrence of puberty and explain some of these relationships (Graber et al. 2010; Bleil et al. 2013), and, on the other hand, such correlations may be observed well beyond the period of puberty itself (Michaud et al. 2006). Given the emerging evidence that we have of the role of hormones and brain development in determining adolescent behavior, such issues should be kept in mind. Also, the measurement of pubertal timing is often difficult, as many if not most of the published literature focuses on a self-evaluation of pubertal stage by the adolescents themselves and not on an actual clinical assessment or a dosage of hormones. While the developmental staging in five clinically observable steps proposed by Tanner (1962) is largely used by clinicians and represents a good appreciation of the progress of puberty, a fairly large literature has used self-reported measures of puberty, using questions or drawings or

comparisons with peers, without any clinical examination. These studies show that both girls' and boys' reports tend to be biased by their own individual representations, with some subjects overestimating or underestimating the progress of their own puberty (Brooks-Gunn et al. 1987; Berg-Kelly and Erdes 1997; Bond et al. 2006).

Another area of conceptual discussion is that of so-called "risk behaviors" (Jessor 1991); the literature, especially that of Anglo-Saxon origin, is filled with papers pertaining to risky behaviors or risk-taking behavior. As of November 2013, Medline provided around 34,000 references on this topic. There are several reasons why this wording should be used cautiously:

1. Many studies tend to confuse risk factors and risk behaviors, which are two distinct aspects of the general concept of risk;
2. Conduct that is considered risky is often not well defined. Indeed, while it is clear that unprotected sex can potentially result in the risk of transmission of sexually transmitted infections or unintended pregnancy, sexual activity per se should not be considered a risk-taking behavior; and
3. Confusion often exists between the behaviors and the individuals, inducing a kind of "labeling" of individuals as risk-taking teenagers. This amalgam ignores the fact that many behaviors during adolescence are transitory. Moreover, it is stigmatizing and unfair to individuals who actually adopt deviant behavior not so much because of their personality but as a consequence of an unfavorable environment.

For these reasons, we have repeatedly suggested the use of expressions such as experimental or exploratory behavior instead (Michaud et al. 1998; Michaud 2006), underscoring the fact that a process of "try and retry" is part of the adolescent developmental process (as it is for children). For extreme behavior that potentially exposes individuals to short- and long-term consequences, the expression "health-compromising behavior" is also probably more appropriate and less stigmatizing than risk-taking behavior.

Pubertal Timing, Physical and Mental Health, Exploratory Behavior

It is now recognized that precocious puberty is linked with several physical disorders, such as polycystic ovary syndrome (PCOS) or a higher risk for breast cancer, probably due to exposure to environmental toxic agents (Buck Louis et al. 2008; Golub et al. 2008). Early puberty may also be linked with a higher risk for the persistence of asthma into adulthood (Varraso et al. 2005). Still other studies suggest that early puberty is linked with a higher rate of somatic burden, such as muscular disorders or headaches (Rhee 2005; Patton and Viner 2007).

Mental health problems and diseases occur earlier and at a higher rate among early maturers, especially among girls (Kaltiala-Heino et al. 2003b; Graber et al. 2004, 2010; Johansson and Ritzen 2005; Mendle et al. 2007; DeRose et al. 2011; Oldehinkel et al. 2011), including a higher risk for deliberate self harm and suicidal conduct (Wichstrom 2000; Michaud et al. 2006). However, the relationship is far from clear, as one study found a higher risk for self harm among late-maturing girls (Patton et al. 2007). An interesting question is whether the fact that the rate of depression is higher among girls than among boys is linked to gender/sex issues or to the fact that puberty begins earlier among females (Wade et al. 2002). It should be stressed, however, that early puberty among boys may also in some instances be linked with a higher risk for mental health problems (Mendle et al. 2012). Also, it is well known that schizophrenia and psychotic disorders often appear during adolescence, although the relationships between the increase in psychotic disorders, the surge of hormones and the development of the brain are still not well understood (Cohen et al. 1999).

Early puberty among girls and delayed puberty among boys have both been showed to be correlated with lower self image and self esteem, with a higher risk for eating disorders (Striegel-Moore et al. 2001; McCabe and Ricciardelli 2004; Zehr et al. 2007; Graber et al. 2010). In a cross-sectional study involving 16–20 years adolescents of both sexes, we have shown that such a correlation is still observable beyond the period of puberty until late adolescence (Michaud et al. 2006). Much of the available literature focuses on the correlations between early puberty and risk/exploratory behaviors, such as early unprotected sexual intercourse or substance use and misuse or antisocial behavior. The effect of early puberty on sexual behavior, both among girls and boys, is well documented. In all studies focusing on this issue, the rate of teenagers engaging in active sexual relations is higher among early maturers and that this correlation persists into early adulthood (Lam et al. 2002; Kaltiala-Heino et al. 2003a; Parent et al. 2003; Johansson and Ritzen 2005; Michaud et al. 2006). To some extent, the correlation may be higher among boys than among girls (Ostovich and Sabini 2005). In most of these publications, it has also been shown that late maturers of both sex conversely engage in an active sexual life at a lower pace/later. The age and rate at which young people engage in the use/misuse of psychotropic drugs is also linked with pubertal timing (Dick et al. 2000; Lanza and Collins 2002; Patton et al. 2004; Michaud et al. 2006); both early maturing boys and girls engage in the consumption of substances (e.g., cigarette, alcohol and illegal drugs) at a higher rate, with the difference persisting into young adulthood. Conversely, delayed puberty may constitute a protective factor against substance use and misuse (Orr and Ingersoll 1995; Waylen and Wolke 2004).

In summary, among girls, the relationship between early versus late puberty is fairly uniform, e.g., early maturing girls display a lower self image, a higher rate of mental health problems and a higher and more precocious rate of exploratory behavior than their mates of similar chronological age but who mature slower. Among boys, the relationship is more complex; for example, in the field of mental health, the relationship is U-curved, that is, both early- and late-maturing boys

suffer from mental health problems and low self esteem at a higher rate, whereas the correlation, as far as experimental/health-compromising behavior is concerned, is similar to that of the girls (Michaud et al. 2006).

Hypotheses

As shown in Fig. 1, the interplay between pubertal timing and mental/behavioral outcomes is extremely complex and probably still not well understood, involving genetic and epigenetic factors, the hormonal climate, brain development, and the influence of education, peers, and so on. Two examples of this complexity can be provided. Eating disorders have been shown to be more prevalent among early-maturing girls (Cooley and Toray 2001; Striegel-Moore et al. 2001; Zehr et al. 2007; McNicholas et al. 2012). Is this linked with the fact that puberty has a physiological impact on the amount of fat in body composition, or is it mainly linked with the fact that these girls feel ashamed of their growing body as compared to their mate? Or is the phenomenon linked directly with the hormones in the brain, or is eating behavior mediated by modifications of the brain structure and functioning, or are all these factors contributing, directly or indirectly ? In the field of substance use, one author elaborated some years ago two hypotheses to explain the higher rate of substance use among early maturers (Tschann et al. 1994): the maturational-deviance hypothesis was used to explain why early-maturing girls and late-maturing boys experienced heightened emotional distress, which in turn influenced initiation and use of substances. Alternatively, an early-maturation hypothesis was suggested as a cause for early-maturing girls to engage in more substance use than all other groups, independent of any emotional distress.

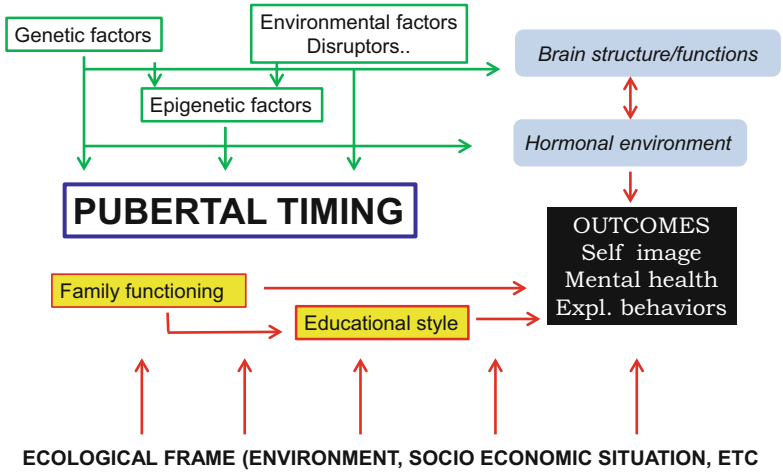

Fig. 1 The interplay between pubertal timing and mental and behavioral outcomes

In the same perspective, another question that has to be addressed is whether it is the actual pubertal stage that impacts on one's behavior or rather the image that an adolescent has of his pubertal stage that influences his self image and behavior. Two articles have focused on this question and have indeed shown that it is the perception that adolescents have of their pubertal stage rather than their actual stage that may impact on their behavior (Bratberg et al. 2007; Deppen et al. 2012). In the Bratberg paper, the authors found that perceived early maturation was associated, in both genders, with increased involvement in binge drinking and cigarette use. Interestingly, among late-maturing boys cigarette smoking was also more prevalent, as compared with "on time" maturers. In other words, the relationship between cigarette smoking and pubertal timing among boys again displays a U-curved aspect. The Deppen paper focused on a different issue among girls only, that is, assessing the relationship between girls who have objectively an 'on-time' pubertal status and who feel on time with those who are objectively on time but feel as early maturers. The authors found that a perception of pubertal precocity was associated with early sexual intercourse (before age 16) and early use of illegal drugs other than cannabis, as compared with those who felt on time. Conversely, girls perceiving their puberty as late are less likely to report intercourse before age 16, as compared with those who are and feel on time. These two papers suggest that, among the factors that explain the differences in exploratory behaviors found between early, on time, and late-maturing adolescents, the role of perceived pubertal stage is critical.

Another set of researchers focused on developmental pathways that antedate the start of puberty. In a recent publication (Mensah et al. 2013), an Australian team investigating prospectively a cohort of children that were followed from the age of 4–5. They found that those who had already entered their puberty by age 8–9 had experienced poor social adjustment (using a Pediatric Quality of Life Inventory) already at an earlier age, that is, as they were 4–5 years old. Moreover, among boys, behavioral difficulties at an earlier age also predicted early puberty. In other words, this study suggests that children experiencing stressful situations as children may mature at an earlier age, as compared with those who do not. The authors conclude that "at least in part, the association between early-onset puberty and poor mental health appears to result from processes under way," and not the reverse. This correlation between stressful experiences during early childhood and pubertal timing has also been found by other authors (Bleil et al. 2013), but another study has found the reverse, that is that depressive symptoms during the childhood among girls may predict late pubertal timing (Black and Klein 2012). The differences found between these different investigations may well be linked not only to the design of the studies but also to the context in which adolescents live. It can be hypothesized that the human environment directly impacts on the kind of relationship found: indeed, in a cohort study published in December 2013, White et al. (2013) found that the impact of early puberty on both externalizing and internalizing symptoms of Mexican-origin boys differed, depending on the ethnicity of their neighborhood; that is, environments providing a high number of peers of similar ethnicity seemed to buffer the effect of early pubertal timing (White

et al. 2013). More epidemiological and neuroendocrinological studies will be needed to elucidate the complex relationships between environment, hormonal changes, brain development and mental health and behavior (Romeo et al. 2002; Sisk and Foster 2004; Sisk and Zehr 2005; Natsuaki 2013).

Implications for Clinical Practice

From the preceding sections, it can be understood that the relationships between pubertal timing and both mental health and experimental behaviors are complex and probably imply bidirectional causality, e.g., the impact of pubertal timing on development and the impact of developmental issues on pubertal timing, as tentatively described in Fig. 1. What can be done to address the issue of mental health and experimental behaviors in the clinical care of adolescents and how should these developmental issues be accounted for?

The World Health Organization, along with other UN agencies, has developed a conceptual framework that should inspire health professionals in their approach to adolescent health, as shown in Fig. 2 (World Health Organization 1989). Although somehow arbitrary, the developmental process of adolescence can be divided into three main phases, which are early (e.g., ~10–13 years), middle (e.g., ~14–16 or

Development	Early adolescence	Mid adolescence	Late adolescence
Biological	pubertal development	increase in pilosity breaking	increase in muscle & bone mass, modific. of body shape
Cognitive & Emotional	concrete thinking focus on body changes need for intimacy	increase in abstract thinking lack of control Sensation seeking Conflicts with parents	Increase in abstract thinking perspectives on one's future consolidation of sexual orientation Acquiring autonomy
Social	separating from the parents staying with friends (of same sex)	peer activities going out Experimentation (sex/drugs..)	acceptance of parents' support Intimate relationships Capacity for compromise

Fig. 2 A grid assisting professionals to tailor their interventions in adolescents' developmental stages

17 years) and late adolescence (e.g., ~17–19 or 20 years), with each phase characterized by specific aspects along the biological, cognitive/emotional and social dimensions. While for many individuals the three dimension stages of the adolescent process are more or less synchronized, it can be understood from the preceding section that the three dimensions overlap, especially among teenagers who start their puberty early or late.

Health professionals who deliver health care to adolescents have to take into account these developmental stages: for instance, the physiological changes linked with the surge in growth has a clear impact on hepatic function and the metabolism of drugs and medicine, so that what is often suspected as poor adherence or weak effect of the treatment is just linked with too low doses of medication. Similarly, it is often not relevant to warn young adolescents with a chronic disease (e.g., cystic fibrosis or diabetes) about the long-term effects of poor adherence as they lack the cognitive capacity to analyze the long-term consequences of their behavior. Whatever her chronological age, the investigation of abdominal pain in a female adolescent will all depend on whether she has started puberty and may potentially be pregnant or whether she has not yet had any pubertal symptoms. We also know from epidemiological research that young people who have engaged in the use of psychotropic drugs (cigarette, alcohol, cannabis. . .) early, that is before the age of 14 or 15 years, have a higher risk of becoming dependent (Ellickson et al. 2004; Swift et al. 2008), which may be linked with the immaturity of the adolescent brain during early adolescence. Both cross-sectional and longitudinal studies have confirmed this phenomenon (Tschann et al. 1994; Patton et al. 2004; Michaud et al. 2006; Westling et al. 2008), and it seems as if not only individuals with early puberty but also those with perceived early puberty engage at an earlier age in the use of legal and illegal drugs (Arbeau et al. 2007; Deppen et al. 2012; Cance et al. 2013). It thus can be easily understood that practitioners must take into account the actual and perceived pubertal stage in assessing their young patients' lifestyles, and deliver proper responses to situations that threaten their patients' health and development: substance use and other exploratory/health-compromising behavior should be assessed systematically among early-maturing adolescents (and older adolescents as well).

Implications in the Field of Public Health

In a fairly recent paper, Steinberg reviews the extent to which neuroscience and research on the adolescent brain inform decision makers in the field of public health (Steinberg 2009). It does indeed make sense to consider that policies and preventive strategies should be developmentally appropriate, and we have learned from both behavioral and neuro-developmental studies that younger adolescents tend to lack specific control of their emotional arousals and sensation-seeking situations (Giedd 2008, 2012). It can be thus understood how an individual who matures early finds himself in the position of a 1,000 cm^3 motorcycle driver who has not learned how to

use a brake: in other words, one can hypothesize that the adolescent brain does not necessarily mature at the same pace as the endocrine system does, so that early maturers are particularly at risk of not being able to measure the short- and long-term impacts of their exploratory behavior. This overlap of pubertal maturation paces within groups of the same chronological age raises several issues: for instance, physical education courses at school and training sessions with young people on sports team must be tailored to the size and strength of the participants, which is difficult in team sports activities that gather individuals of the same chronological age. Another issue is the one of school sexual education: by age 13, educators find themselves in front of a mixed audience of pre-pubertal boys who have little interest in sex and mature girls who may have already had sexual intercourse.

In the fields of health education, prevention and health promotion, many programs attempt to shape adolescent behaviors; for example, programs such as those pertaining to the prevention of injuries, unintended pregnancy, and substance misuse are driven by the assumption that teaching teenagers how to adopt healthy lifestyles is enough and efficient. Health education – providing young people with information about the risks linked with various life experiences – is useful, but certainly not enough. Expecting adolescents to simply learn from adults (parents, teachers, educators, etc.) is unreasonable and incorrect for at least two reasons.

First, it disregards the impact of the emotional context of such experiences and it often overlooks the influence of peer pressure (Steinberg 2008). Second, it is ethically unacceptable, as it ignores the influence of the environment (Michaud 2006): in a world filled with medications for every mood and difficulty that adults may experience, why should we expect teenagers to refrain from experimenting with drugs? In a society that promotes sensation seeking and praises fast cars, why should we ask young people to be careful while driving their mopeds? In other words, when adolescents fail to take into account our warnings and end up in the hospital, many adults and health care professionals see the etiology as a function of their risk-taking behavior rather than a consequence of the social background in which we live. Indeed, their language is one of victim-blaming rather than reflecting collective responsibility. On an individual basis, most of the so-called risk-taking behaviors should be understood as normative behaviors and considered as experimental conduct that inspires the adolescent's psychosocial maturation. As such, they should be assessed by taking into account the maturational stage of the individual (Goldenring and Rosen 2004). On a societal level, prevention strategies should attempt to create safe environments, a concept that is largely supported by the World Health Organization (2009). Such an environment is one in which young people are offered attractive leisure activities to deter them from drug misuse, in which the organization of the traffic is pedestrian- or cyclist-friendly and in which communities provide safe public transportation late in the night, or in which adolescents have easy access to contraception and protection instead of the "just say no" useless message. In other words, recent endocrinological and neurodevelopmental research should inform the activities and decisions of both health professionals and policy makers.

References

Arbeau KJ, Galambos NL, Mikael Jansson S (2007) Dating, sex, and substance use as correlates of adolescents' subjective experience of age. J Adolesc 30:435–447

Bellis MA, Downing J, Ashton JR (2006) Adults at 12? Trends in puberty and their public health consequences. J Epidemiol Commun Health 60:910–911

Berg-Kelly K, Erdes L (1997) Self-assessment of sexual maturity by mid-adolescents based on a global question. Acta Paediatr 86:10–17

Black SR, Klein DN (2012) Early menarcheal age and risk for later depressive symptomatology: the role of childhood depressive symptoms. J Youth Adolesc 41:1142–1150

Bleil ME, Adler NE, Appelhans BM, Gregorich SE, Sternfeld B, Cedars MI (2013) Childhood adversity and pubertal timing: understanding the origins of adulthood cardiovascular risk. Biol Psychol 93:213–219

Bogin B (1994) Adolescence in evolutionary perspective. Acta Paediatr Suppl 406:29–35; discussion 36

Bond L, Clements J, Bertalli N, Evans-Whipp T, McMorris BJ, Patton GC, Toumbourou JW, Catalano RF (2006) A comparison of self-reported puberty using the Pubertal Development Scale and the Sexual Maturation Scale in a school-based epidemiologic survey. J Adolesc 29:709–720

Bourguignon JP, Rasier G, Lebrethon MC, Gerard A, Naveau E, Parent AS (2010) Neuroendocrine disruption of pubertal timing and interactions between homeostasis of reproduction and energy balance. Mol Cell Endocrinol 324:110–120

Bourguignon JP, Franssen D, Gerard A, Janssen S, Pinson A, Naveau E, Parent AS (2013) Early neuroendocrine disruption in hypothalamus and hippocampus: developmental effects including female sexual maturation and implications for endocrine disrupting chemical screening. J Neuroendocrinol 25:1079–1087

Bratberg GH, Nilsen TI, Holmen TL, Vatten LJ (2007) Perceived pubertal timing, pubertal status and the prevalence of alcohol drinking and cigarette smoking in early and late adolescence: a population based study of 8950 Norwegian boys and girls. Acta Paediatr 96:292–295

Brooks-Gunn J, Warren MP, Rosso J, Gargiulo J (1987) Validity of self-report measures of girls' pubertal status. Child Dev 58:829–841

Buck Louis GM, Gray LE Jr, Marcus M, Ojeda SR, Pescovitz OH, Witchel SF, Sippell W, Abbott DH, Soto A, Tyl RW, Bourguignon JP, Skakkebaek NE, Swan SH, Golub MS, Wabitsch M, Toppari J, Euling SY (2008) Environmental factors and puberty timing: expert panel research needs. Pediatrics 121(Suppl 3):S192–S207

Cance JD, Ennett ST, Morgan-Lopez AA, Foshee VA, Talley AE (2013) Perceived pubertal timing and recent substance use among adolescents: a longitudinal perspective. Addiction 108:1845–1854

Cohen RZ, Seeman MV, Gotowiec A, Kopala L (1999) Earlier puberty as a predictor of later onset of schizophrenia in women. Am J Psychiatry 156:1059–1064

Cooley E, Toray T (2001) Body image and personality predictors of eating disorder symptoms during the college years. Int J Eat Disord 30:28–36

Deppen A, Jeannin A, Michaud PA, Alsaker F, Suris JC (2012) Subjective pubertal timing and health-compromising behaviours among Swiss adolescent girls reporting an on-time objective pubertal timing. Acta Paediatr 101:868–872

DeRose LM, Shiyko MP, Foster H, Brooks-Gunn J (2011) Associations between menarcheal timing and behavioral developmental trajectories for girls from age 6 to age 15. J Youth Adolesc 40:1329–1342

Dick DM, Rose RJ, Viken RJ, Kaprio J (2000) Pubertal timing and substance use: associations between and within families across late adolescence. Dev Psychol 36:180–189

Ellickson PL, Tucker JS, Klein DJ, Saner H (2004) Antecedents and outcomes of marijuana use initiation during adolescence. Prev Med 39:976–984

Giedd JN (2008) The teen brain: insights from neuroimaging. J Adolesc Health 42:335–343

Giedd JN (2012) The digital revolution and adolescent brain evolution. J Adolesc Health 51:101–105
Gluckman PD, Hanson MA (2006) Evolution, development and timing of puberty. Trends Endocrinol Metab 17:7–12
Gluckmann P (2005) An evolutionary and life history perspective on the nature of puberty. In: Puberty: a sensor of genetic and environmental interactions throughout development. Evian
Goldenring J, Rosen D (2004) Getting into adolescent heads: an essential update. Contemp Pediatr 21:64–90
Golub MS, Collman GW, Foster PM, Kimmel CA, Rajpert-De Meyts E, Reiter EO, Sharpe RM, Skakkebaek NE, Toppari J (2008) Public health implications of altered puberty timing. Pediatrics 121(Suppl 3):S218–S230
Graber JA, Lewinsohn PM, Seeley JR, Brooks-Gunn J (1997) Is psychopathology associated with the timing of pubertal development? J Am Acad Child Adolesc Psychiatry 36:1768–1776
Graber JA, Seeley JR, Brooks-Gunn J, Lewinsohn PM (2004) Is pubertal timing associated with psychopathology in young adulthood. J Am Acad Child Adolesc Psychiatry 43:718–726
Graber JA, Nichols TR, Brooks-Gunn J (2010) Putting pubertal timing in developmental context: implications for prevention. Dev Psychobiol 52:154–262
Jessor R (1991) Risk behavior in adolescence: a psychosocial framework for understanding and action. J Adolesc Health 12:597–605
Johansson T, Ritzen EM (2005) Very long-term follow-up of girls with early and late menarche. Endocr Dev 8:126–136
Kaltiala-Heino R, Kosunen E, Rimpela M (2003a) Pubertal timing, sexual behaviour and self-reported depression in middle adolescence. J Adolesc 26:531–545
Kaltiala-Heino R, Marttunen M, Rantanen P, Rimpela M (2003b) Early puberty is associated with mental health problems in middle adolescence. Soc Sci Med 57:1055–1064
Lam TH, Shi HJ, Ho LM, Stewart SM, Fan S (2002) Timing of pubertal maturation and heterosexual behavior among Hong Kong Chinese adolescents. Arch Sex Behav 31:359–366
Lanza ST, Collins LM (2002) Pubertal timing and the onset of substance use in females during early adolescence. Prev Sci 3:69–82
McCabe MP, Ricciardelli LA (2004) A longitudinal study of pubertal timing and extreme body change behaviors among adolescent boys and girls. Adolescence 39:145–166
McNicholas F, Dooley B, McNamara N, Lennon R (2012) The impact of self-reported pubertal status and pubertal timing on disordered eating in Irish adolescents. Eur Eat Disord Rev 20:355–362
Mendle J, Turkheimer E, Emery RE (2007) Detrimental psychological outcomes associated with early pubertal timing in adolescent girls. Dev Rev 27:151–171
Mendle J, Harden KP, Brooks-Gunn J, Graber JA (2012) Peer relationships and depressive symptomatology in boys at puberty. Dev Psychol 48:429–435
Mensah FK, Bayer JK, Wake M, Carlin JB, Allen NB, Patton GC (2013) Early puberty and childhood social and behavioral adjustment. J Adolesc Health 53:118–124
Michaud PA (2006) Adolescents and risks: why not change our paradigm? J Adolesc Health 38:48183
Michaud PA, Blum RW, Ferron C (1998) "Bet you I will!" risk or experimental behavior during adolescence? Arch Pediatr Adolesc Med 152:224–226
Michaud PA, Suris JC, Deppen A (2006) Gender-related psychological and behavioural correlates of pubertal timing in a national sample of Swiss adolescents. Mol Cell Endocrinol 254–255:172–178
Moffitt TE (1993) Adolescence-limited and life-course-persistent antisocial behavior: a developmental taxonomy. Psychol Rev 100:674–701
Natsuaki MN (2013) Puberty in context: toward a more nuanced understanding of early maturation. J Adolesc Health 53:677–678
Oldehinkel AJ, Verhulst FC, Ormel J (2011) Mental health problems during puberty: Tanner stage-related differences in specific symptoms. The TRAILS study. J Adolesc 34:73–85
Orr DP, Ingersoll GM (1995) The contribution of level of cognitive complexity and pubertal timing to behavioral risk in young adolescents. Pediatrics 95:528–533

Ostovich JM, Sabini J (2005) Timing of puberty and sexuality in men and women. Arch Sex Behav 34:197–206
Parent AS, Teilmann G, Juul A, Skakkebaek NE, Toppari J, Bourguignon JP (2003) The timing of normal puberty and the age limits of sexual precocity: variations around the world, secular trends, and changes after migration. Endocr Rev 24:668–693
Patton GC, Viner R (2007) Pubertal transitions in health. Lancet 369:1130–1139
Patton GC, McMorris BJ, Toumbourou JW, Hemphill SA, Donath S, Catalano RF (2004) Puberty and the onset of substance use and abuse. Pediatrics 114:e300–e306
Patton GC, Hemphill SA, Beyers JM, Bond L, Toumbourou JW, McMorris BJ, Catalano RF (2007) Pubertal stage and deliberate self-harm in adolescents. J Am Acad Child Adolesc Psychiatry 46:508–514
Rhee H (2005) Relationships between physical symptoms and pubertal development. J Pediatr Health Care 19:95–103
Romeo RD, Richardson HN, Sisk CL (2002) Puberty and the maturation of the male brain and sexual behavior: recasting a behavioral potential. Neurosci Biobehav Rev 26:381–391
Sisk CL, Foster DL (2004) The neural basis of puberty and adolescence. Nat Neurosci 7:1040–1047
Sisk CL, Zehr JL (2005) Pubertal hormones organize the adolescent brain and behavior. Front Neuroendocrinol 26:163–174
Steinberg A (2008) Risk taking in adolescence: what changes and why? Ann N Y Acad Sci 1021:51–58
Steinberg L (2009) Should the science of adolescent brain development inform public policy? Am Psychol 64:739–750
Striegel-Moore RH, McMahon RP, Biro FM, Schreiber G, Crawford PB, Voorhees C (2001) Exploring the relationship between timing of menarche and eating disorder symptoms in black and white adolescent girls. Int J Eat Disord 30:421–433
Swift W, Coffey C, Carlin JB, Degenhardt L, Patton GC (2008) Adolescent cannabis users at 24 years: trajectories to regular weekly use and dependence in young adulthood. Addiction 103:1361–1370
Tanner J (1962) Growth at adolescence, 2nd edn. Blackwell, Oxford
Tschann JM, Adler NE, Irwin CE Jr, Millstein SG, Turner RA, Kegeles SM (1994) Initiation of substance use in early adolescence: the roles of pubertal timing and emotional distress. Health Psychol 13:326–333
Varraso R, Siroux V, Maccario J, Pin I, Kauffmann F, Epidemiological Study on the G, Environment of A (2005) Asthma severity is associated with body mass index and early menarche in women. Am J Respir Crit Care Med 171:334–339
Wade TJ, Cairney J, Pevalin DJ (2002) Emergence of gender differences in depression during adolescence: national panel results from three countries. J Am Acad Child Adolesc Psychiatry 41:190–198
Waylen A, Wolke D (2004) Sex 'n' drugs 'n' rock 'n' roll: the meaning and social consequences of pubertal timing. Eur J Endocrinol 151(Suppl 3):U151–U159
Westling E, Andrews JA, Hampson SE, Peterson M (2008) Pubertal timing and substance use: the effects of gender, parental monitoring and deviant peers. J Adolesc Health 42:555–563
White RM, Deardorff J, Liu Y, Gonzales NA (2013) Contextual amplification or attenuation of the impact of pubertal timing on Mexican-origin boys' mental health symptoms. J Adolesc Health 53:692–698
Wichstrom L (2000) Predictors of adolescent suicide attempts: a nationally representative longitudinal study of Norwegian adolescents. J Am Acad Child Adolesc Psychiatry 39:603–610
World Health Organization (1989) The health of youth. World Health Organization, Geneva
World Health Organization (2009) Strengthening the health sector response to adolescent health and development. World Health Organization, Geneva
Zehr JL, Culbert KM, Sisk CL, Klump KL (2007) An association of early puberty with disordered eating and anxiety in a population of undergraduate women and men. Horm Behav 52:427–435

Puberty, the Brain and Mental Health in Adolescence

Russell Viner

Abstract There is increasing evidence that puberty influences mental health and emotional well-being in humans in multiple ways. These effects begin with adrenarche in late childhood and continue with gonadarche in early adolescence.

Puberty itself is associated with increased behavioral problems in boys and increased social anxiety, depression and self-harm in girls. It is also associated with a lower sense of well-being and with increased reports of fatigue, irritability and somatic complaints.

The mechanisms by which puberty influences mental health are unclear, and likely encompass biological and sociological pathways. Pubertal timing appears to have effects that are separate from the processes of puberty itself, although it is uncertain whether those with earlier puberty simply enter this risk period earlier than peers. Developmental mismatch may be a key mechanism for the effects of early puberty, i.e., a mismatch between emotional challenges and the cognitive capacities of young adolescents. Hyperactivation of HPA stress responses with puberty may play a role in mediating these associations.

However, it is also possible that early puberty and adolescent mental health problems share a range of common risk factors. These may include stressful family environments, early childhood adversity, sexual abuse and lack of parental investment or warmth. In support of this possibility are recent findings that children with early puberty can be identified as having increased psychosocial problems in early childhood before puberty (Mensah et al. J Adolesc Health 53:118–124, 2013). Others have found that higher levels of mental health problems are associated with early puberty only in those with high family risk. This finding has led to hypotheses that early puberty may be an evolutionary response to early-life adversities and, in turn, may be linked to a suite of risk-taking behaviors linked with early reproduction.

Life-course studies of the associations of early puberty and mental health have not been undertaken. I present findings from two British Birth Cohort studies (1958 and 1970 birth cohorts) that examined the associations between childhood adversity

R. Viner (✉)
General and Adolescent Paediatrics Unit, UCL Institute of Child Health, 30 Guildford Street, London WC1N 1EH, UK
e-mail: r.viner@ucl.ac.uk

J.-P. Bourguignon et al. (eds.), *Brain Crosstalk in Puberty and Adolescence*, Research and Perspectives in Endocrine Interactions 13,
DOI 10.1007/978-3-319-09168-6_5

and mental health in adult life. I also discuss a new Australian cohort study, the Childhood to Adolescence Transition Study (CATS; Mundy et al. BMC Pediatr 13:160, 2013) that was set up to examine the effects of early life experience and the earliest changes of adrenarche and gonadarche on mental health, behavior and well-being in Australian children.

Introduction

There is increasing recognition of the importance of mental health in adolescence for mental health and well-being across the life-course, driven by recognition that adolescence is a key period of onset of mental disorders and spurred by rapid advances in the neuroscience of the developing brain. In US samples, it is estimated that three-quarters of all lifetime mental health disorders (with the exception of dementia) onset by age 24 years (Kessler et al. 2005).

Mental health problems among young people have increased in the past four decades, including both emotional and behavioral problems (Nuffield Foundation 2009), in both the USA (Achenbach et al. 2003) and UK (Collishaw et al. 2004). While a large part of adolescent mental health problems can be attributed to the social transitions inherent in adolescent development, processes that were exacerbated by rapid social change over the past three decades (Nuffield Foundation 2009), there is increasing evidence that biological mechanisms may also be responsible.

Dramatic advances in adolescent neuroscience have shown the rapid development of both cortical and sub-cortical structures continuing through adolescence. There is increasing evidence that abnormalities of adolescent neural developmental processes are related to the typical onset of conditions such as psychotic disorders (Holtzman et al. 2013), eating disorders (Frank and Kaye 2012) and Attention Deficit Hyperactivity Disorder (ADHD; Frodl and Skokauskas 2012).

Increasing evidence of the actions of sex steroids in the risk for mental disorder, particularly in psychoses (Trotman et al. 2013), and recent evidence that puberty influences brain developmental trajectories (Goddings et al. 2012), has focused attention on puberty as a potential modifier of the impacts of adolescent neurocognitive and sociological development on mental health risk.

In this chapter, I will review the evidence and mechanisms for an association between puberty and mental health in adolescence and across the life-course and will present novel findings from a large-scale longitudinal examination of this association.

Puberty and Turbulence

Puberty has long been seen as a turbulent time in human life. William Shakespeare famously declared, in The Winter's Tale, "I would there were no age between sixteen and three-and-twenty, or that youth would sleep out the rest; for there is nothing in the between but getting wenches with child, wronging the ancientry, stealing, fighting"(Shakespeare 1623). Similar quotations can be found dating back to Roman and Greek times. The idea of adolescence as a time of Sturm und Drang (storm and stress) was common from the late eighteenth century and, throughout much of the modern period, Goethe's *Young Werther* symbolized the difficult struggles of young people becoming adults within the constraints of conventional society.

By the early twentieth century, such ideas were being incorporated into scientific theories of adolescence. The American psychologist G. Stanley Hall, following Ernst Haeckel in believing that 'ontogeny recapitulates phylogeny,' saw adolescence as a naturally turbulent period recapitulating in each individual the 'savage' stage of human evolution (Hall 1904). Sigmund Freud and his disciples added to the perception of puberty as a difficult period, understanding it to result from the need for individuals to come to terms with their hitherto-suppressed sexual urges (Holder 2005).

There have been, of course, a number of counter-arguments suggesting that the apparent turbulence of puberty is a Western social construction. Margaret Mead claimed, in *Coming of Age in Samoa* (1928), that young people in Samoa experienced no additional problems during adolescence, and she concluded that the rebelliousness and emotional or behavioral problems increasingly seen in US adolescents were the products of culture rather than biology (Scheper-Hughes 1984). This notion of adolescence as almost entirely a social construction still retains some currency today (Mayall 2002).

Yet there is increasing evidence cross-culturally that the timing of puberty influences the risk of a range of mental health problems in young people.

Secular Trends

First it is worthwhile noting that there have been notable trends in mental health among young people as well as the well-known 'secular trend' in pubertal timing.

Michael Rutter and others showed in the 1990s that there had been concerning increases in adolescent mental health problems across the middle and end of the twentieth century in many countries (Rutter and Smith 1995). In-depth studies have shown that mental health disorders increased in young people over the past 30 years in the UK (Collishaw et al. 2004, 2010; Nuffield Foundation 2009), the USA (Achenbach et al. 2003) and even in developing countries such as China (Xin et al. 2012).

The secular trend for the falling age of puberty is well described, particularly in girls, and is largely thought to relate to improved nutrition. The secular trend was largely complete by the 1950s in high income Western countries (Eveleth 1998), although it remains controversial whether there have been further falls in the onset of puberty over the past 20 years (Viner 2002; Parent et al. 2003; Sorensen et al. 2012). The evidence suggests that there have been no real changes in age at menarche or in age of gonadotropin and sex steroid surges in Western populations (Viner 2002; Sorensen et al. 2012), although there may be increasing numbers with early gonadotropin-independent thelarche (Sorensen et al. 2012). The vast majority of data on secular trends in puberty relate to age of menarche in girls, as pubertal events in boys are less amenable to study in large populations. An interesting approach to studying pubertal trends in boys has been the study of changes in injury mortality, given assumptions that puberty drives much of the rise in risky behaviors seen in early to mid-adolescent males. Injury mortality creates an appreciable 'hump' in male mortality during adolescence, and the age of onset of this hump has been shown to have fallen steadily since 1750 through to the mid-twentieth century, with stabilization after 1950 (Goldstein 2011).

Given the differences in timing of the respective secular trends in puberty and in adolescent mental health, it is unlikely that changes in the timing of puberty contribute significantly to increases in adolescent mental health problems.

Evidence for the Association of Puberty and Mental Health Problems in Adolescence

There is increasing evidence that puberty influences mental health and emotional well-being in humans in multiple ways. These effects begin with adrenarche in late childhood and continue with gonadarche in early adolescence.

Studies of Adrenarche

The impact of adrenarche on mental health has been little studied. Existing studies are uniformly small and of variable quality. Studies also largely focus on those with premature adrenarche, and little is known about the effects of normal adrenarcheal timing on mental health and well-being. Girls with premature adrenarche have been reported to have more behavioral disorders and higher symptom counts for anxiety and mood problems (Dorn et al. 2008). A number of very small studies have found that children who are early into adrenarche reported higher externalizing, depression and anxiety symptoms and somatic complaints compared to on-time children (Dorn et al. 1999; Sontag-Padilla et al. 2012).

Others have found that levels of adrenal androgens such as dehydroepiandrosterone (DHEA) are related to depression in children and adolescents and postulate that adrenal androgens may modulate the actions of cortisol in predisposing to depression (Goodyer et al. 1996, 2001).

Aggression and Violence

Aggressive behaviors, including bullying and violence, increase dramatically and peak in mid adolescence, coincident with mid to late puberty (Krug et al. 2002; Patton and Viner 2007). This finding, together with the known effects of testosterone on aggression in experimental animals and in humans (Archer 1991), focused early attention on the relationship of puberty and aggression in males (Olweus et al. 1988). However, there is also good evidence from large-scale studies that increasing pubertal stage is associated with a two- to three-fold increase in risk of violence and relational aggression in boys, compared with earlier pubertal stages. There is a dose-response across puberty, with violence/aggression increasing from early to mid to late puberty (Hemphill et al. 2010).

Depressive Disorders

There is considerable evidence that early puberty increases the risk of depression in girls (Hayward et al. 1997; Kaltiala-Heino et al. 2003a, b; Mendle et al. 2007). Concerns about an association between puberty and emotional problems in adolescence gained greater prominence when Angold and colleagues (1998) showed that pubertal stage predicted risk of major depression in adolescents better than age. Rates of depression are higher in boys than girls before puberty, but adult females have two- to three-fold higher rates than adult males. Angold et al. found that it appeared to be in stage 3 puberty that girls began to transition towards female preponderance in depression. There was also a suggestion that puberty may actually reduce the prevalence of depression in males (Angold et al. 1998).

Anxiety

Adolescence is a time when anxiety disorders markedly increase in both sexes, suggesting an association with puberty. However, the evidence for an association between anxiety and pubertal timing is much less clear than for depression.

A recent review of over 45 empirical studies found that there is only moderate quality evidence in girls that both earlier timing and more advanced pubertal stage increase anxiety scores or symptoms after adjusting for age. Findings for boys are less

robust (Reardon et al. 2009). There is little evidence of anxiety disorders per se. While one study reported a 2.3-fold increase (OR 2.3) in the likelihood of having had a panic attack for each greater Tanner stage of puberty (Hayward et al. 1992), others have found no association between anxiety disorders and pubertal timing in boys or girls (Graber et al. 1997).

Deliberate Self-Harm

Deliberate self-harm (DSH), a major risk factor for suicide, is another problem that rises sharply in early adolescence. In young women, DSH appears to peak around 15–16 years of age and prevalence falls thereafter (Hawton et al. 2002; Madge et al. 2008). The literature on DSH and puberty is much smaller than that for depression or anxiety. In a large population-based study, apparent strong associations between DSH and pubertal stage (adjusted for age) were attenuated when models were adjusted for depressive symptoms, showing that this association was largely or entirely mediated by depression (Patton et al. 2007).

Eating Disorders

There is strong evidence of an association between puberty and eating disorders, at least in girls. A recent systematic review showed that more than 40 studies identified advanced pubertal status and/or early pubertal timing as a risk factor for eating disorders or disordered eating in girls; similar results were seen in over 20 studies in boys. Early maturing girls and boys have a higher risk of a range of eating problems, including anorexia nervosa, bulimia nervosa and eating disorder symptoms, including body dissatisfaction and weight and shape concerns (Klump 2013). However, a number of studies found no association between eating disorders and puberty, particularly in boys, and others have reported that early or advanced puberty was associated with improved body image (McCabe et al. 2001).

Mechanisms for the Association of Puberty and Mental Health

The evidence is robust that early pubertal timing increases the risk for psychopathology during adolescence in girls (Mendle et al. 2007), with good but less robust evidence for the same association in boys (Mendle and Ferrero 2012). However, evidence that late pubertal maturation is also associated with some psychological problems in boys (Huddleston and Ge 2003) suggests that it may be off-time

maturation that is the source of problems in boys. There is little evidence that this is the case in girls.

The mechanisms suggested fall into three broad categories:

(A) Psychosocial mechanisms

1. Psychosocial impact of differentiation from peers
2. Early adoption of more adult roles. The stage-termination hypothesis suggests that earlier maturers face the adolescent transition before they have successfully completed prior developmental tasks and developed the skills necessary to cope with the challenges of adolescence (Caspi and Moffitt 1991).
3. Early limiting of adult educational trajectories. Early maturation may also orient girls toward early motherhood, with limited educational and occupational prospects, increasing their risk for select psychiatric outcomes (e.g., emotional and substance-related).

(B) Biological mechanisms

1. Direct effects of pubertal hormones on the CNS
2. Genetic mechanisms
   (i) Hormonal activation of genetic influences
   (ii) Common genetic factors responsible for both early puberty and psychopathology

(C) Bio-psycho-social mechanisms related to stress

Early childhood stress is well established as a key factor for psychopathology across the life-course. (Grant et al. 2003). It is postulated that early developmental experience plays a role in calibrating the response dynamics of central neuroendocrine systems, with early adversity resulting in a profile of heightened or prolonged reactivity to stressors. This response conventionally has been seen as an atavistic and pathogenic legacy of an evolutionary past in which threats to survival were more prevalent and severe (Boyce and Ellis 2005). Alternatively, the developmental plasticity of the stress response systems can be understood as evolved mechanisms that monitor childhood environments as a way of calibrating the development of stress response systems to best match those environments (Boyce and Ellis 2005). Vulnerable young people exposed to childhood adversity may later be particularly sensitive to stress sensitization during disruptive periods such as puberty (Rudolph and Flynn 2007).

Obesity and the Metabolic Syndrome

One potential additional mechanism that has not been examined is potential mediation through obesity. There is strong evidence that early puberty is associated with

later obesity in adolescence and in later adult life, particularly in women (Prentice and Viner 2013). There is some evidence that this association is causal, i.e., it persists after adjustment for childhood body mass index (Prentice and Viner 2013). However, obesity and the metabolic syndrome are associated with psychological disorders in adulthood through psychological as well as potentially biological mechanisms (Shefer et al. 2013). It is possible that the association between early puberty and adult psychopathology is, in fact, mediated by obesity. This has not yet adequately examined.

Depression

Angold et al. (1998) found that it was pubertal stage rather than the timing of puberty – found (no association with early menarche) or a transition into a new stage that was important, i.e., that prevalence of depression was not associated with recentness of transition between stages (Angold et al. 1998).

Alternatively, the stress of the transition through puberty may activate stress-sensitization processes linked to depression. This is particularly likely in girls, given the evidence of gender differences in stress sensitization (Rudolph and Flynn 2007). Imaging studies suggest that there are unique pubertal influences on white matter volumes and structures in a sexually dimorphic fashion that are independent of age, although data are limited (Ladouceur 2012). Given other data suggesting that the development of white matter tracts influences the risk for depression, it is plausible that pubertal influences on white matter development help explain the female rise of depression during adolescence (Ladouceur et al. 2012).

Anxiety

Potential reverse causality has been examined in a small number of studies, (i.e., does childhood anxiety lead to earlier puberty onset?) There is weak evidence from small studies that higher childhood anxiety may delay the onset of puberty in girls (Reardon et al. 2009). This is likely to relate to earlier stressors. . ..

Evidence that pubertal hormones are associated with anxiety is weak, with inconsistent findings from very small studies (Reardon et al. 2009).

Behavioral Disorders

It is widely believed that increases in testosterone in boys during puberty explain the increase seen in aggression and violence during adolescence. However,

evidence that aggression and behavioral disorders are directly related to testosterone in humans is inconclusive. Indeed, the relationship may be reverse-causal, as there is evidence from animal studies that behaviors may also modulate testosterone levels (Archer 1991).

Suggested other mechanisms for the association of higher pubertal stage and aggression or violence range from simple capacity issues, i.e., the increase in physical size and strength at puberty allows greater capacity for violence, to psychosocial factors, including conflict with parents, school disengagement and increased substance use seen in puberty, to peer factors. Asynchrony between brain maturation and emotional and behavioral regulation within a body rapidly developing greater strength may also increase risk for aggression.

Other mechanisms have focused on stress – acknowledging the increased stress of growth, development and social role transitions during puberty (Stroud et al. 2009) – but also the increasing evidence that biological stress responses are exaggerated during puberty. There is a growing literature examining the role of variations in stress responses, e.g., hypo- or hyper-arousal of the HPA axis, on the association of puberty and aggressive behaviors; however, findings are contradictory (Susman et al. 2010).

Further, it is possible that common genetic factors predispose to both aggression and early puberty – evolutionary ideas. . .. – (Harden 2012)

There is evidence that links between puberty and violence/aggression are stronger in boys with more anti-social friends, suggesting that puberty produces either a change in peer group or greater susceptibility to peer influence (Patton and Viner 2007).

Other mechanisms may operate through associations of puberty with other mental health problems, e.g., with anxiety, as this may increase aggression, particularly in males (Susman et al. 2010).

Eating Disorders

For eating disorders, mechanisms have focused on the association of puberty with body dissatisfaction, a strong risk factor for eating disorders in girls. The physical changes associated with puberty in girls, such as increased weight and fat deposition on the thighs and bust, are postulated to increase body dissatisfaction and, therefore, disorder risk. Girls with early puberty are thought to be at particular risk, as they may experience even more body dissatisfaction than their developmentally on-time counterparts (Klump 2013). In boys, mechanisms are less clear, but it has been postulated that eating disorders may relate to fear of adulthood. Significant weight loss results in gonadotrophin suppression and pubertal arrest or delay, probably mediated by adipose mass sensing systems.

There is also considerable evidence for hormonal and genetic effects. Twin studies provide strong support for puberty as a period of significant genetic risk for eating disorders, with evidence suggesting that genetic factors become

"activated" during puberty to increase risk. Heritability of eating disorders in girls is 0 % before puberty but rises to 50 % during and after puberty, whereas heritability in boys is around 50 % before and during puberty (Klump et al. 2012). There is also evidence that rises in estrogen during puberty may increase eating disorder risk, potentially through effects on modification of genetic risk (Klump et al. 2010). When girls in a pilot study of 99 female twin pairs aged 10–15 years were dichotomized into low or high estradiol pairs, based upon salivary estradiol, correlations in eating disorder scores were similar (around 0.5) for monozygotic (MZ) and dizygotic (DZ) twins in the low estradiol group, suggesting that genetic factors played little role. In contrast, twin correlations were high and significant (0.47) in the MZ group but low and non-significant (0.04) in the DZ group, indicating the presence of genetic effects (Klump et al. 2010). As heritability in boys is not related to puberty (i.e., is stable across pre-puberty, mid and late puberty), this finding adds weight to the hypothesis that rises in estrogen somehow activate genetic risk (Klump et al. 2012).

Problems with Adjustment for Age

Nearly all studies adjusted models for both puberty and age and did not discuss issues of co-linearity between puberty and age.

Long-Term Effects of Puberty on Mental Health

Despite the wealth of data on puberty and psychological function in adolescence, very few studies have examined whether these associations persist across the life-course. While puberty may be important for mental health during the brief years of adolescence, it is its effects across the life-course that will determine whether pubertal timing is of major developmental and public health importance.

While a number of studies of adults have retrospectively collected data on pubertal timing, few prospective studies have examined the effects of pubertal timing on function after the age of 20 years.

In a follow-up of Angold's seminal study, Copeland et al. (2010) found that, at age 19–21 years, the majority of associations found between puberty and psychological function in adolescence were attenuated, with the exception of depression in girls with earlier conduct disorder. They found that early puberty ceased to be a risk factor for depression in the wider group, and for crime, substance use and educational and employment outcomes (Copeland et al. 2010).

A follow-up of young people from the Oregon Depression project at age 24 years found that early maturing girls were more likely to have significant depression. However, young men who had been late maturers had higher rates of disruptive behavior and substance use disorders during the transition to adulthood (Graber et al. 2004).

However, no studies have examined outcomes into middle adulthood. Furthermore, no studies have examined whether the effects of early puberty on later psychopathology are mediated through obesity or, in fact, reflect common factors causing both early puberty and psychopathology, such as early adversity.

Findings from the 1970 British Birth Cohort

We examined longitudinal outcomes of pubertal timing in individuals from the 1970 British Birth cohort, a nationally representative birth cohort that initially recruited over 16,000 individuals born in one week in April of 1970. A total of 16,567 babies were enrolled, with follow-up at 5 years (N = 13,135), 10 years (N = 14,875), 16 years (N = 11,622), 30 years (N = 11,261) and 34 years (N = 9,665). We used data from the birth survey and the 5-, 10-, 16- and 34-year follow-ups (Butler and Golding 1986; Butler et al. 2000; Bynner et al. 2002).

Data on puberty were taken from the 10-year survey (N = 12,981) when children were examined by a school medical officer and physical signs of puberty were noted between age 10 and 11 years. Signs of puberty for girls were defined as significant breast, pubic hair or axillary hair development or a report from the child or mother of having achieved menarche. Data on age of menarche were combined from the 16-year survey (when girls and mothers both reported on age at menarche) and the 10-year survey.

At 10 years, data on pubertal status were available on 6,296 girls, with 25.9 % (N = 1,696) recorded as having any signs of puberty. This finding accords well with expectations for age 10 years according to British normative data (see Table 1). We were unable to stage puberty accurately at age 10 years as data were only recorded as present or absent. We therefore defined those girls who had any signs of puberty at age 10 as having early puberty, representing the earliest quartile of pubertal timing.

Data on age at menarche were available on 5,123 girls. Mean age of menarche in this sample was 13.0 years, with a standard deviation of 1.0 years, which accords very well with contemporary British reference data (mean age of menarche, 13.2 years, SD 1.0 year). We defined early menarche as achieving menarche before the 11th birthday, as this indicates that Tanner stage 3 to 4 had been attained by this time. This group included 17.5 % (N = 895) of the girls.

Table 1 Frequency of signs of puberty at 10 years and age at menarche compared with contemporary UK reference data

	Frequency in cohort % (N)	Expected frequency from UK reference data %
Signs of puberty at 10 years	N = 6,296	
Breast	23 (144)	25
Pubic hair	8 (514)	9
Axillary hair	3 (177)	–
Menarche	0.4 (25)	0.04
Any signs of puberty	27 (1,696)	–
Age at menarche reported at 16 years	N = 5,123	
<11th birthday	18 (895)	2
11 years	13 (641)	13
12 years	22 (1,126)	35
13 years	25 (1,290)	35
14 years	15 (770)	13
≥15 years or not yet started	8 (401)	2

Analyses

Analyses were undertaken in Stata 12. We first examined differences in proportions of adult outcomes in those in early or normal/late puberty groups. We then calculated odds ratios for adult outcomes related to early puberty, adjusting for the likely confounders of socioeconomic status in childhood and adulthood, maternal educational status, childhood height and adult body mass index.

Findings

Data on frequency of signs of early puberty and age at menarche are given in Table 1. Table 2 shows associations of early puberty (any signs of puberty at 10 years) with adult outcomes. Table 3 shows associations of early menarche (menarche <11 years) with adult outcomes.

We found that early puberty was associated with higher risk of serious mental health problems at age 34 years.

Table 2 Associations of pubertal status at age 10 years with outcomes at age 34 years

Adult outcome variables at age 34 years	Proportions with adult outcome by pubertal timing		Adjusted OR (95 % CI)	p
	Early puberty	Normal/later puberty		
Low life satisfaction (lowest quartile)	24.2 (278)	21.5 (659)	1.1 (0.9, 1.4)	0.16
Psychological disorder (Malaise Inventory high scorer, indicating likely depression)	19.8 (17.6, 22.2)	17.6 (16.3, 19.02)	1.2 (1.0, 1.4)	0.11
Serious mental illness (Kessler 6 inventory high score)	5.6 (4.4, 7.1)	4.0 (3.4, 4.8)	1.4 (1.1, 1.9)	0.03
Alcohol misuse or dependency (CAGE high scorer)	13.6 (11.7, 15.8)	13.2 (12.0, 14.5)	1.1 (0.9, 1.3)	0.7
Cautioned or criminal conviction	1.5 (1.0, 2.4)	1.7 (1.3, 2.3)	0.8 (0.5, 1.5)	0.6
Unemployed and seeking work	0.9 (0.5, 1.6)	1.8 (1.3, 2.3)	0.5 (0.2, 0.9)	0.04

OR, Odds ratios adjusted for social class at 10 and 34 years and body mass index (BMI) at 34 years
p-values for differences between proportions with adult outcome by pubertal timing, Fishers exact test $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Table 3 Associations of early menarche with outcomes at age 34 years

Adult outcome variables at age 34 years	Proportions with adult outcome by menarcheal timing		Adjusted OR (95 % CI)	p
	Early menarche	Normal/later menarche		
Low life satisfaction (lowest quartile)	22.4 (19.3, 25.9)	22.4 (20.9, 23.9)	1.0 (0.8, 1.2)	0.7
Psychological disorder (Malaise Inventory high scorer, indicating likely depression)	18.5 (15.6, 21.8)	17.8 (16.5, 19.2)	1.0 (0.8, 1.3)	0.9
Serious mental illness (Kessler 6 inventory high score)	5.0 (3.6, 7.2)	4.3 (3.6, 5.1)	1.3 (0.8, 1.9)	0.3
Alcohol misuse or dependency (CAGE high scorer)	13.6 (11.1, 16.7)	13.8 (12.6, 15.1)	1.0 (0.8, 1.4)	0.8
Cautioned or criminal conviction	2.3 (1.4, 3.9)	1.3 (1.0, 1.8)	1.8 (0.9, 3.4)	0.09
Unemployed and seeking work	1.5 (0.8, 2.8)	1.1 (0.9, 1.1)	1.3 (0.6, 3.0)	0.5

Odds ratios adjusted for social class at 10 and 34 years and body mass index (BMI) at 34 years
p-values for differences between proportions with adult outcome by pubertal timing, Fishers exact test $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Conclusions and Future Directions

There is strong evidence that early puberty in girls is associated with poorer psychological function and increased risk of a range of psychiatric disorders during adolescence. The evidence for boys is notably weaker, and there is some evidence that late pubertal maturation similarly increases risk.

While the literature on the long-term effects of puberty on psychological function is sparse, and there is some evidence of limited persistent effects on psychopathology in females, data suggest that the effects of puberty on psychological, social and educational function are predominantly limited to adolescence. This argues for psychosocial rather than biological factors being the predominant mechanisms by which early puberty influences psychological function. We did not find evidence to support childhood adversity as a common factor underlying early puberty and later psychological function.

References

Achenbach TM, Dumenci L, Rescorla LA (2003) Are American children's problems still getting worse? A 23-year comparison. J Abnorm Child Psychol 31:1–11

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

Archer J (1991) The influence of testosterone on human aggression. Br J Psychol 82(Pt 1):1–28

Boyce WT, Ellis BJ (2005) Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. Dev Psychopathol 17:271–301

Butler NR, Golding J (1986) From birth to five: a study of the health and behaviour of Britain's 5-year-olds. Pergamon Press, Oxford

Butler NR, Despotidou S, Shepherd P (2000) The 1970 British Birth Cohort Study: ten year follow-up: a guide to the BCS70 Ten-year data available at the Economic and Social Research Council Data Archive, London

Bynner J, Butler N, Ferri E, Shepherd P, Smith K (2002) The design and conduct of the 1999–2000 surveys of the National Child Development Study and the 1970 British Birth Cohort Study. Centre for Longitudinal Studies, Institute of Education, London

Caspi A, Moffitt TE (1991) Individual differences are accentuated during periods of social change: the sample case of girls at puberty. J Pers Soc Psychol 61:157–168

Collishaw S, Maughan B, Goodman R, Pickles A (2004) Time trends in adolescent mental health. J Child Psychol Psychiatry 45:1350–1362

Collishaw S, Maughan B, Natarajan L, Pickles A (2010) Trends in adolescent emotional problems in England: a comparison of two national cohorts twenty years apart. J Child Psychol Psychiatry 51:885–894

Copeland W, Shanahan L, Miller S, Costello EJ, Angold A, Maughan B (2010) Outcomes of early pubertal timing in young women: a prospective population-based study. Am J Psychiatry 167:1218–1225

Dorn LD, Hitt SF, Rotenstein D (1999) Biopsychological and cognitive differences in children with premature vs. on-time adrenarche. Arch Pediatr Adolesc Med 153:137–146

Dorn LD, Rose SR, Rotenstein D, Susman EJ, Huang B, Loucks TL, Berga SL (2008) Differences in endocrine parameters and psychopathology in girls with premature adrenarche versus on-time adrenarche. J Pediatr Endocrinol Metab 21:439–448

Eveleth P (1998) Variation in age at menarche. In: Ulijaszek SJ, Johnston FE, Preece MA (eds) Cambridge encyclopaedia of human growth and development. Cambridge University Press, Cambridge, pp 376–377
Frank GK, Kaye WH (2012) Current status of functional imaging in eating disorders. Int J Eat Disord 45:723–736
Frodl T, Skokauskas N (2012) Meta-analysis of structural MRI studies in children and adults with attention deficit hyperactivity disorder indicates treatment effects. Acta Psychiatr Scand 125:114–126
Goddings A-L, Burnett Heyes S, Bird G, Viner RM, Blakemore S-J (2012) The relationship between puberty and social emotion processing. Dev Sci 15:801–811
Goldstein JR (2011) A secular trend toward earlier male sexual maturity: evidence from shifting ages of male young adult mortality. PLoS One 6:e14826
Goodyer IM, Herbert J, Altham PM, Pearson J, Secher SM, Shiers HM (1996) Adrenal secretion during major depression in 8- to 16-year-olds, I. altered diurnal rhythms in salivary cortisol and dehydroepiandrosterone (DHEA) at presentation. Psychol Med 26:245–256
Goodyer IM, Park RJ, Netherton CM, Herbert J (2001) Possible role of cortisol and dehydroepiandrosterone in human development and psychopathology. Br J Psychiatry 179:243–249
Graber JA, Lewinsohn PM, Seeley JR, Brooks-Gunn J (1997) Is psychopathology associated with the timing of pubertal development? J Am Acad Child Adolesc Psychiatry 36:1768–1776
Graber JA, Seeley JR, Brooks-Gunn J, Lewinsohn PM (2004) Is pubertal timing associated with psychopathology in young adulthood? J Am Acad Child Adolesc Psychiatry 43:718–726
Grant KE, Compas BE, Stuhlmacher AF, Thurm AE, McMahon SD, Halpert JA (2003) Stressors and child and adolescent psychopathology: moving from markers to mechanisms of risk. Psychol Bull 129:447–466
Hall GS (1904) Adolescence: its psychology and its relation to physiology, anthropology, sociology, sex, crime, religion and education. Appleton, New York
Hawton K, Rodham K, Evans E, Weatherall R (2002) Deliberate self harm in adolescents: self report survey in schools in England. BMJ 325:1207–1211
Hayward C, Killen JD, Hammer LD, Litt IF, Wilson DM, Simmonds B, Taylor CB (1992) Pubertal stage and panic attack history in sixth- and seventh-grade girls. Am J Psychiatry 149:1239–1243
Hayward C, Killen JD, Wilson DM, Hammer LD, Litt IF, Kraemer HC, Haydel F, Varady A, Taylor CB (1997) Psychiatric risk associated with early puberty in adolescent girls. J Am Acad Child Adolesc Psychiatry 36:255–262
Hemphill SA, Kotevski A, Herrenkohl TI, Toumbourou JW, Carlin JB, Catalano RF, Patton GC (2010) Pubertal stage and the prevalence of violence and social/relational aggression. Pediatrics 126:e298–e305
Holder A (2005) Anna Freud, Melanie Klein, and the psycho-analysis of children and adolescents. Karnac Books, London
Holtzman CW, Trotman HD, Goulding SM, Ryan AT, Macdonald AN, Shapiro DI, Brasfield JL, Walker EF (2013) Stress and neurodevelopmental processes in the emergence of psychosis. Neuroscience 249:172–191
Huddleston J, Ge X (2003) Boys at puberty: psychosocial implications. In: Hayward C (ed) Gender differences at puberty. Cambridge University Press, New York, pp 113–134
Kaltiala-Heino R, Kosunen E, Rimpela M (2003a) Pubertal timing, sexual behaviour and self-reported depression in middle adolescence. J Adolesc 26:531–545
Kaltiala-Heino R, Marttunen M, Rantanen P, Rimpela M (2003b) Early puberty is associated with mental health problems in middle adolescence. Soc Sci Med 57:1055–1064
Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE (2005) Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 62:593–602
Klump KL (2013) Puberty as a critical risk period for eating disorders: a review of human and animal studies. Horm Behav 64:399–410

Klump KL, Keel PK, Sisk C, Burt SA (2010) Preliminary evidence that estradiol moderates genetic influences on disordered eating attitudes and behaviors during puberty. Psychol Med 40:1745–1753

Klump KL, Culbert KM, Slane JD, Burt SA, Sisk CL, Nigg JT (2012) The effects of puberty on genetic risk for disordered eating: evidence for a sex difference. Psychol Med 42:627–637

Krug EG, Mercy JA, Dahlberg LL, Zwi AB (2002) The world report on violence and health. Lancet 360:1083–1088

Ladouceur CD (2012) Neural systems supporting cognitive-affective interactions in adolescence: the role of puberty and implications for affective disorders. Front Integr Neurosci 6:65

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

Madge N, Hewitt A, Hawton K, de Wilde EJ, Corcoran P, Fekete S, van Heeringen K, De Leo D, Ystgaard M (2008) Deliberate self-harm within an international community sample of young people: comparative findings from the Child & Adolescent Self-harm in Europe (CASE) Study. J Child Psychol Psychiatry 49:667–677

Mayall B (2002) Towards a sociology for childhood: thinking from children's lives. Open University Press, Buckingham

McCabe MP, Ricciardelli LA, Banfield S (2001) Body image, strategies to change muscles and weight, and puberty: do they impact on positive and negative affect among adolescent boys and girls? Eat Behav 2:129–149

Mendle J, Ferrero J (2012) Detrimental psychological outcomes associated with pubertal timing in adolescent boys. Dev Rev 32:49–66

Mendle J, Turkheimer E, Emery RE (2007) Detrimental psychological outcomes associated with early pubertal timing in adolescent girls. Dev Rev 27:151–171

Mensah FK, Bayer JK, Wake M, Carlin JB, Allen NB, Patton GC (2013) Early puberty and childhood social and behavioral adjustment. J Adolesc Health 53:118–124

Mundy LK, Simmons JG, Allen NB, Viner RM, Bayer JK, Olds T, Williams J, Olsson C, Romaniuk H, Mensah F, Sawyer SM, Degenhardt L, Alati R, Wake M, Jacka F, Patton GC (2013) Study protocol: the Childhood to Adolescence Transition Study (CATS). BMC Pediatr 13:160

Nuffield Foundation (2009) Time trends in adolescent well-being: update 2009. Nuffield Foundation, London

Olweus D, Mattsson A, Schalling D, Low H (1988) Circulating testosterone levels and aggression in adolescent males: a causal analysis. Psychosom Med 50:261–272

Parent AS, Teilmann G, Juul A, Skakkebaek NE, Toppari J, Bourguignon JP (2003) The timing of normal puberty and the age limits of sexual precocity: variations around the world, secular trends, and changes after migration. Endocr Rev 24:668–693

Patton GC, Viner R (2007) Pubertal transitions in health. Lancet 369:1130–1139

Patton GC, Hemphill SA, Beyers JM, Bond L, Toumbourou JW, McMorris BJ, Catalano RF (2007) Pubertal stage and deliberate self-harm in adolescents. J Am Acad Child Adolesc Psychiatry 46:508–514

Prentice P, Viner RM (2013) Pubertal timing and adult obesity and cardiometabolic risk in women and men: a systematic review and meta-analysis. Int J Obes (Lond) 37:1036–1043

Reardon LE, Leen-Feldner EW, Hayward C (2009) A critical review of the empirical literature on the relation between anxiety and puberty. Clin Psychol Rev 29:1–23

Rudolph KD, Flynn M (2007) Childhood adversity and youth depression: influence of gender and pubertal status. Dev Psychopathol 19:497–521

Rutter M, Smith DJ (1995) Psychosocial disorders in young people: time trends and their causes. Wiley, New York

Scheper-Hughes N (1984) The Margaret Mead controversy: culture, biology, and anthropological inquiry. Hum Organ 43:85

Shakespeare W (1623) The Winter's Tale. pp Act III, scene iii

Shefer G, Marcus Y, Stern N (2013) Is obesity a brain disease? Neurosci Biobehav Rev 37(10 Pt 2):2489–2503

Sontag-Padilla LM, Dorn LD, Tissot A, Susman EJ, Beers SR, Rose SR (2012) Executive functioning, cortisol reactivity, and symptoms of psychopathology in girls with premature adrenarche. Dev Psychopathol 24:211–223

Sorensen K, Mouritsen A, Aksglaede L, Hagen CP, Mogensen SS, Juul A (2012) Recent secular trends in pubertal timing: implications for evaluation and diagnosis of precocious puberty. Horm Res Paediatr 77:137–145

Stroud LR, Foster E, Papandonatos GD, Handwerger K, Granger DA, Kivlighan KT, Niaura R (2009) Stress response and the adolescent transition: performance versus peer rejection stressors. Dev Psychopathol 21:47–68

Susman EJ, Dockray S, Granger DA, Blades KT, Randazzo W, Heaton JA, Dorn LD (2010) Cortisol and alpha amylase reactivity and timing of puberty: vulnerabilities for antisocial behaviour in young adolescents. Psychoneuroendocrinology 35:557–569

Trotman HD, Holtzman CW, Ryan AT, Shapiro DI, MacDonald AN, Goulding SM, Brasfield JL, Walker EF (2013) The development of psychotic disorders in adolescence: a potential role for hormones. Horm Behav 64:411–419

Viner RM (2002) Splitting hairs: is puberty getting earlier in girls? Arch Dis Child 86:8–10

Xin Z, Niu J, Chi L (2012) Birth cohort changes in Chinese adolescents' mental health. Int J Psychol 47:287–295

The Role of Puberty in Human Adolescent Brain Development

Anne-Lise Goddings

Abstract Many of the physical, social and behavioural changes associated with adolescence are linked with puberty, the physiological process resulting in reproductive competence. Recent research has demonstrated that the human brain undergoes significant change during adolescence, but little is known about the role of puberty in this process. This review summarises findings from current human imaging studies regarding the relationship between both structural and functional brain development and pubertal maturation, and it explores how these occur in the context of changing chronological age and pubertal status. The findings across these structural and functional MRI studies are consistent with the hypothesis that pubertal hormones interact with the neuroanatomical and neurocognitive changes seen during puberty (Blakemore et al., Hum Brain Map 31:926–933, 2010; Sisk and Foster, Nat Neurosci 7:1040–1047, 2004) and that some aspects of brain development in adolescence might be more closely linked to the physical and hormonal changes of puberty than to chronological age.

Introduction

Adolescence is a key stage in human development, incorporating physical, social, and psychological changes and culminating in the attainment of a stable adult role. During adolescence, new behaviours are laid down, educational, socioeconomic and relationship trajectories are canalized, and a new epidemiology of disease burden emerges (Patton and Viner 2007). Many of these changes have been linked with puberty, the biological process that culminates in reproductive competence and a defining event of adolescence (Sisk and Foster 2004). Research has demonstrated that the human brain undergoes significant change during adolescence, as determined by age, but little is known about how puberty influences the development of the human brain.

A.-L. Goddings (✉)
MRC Clinical Training Research Fellow, UCL Institute of Child Health, 30 Guilford St., London WC1N 1EH, UK
e-mail: Anne-lise.goddings@ucl.ac.uk

J.-P. Bourguignon et al. (eds.), *Brain Crosstalk in Puberty and Adolescence*, Research and Perspectives in Endocrine Interactions 13,
DOI 10.1007/978-3-319-09168-6_6

Since there is a normal variation of four to five years in the timing of onset of puberty in healthy humans (Parent et al. 2003), pubertal development is partially dissociable from chronological age. Nevertheless, puberty and age are inevitably highly correlated when considered over the whole age range of adolescence. Most developmental studies have not tried to dissect these two differing developmental variables, either inferring pubertal effects using age-based studies (e.g. Giedd et al. 1999) or focussing on puberty effects in a wide age range (e.g., Peper et al. 2009). Where studies have attempted to dissociate age from pubertal development, different techniques have been employed. One method involves limiting the age range studied to a sufficiently narrow range that puberty is no longer correlated with age (e.g., Forbes et al. 2010). An alternative approach is to statistically incorporate age as a confounding variable within an analysis looking at pubertal stage (e.g., Op de Macks et al. 2011). Different methods may be best suited to different types of study, e.g., size of sample and cross-sectional vs. longitudinal.

This review summarises the findings from current human imaging studies regarding the relationship between both structural and functional brain development and pubertal maturation, and it explores how these occur in the context of changing chronological age and pubertal status.

Structural Brain Development and Puberty

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 magnetic resonance imaging (MRI) studies (Brain Development Cooperative Group 2012; Raznahan et al. 2011; Sowell et al. 2002). To date, studies of brain growth trajectories over adolescence have predominantly considered growth in relation to chronological age, with few exceptions (Paus et al. 2010; Raznahan et al. 2010). It has been hypothesised that the brain restructuring and development seen in adolescence might 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 et al. 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 non-human primates (Ahmed et al. 2008; Hajszan et al. 2008; Sato et al. 2008), with differential effects of androgens and oestrogens on different brain areas, which might be related to hormone receptor distribution (Clark et al. 1988; Sholl and Kim 1989). These differential effects across brain areas might provide an explanation for the diverging growth trajectories of particular brain structures between males and females that has been documented across studies and the resultant increasing sexual dimorphism in adolescence reported in some regions (Brain Development Cooperative Group 2012; Lenroot et al. 2007; Neufang et al. 2009; Sowell et al. 2002).

An early cross-sectional study by De Bellis and colleagues (2001) found significant correlations between Tanner stage (Marshall and Tanner 1969, 1970), a marker of physical pubertal development, and changes in grey matter (GM) volume, white matter (WM) volume and corpus callosum (CC) area in a sample of 118 children and adolescents aged 6.9–17 years. They reported a Tanner stage by age interaction in development and hypothesised that this might relate to differential hormone exposure in males and females in puberty (De Bellis et al. 2001). Subsequent cross-sectional studies have investigated pubertal development by considering both the physical changes of puberty and by measuring sex steroid hormone levels. A study looking at cortical GM and pubertal measures found region-specific correlations between GM density and both pubertal stage and oestradiol concentration in girls (Peper et al. 2009). Neufang and colleagues (2009) studied a sample of 46 healthy participants aged between 8 and 15 years of age, focussing on the association between brain volumes and both pubertal stage ($n = 46$) and testosterone concentration ($n = 30$). They 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). A second study ($n = 80$; 10.75–13.48 years) investigating puberty and pubertal hormone correlations with GM volume again showed larger amygdala volumes in more pubertally mature males than in their less mature counterparts but showed the opposite trend in females, with decreasing amygdala volume with increasing testosterone levels (Bramen et al. 2011). They also studied other brain regions, including the hippocampus, thalamus, caudate and cortex, and reported significant decreases in volume in the right hippocampus and cerebral cortex in females, but no significant changes in males. The discrepant findings between these two latter papers could result from the relatively small sample sizes or the differing age ranges of the two studies, which also used different methods to extract the volumes of interest (Neufang et al. 2009: voxel-based morphometry; Bramen et al. 2011: surface-based reconstruction). This final study conducted multiple regression analyses incorporating both age and puberty measurements to study the relative contributions of both variables to the changing brain structure seen (Bramen et al. 2011). Puberty and age are highly correlated when considering adolescence as a whole, since puberty is a progressive process that usually begins during early-mid adolescence and develops over time. However, since there is a four- to five-year normal variation in the timing of onset of puberty (Parent et al. 2003), careful study design, appropriate age ranges of participants, and suitable analytical tools can allow puberty and age to be teased apart so that the differing contributions of each variable to development can be studied.

These studies have limited power due to relatively small sample sizes, which constrain our ability to attribute causality to this association or to investigate the effect of sex steroids on brain developmental trajectories during adolescence. Longitudinal analysis allows comparison of brain volumes both between participants and also within each participant 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 regions by following what happens to each participant. This approach is particularly advantageous when looking at brain volumes, which vary substantially between individuals (Brain Development Cooperative Group 2012; Lenroot et al. 2007; Tamnes et al. 2013).

We recently conducted a study using longitudinal data to address some of these issues and to further explore the relationship between structural brain development and the correlated but physiologically distinct variables of chronological age and pubertal stage. This study included 275 individuals aged between 7 and 20 years, each of whom was scanned at least twice, with a total of 711 scans used in the study (Goddings et al. 2013). We focussed on the developmental trajectories of subcortical regions across adolescence, and we used multi-level modelling analyses to tease apart the effects of pubertal status (measured by Tanner stage) and chronological age. In this study, we found that the development of all regions examined was associated with pubertal maturation (Fig. 1; Goddings et al. 2013), and for many structures (females: amygdala, hippocampus, caudate, putamen; males: amygdala, putamen), models including both Tanner stage and age best described volume change over adolescence. Despite the close proximity of the subcortical structures explored, there were clear differences in their structural development during adolescence. These results might reflect the different mechanisms that influence macroscopic volume changes between structures, with varying influences

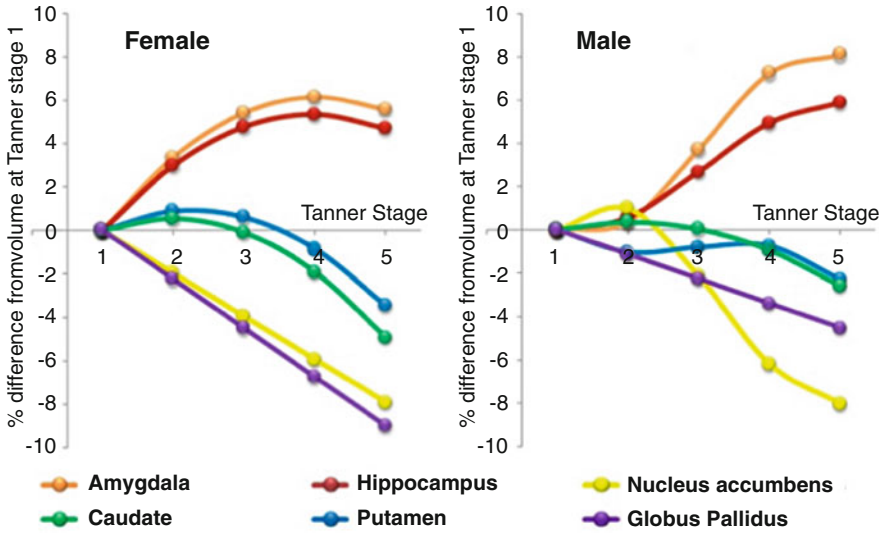

Fig. 1 Growth trajectories (in terms of % volume change) for subcortical regions in females and males across puberty. For each structure, the percentage volume was calculated for each pubertal stage as a proportion of prepubertal volume (at Tanner stage 1). This allows comparison between structures for relative changes in volume across puberty. For both sexes, the amygdala and hippocampus increased in volume over puberty, while the nucleus accumbens, caudate, putamen and globus pallidus decreased in volume (From Goddings et al. 2013)

of age and puberty between regions. Alternatively, the regions might undergo similar growth patterns but do so at different chronological time points.

These studies provide evidence for a role for puberty in the development of the human adolescent brain. Further large longitudinal studies incorporating both hormonal and physical indicators of puberty are needed to further explore this relationship and to tease apart puberty and age effects on structural brain maturation.

Functional Brain Development in Puberty

In addition to assisting in the investigation of changing brain structure over adolescence, MRI techniques can be used to assess changing brain function with development. Functional MRI (fMRI) techniques have allowed researchers to investigate the patterns of neural activation in subjects, as measured by recording blood-oxygen-level dependent (BOLD) signal changes while they perform specific tasks within the scanner, and to establish how these patterns develop from childhood to adulthood. Only a small number of functional neuroimaging studies of the adolescent brain have included puberty measures. These studies have focussed on a range of different behavioural tasks pertinent to adolescent development.

Two fMRI studies have been published assessing changes in face processing with puberty. Reading emotions from faces is an important skill for perceiving emotional states in others and is, therefore, likely to be of particular importance during adolescence (Dahl and Gunnar 2009). Both studies showed differential patterns of neural activation across puberty within the network of regions known to be involved in perception and reaction to emotional expressions. One study showed evidence of 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, with 10 and 13 year olds, Moore and colleagues (2012) found that participants in later stages of pubertal development showed increased signal in face processing regions when looking at affective facial expressions.. These studies reported some discrepant findings, which might reflect the use of different methods of assessing pubertal development or the administration of different tasks (Moore et al. 2012; Op de Macks et al. 2011).

A further aspect of emotional development that is likely to be important during adolescence is the ability to process social emotions. Social emotions require an individual to understand that other people have different mental states to their own and to be able to represent those mental states, a process known as mentalising. Examples of social emotions include ‘guilt’ and ‘embarrassment’. In contrast, basic emotions, e.g., ‘disgust’ and ‘fear,’ do not require this mentalising ability (Burnett et al. 2009). A network of regions involved in mentalising has been robustly demonstrated across a range of fMRI studies (see Blakemore 2012, for review), and the ongoing structural and functional development of this mentalising network

has been shown to continue across adolescence, as determined by chronological age (Mills et al. 2014). We conducted an fMRI study to explore whether the processing of social emotions by this mentalising network changes with puberty in adolescence. The study showed that girls (aged 11 to 13 years) with higher levels of pubertal hormones had increased activation of the anterior temporal cortex - one of the regions of the mentalising network - than their age-matched peers with lower levels of pubertal hormones (Fig. 2; Goddings et al. 2012). In contrast, activation in the prefrontal cortex, which is also involved in mentalising, showed no correlation with pubertal status but showed decreasing activation with increasing chronological age (Goddings et al. 2012), a finding that has been replicated in a number of studies (e.g., Burnett et al. 2009; see Blakemore 2012 for review).

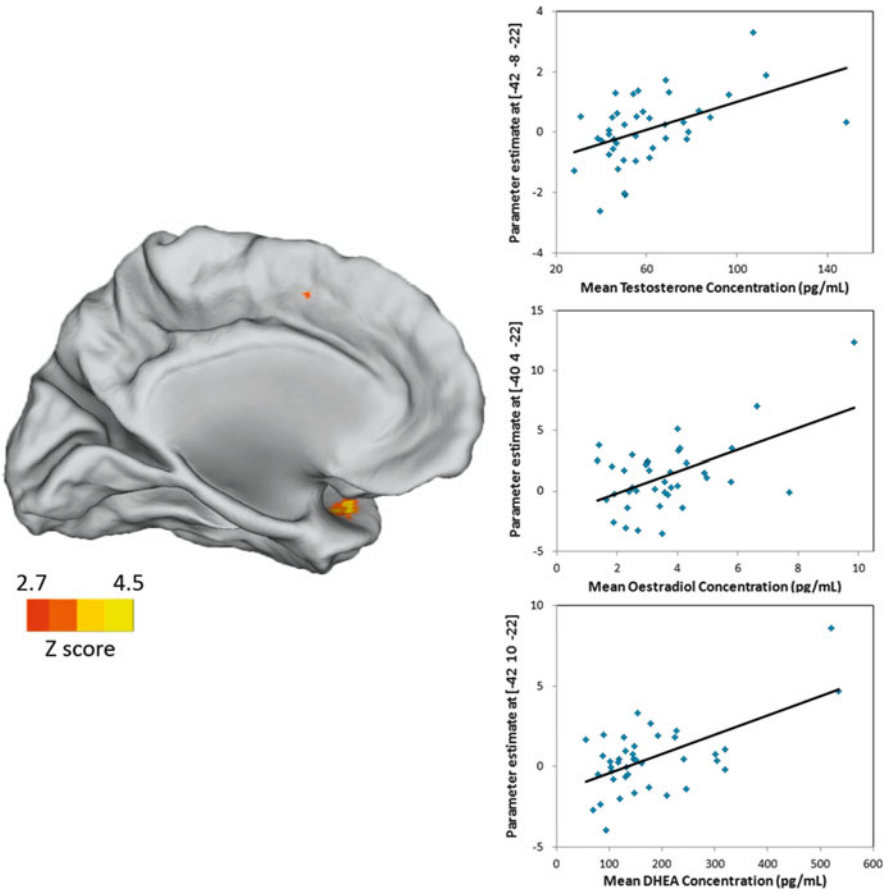

Fig. 2 There was a positive association between level of puberty hormones and BOLD signal in the anterior temporal cortex during a Social>Basic emotion condition of an emotion task (From Goddings et al. 2012) The graphs on the right show the positive correlation at the peak voxel between each puberty hormone and adjusted BOLD signal in the Social>Basic contrast in the left anterior temporal cortex

A different aspect of adolescent cognitive development that has been studied from the perspective of pubertal changes is that of reward processing. Two studies have tackled this topic, with both showing significant differences in patterns of neural activation with advancing puberty. 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 significant correlation between testosterone level and activation in the ventral striatum (caudate in girls and putamen in both genders; Op de Macks et al. 2011). Both of these studies focussed on risk-taking tasks with a binary choice (risky option vs. safer option) and found significant patterns of activation and change with puberty, adding to the evidence for an underlying role of puberty in functional brain development.

Summary

The findings across these structural and functional MRI studies are consistent with the hypothesis that pubertal hormones interact with the neuroanatomical and neurocognitive changes seen during puberty (Blakemore et al. 2010; Sisk and Foster 2004) and that aspects of brain development in adolescence might be more closely linked to the physical and hormonal changes of puberty than to chronological age. Further work is needed to understand these complex relationships. The current set of findings suggests that changes during adolescence in brain structure and 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 could have multiply-specified biological and environmental drivers. Sex hormone receptors are found throughout the brain, with differing concentrations across regions. Thus, the increase in sex hormones at puberty might have direct effects on structural development and on activation of specific brain regions during cognitive tasks. A second potential mechanism to explain differing patterns of neural activation could be that increases in pubertal hormones cause a developmental shift in cognitive strategy, which is then measured as changing patterns of BOLD signals during the task. Ongoing work testing these theories using different methods and paradigms will help to further ascertain the precise role of puberty in ongoing brain development during adolescence.

References

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

Blakemore SJ (2012) Development of the social brain in adolescence. J Roy Soc Med 105:111–116. doi:10.1258/jrsm.2011.110221

Blakemore S-J, Burnett S, Dahl RE (2010) The role of puberty in the developing adolescent brain. Hum Brain Map 31:926–933. doi:10.1002/hbm.21052

Brain Development Cooperative Group (2012) Total and regional brain volumes in a population-based normative sample from 4 to 18 years: the NIH MRI Study of Normal Brain Development. Cereb Cort (1991) 22:1–12. doi: 10.1093/cercor/bhr018

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

Burnett S, Bird G, Moll J, Frith C, Blakemore S-J (2009) Development during adolescence of the neural processing of social emotion. J Cogn Neurosci 21:1736–1750. doi:10.1162/jocn.2009.21121

Clark AS, MacLusky NJ, Goldman-Rakic PS (1988) Androgen binding and metabolism in the cerebral cortex of the developing rhesus monkey. Endocrinology 123:932–940

Dahl RE, Gunnar MR (2009) Heightened stress responsiveness and emotional reactivity during pubertal maturation: implications for psychopathology. Dev Psychopathol 21:1–6

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

Forbes EE, Ryan ND, Phillips ML, Manuck SB, Worthman CM, Moyles DL, Tarr JA, Sciarrillo SR, Dahl RE (2010) Healthy adolescents' neural response to reward: associations with puberty, positive affect, and depressive symptoms. J Am Acad Child Adolesc Psychiatry 49:162–172e1–5

Forbes EE, Phillips ML, Silk JS, Ryan ND, Dahl RE (2011) Neural systems of threat processing in adolescents: role of pubertal maturation and relation to measures of negative affect. Dev Neuropsychol 36:429–452

Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, Paus T, Evans AC, Rapoport JL (1999) Brain development during childhood and adolescence: a longitudinal MRI study. Nat Neurosci 2:861–863

Goddings A-L, Burnett Heyes S, Bird G, Viner RM, Blakemore S-J (2012) The relationship between puberty and social emotion processing. Dev Sci 15:801–811. doi:10.1111/j.1467-7687.2012.01174x

Goddings A-L, Mills KL, Clasen LS, Giedd JN, Viner RM, Blakemore S-J (2013) The influence of puberty on subcortical brain development. NeuroImage 88:242–251

Hajszan T, MacLusky NJ, Leranth C (2008) Role of androgens and the androgen receptor in remodeling of spine synapses in limbic brain areas. Horm Behav 53:638–646. doi:10.1016/j.yhbeh.2007.12.007

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

Marshall WA, Tanner JM (1969) Variations in pattern of pubertal changes in girls. Arch Dis Child 44:291–303

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

Mills KL, Lalonde F, Clasen LS, Giedd JN, Blakemore S-J (2014) Developmental changes in the structure of the social brain in late childhood and adolescence. Soc Cogn Affect Neurosci 9:123–131

Moore WE 3rd, Pfeifer JH, Masten CL, Mazziotta JC, Iacoboni M, Dapretto M (2012) Facing puberty: associations between pubertal development and neural responses to affective facial displays. Soc Cogn Affect Neurosci 7:35–43. doi:10.1093/scan/nsr066

Neufang S, Specht K, Hausmann M, Güntürkün O, Herpertz-Dahlmann B, Fink GR, Konrad K (2009) Sex differences and the impact of steroid hormones on the developing human brain. Cereb Cortex 19:464–473. doi:10.1093/cercor/bhn100

Op de Macks ZA, Gunther Moor B, Overgaauw S, Güroğlu B, Dahl RE, Crone EA (2011) Testosterone levels correspond with increased ventral striatum activation in response to monetary rewards in adolescents. Dev Cogn Neurosci 1:506–516. doi:10.1016/j.dcn.2011.06.003

Parent A-S, Teilmann G, Juul A, Skakkebaek NE, Toppari J, Bourguignon J-P (2003) The Timing of normal puberty and the age limits of sexual precocity: variations around the world, secular trends, and changes after migration. Endocr Rev 24:668–693. doi:10.1210/er.2002-0019

Patton GC, Viner R (2007) Pubertal transitions in health. Lancet 369:1130–1139. doi:10.1016/S0140-6736(07)60366-3

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

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

Peper JS, Hulshoff Pol HE, Crone EA, van Honk J (2011) Sex steroids and brain structure in pubertal boys and girls: a mini-review of neuroimaging studies. Neuroscience 191:28–37. doi:10.1016/j.neuroscience.2011.02.014

Raznahan A, Lee Y, Stidd R, Long R, Greenstein D, Clasen L, Addington A, Gogtay N, Rapoport JL, Giedd JN (2010) Longitudinally mapping the influence of sex and androgen signaling on the dynamics of human cortical maturation in adolescence. Proc Natl Acad Sci U S A 107:16988–16993. doi:10.1073/pnas.1006025107

Raznahan A, Lerch JP, Lee N, Greenstein D, Wallace GL, Stockman M, Clasen L, Shaw PW, Giedd JN (2011) Patterns of coordinated anatomical change in human cortical development: a longitudinal neuroimaging study of maturational coupling. Neuron 72:873–884

Sato SM, Schulz KM, Sisk CL, Wood RI (2008) Adolescents and androgens, receptors and rewards. Horm Behav 53:647–658. doi:10.1016/j.yhbeh.2008.01.010

Sholl SA, Kim KL (1989) Estrogen receptors in the rhesus monkey brain during fetal development. Brain Res Dev Brain Res 50:189–196

Sisk CL, Foster DL (2004) The neural basis of puberty and adolescence. Nat Neurosci 7:1040–1047. doi:10.1038/nn1326

Sowell ER, Trauner DA, Gamst A, Jernigan TL (2002) Development of cortical and subcortical brain structures in childhood and adolescence: a structural MRI study. Dev Med Child Neurol 44:4–16

Tamnes CK, Walhovd KB, Grydeland H, Holland D, Østby Y, Dale AM, Fjell AM (2013) Longitudinal working memory development is related to structural maturation of frontal and parietal cortices. J Cogn Neurosci 25:1611–1623

The Adolescent Brain: Insights from Neuroimaging

Jay N. Giedd and Alexander H. Denker

Abstract Adolescence is a time of dramatic changes in body, behavior, and brain. Although the adolescent brain has different features than those of a child or mature adult, it is not broken or defective. The changes in the teen brain have been exquisitely forged by evolution to facilitate the survival of our species. During the second decade of life the brain does not mature by becoming larger; it matures by becoming more specialized and its subcomponents becoming more "interconnected." Recent advances in neuroimaging and the application of graph theoretical methods of analysis are enabling scientists to characterize these changes in connectivity and how they vary by age, sex, health/illness, and other cognitive or behavioral measures.

Introduction

Adolescence is the transition from our caregiver-dependent childhood to independent functioning as an adult; thus it is defined by both biological and social factors. The timing of this transition may vary across individuals and cultures but in general is thought to begin around the onset of puberty and encompasses the teenage years. Adolescence is a time of change, both physical and psychological. The path to independence is usually accompanied by behaviors and attitudes that are different from their parents, and often these differences are ridiculed as evidence that the teenage brain is "broken" or "defective." Although adolescence is a peak time for the emergence of psychopathology, the many dynamic brain changes occurring during the second decade are also part of the pattern of healthy development and are integral to our success as a species.

In this chapter, we will review aspects of adolescent brain changes derived from our ongoing longitudinal magnetic resonance imaging (MRI) study of brain development that has been conducted since 1989 at the Child Psychiatry Branch of the

J.N. Giedd (✉) • A.H. Denker
Child Psychiatry Branch, National Institute of Mental Health, Bethesda, MD, USA
e-mail: jg@nih.gov

J.-P. Bourguignon et al. (eds.), *Brain Crosstalk in Puberty and Adolescence*,
Research and Perspectives in Endocrine Interactions 13,
DOI 10.1007/978-3-319-09168-6_7

National Institute of Mental Health. The data set encompasses approximately 8,000 images from 3,000 subjects (longitudinal data acquired at approximately 2-year intervals): ¼ from healthy singletons, ¼ from healthy twins, and ½ from one of 25 clinical populations (e.g., autism, ADHD, childhood-onset schizophrenia). Our focus on this data set is not meant to slight the many other excellent studies but to provide one perspective from a single, fairly large project that has used standardized methods of image acquisition and analysis.

The organization of the chapter is to first describe the developmental trajectories of specific brain regions during typical childhood and adolescence and then to discuss the implications of the findings for clinicians, teachers, parents, and the youth themselves.

Total Cerebral Volume

In the NIMH Child Psychiatry Branch study data, total cerebral volume increases during childhood, reaches a peak at age 10.5 in girls and 14.5 in boys, and subsequently slightly declines through the second and third decades (Lenroot et al. 2007). Total brain volume is 95 % of its peak size by age 6. Brain size varies markedly from person to person, with as much as a two-fold difference among healthy subjects of the same age. This high variability extends to measures of brain substructures and has important implications for the interpretation and utility of brain imaging results.

Group-average brain size is approximately 10 % larger in males (see Fig. 1a). The magnitude of this difference is relatively stable from birth across the life span. This difference in brain size should not be interpreted as necessarily imparting any sort of functional advantage or disadvantage, as large-scale brain measures may not reflect sexually dimorphic differences in functionally relevant factors such as neuronal connectivity and receptor density.

The sex difference in total brain volume is not solely attributable to the larger body size of males. This is especially evident in our pediatric population, where the brain size difference persists despite little difference in body size. In fact, both in our sample and according to published normative data from the Centers for Disease Control, because of an earlier growth spurt, females tend to be slightly taller than males from ages 10–13 years. Across species there is a relationship between brain size and body size, but in individual humans the growth trajectories are quite dissimilar, with body size increasing through approximately age 17.

Total brain size difference between males and females has broad implications for studies of sexual dimorphism. Many of the reported findings of brain sexual dimorphism are influenced strongly by whether, or how, the size of subcomponents of the brain are adjusted for the ~10 % difference in total brain volume. Without adjustment, the absolute size of most structures is larger in males. If adjustments are made to subcomponents (via covariation or the use of ratios to total brain volume),

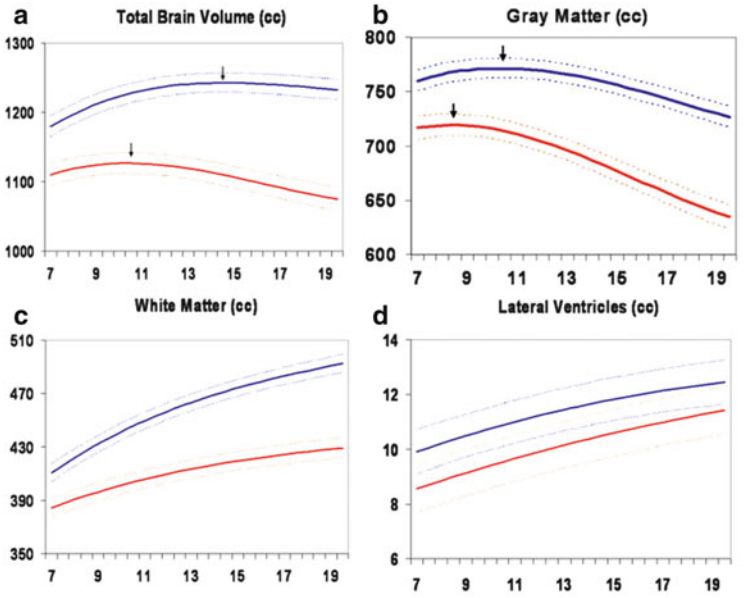

Fig. 1 Mean volume by age in years for males ($N = 475$ scans) and females ($N = 354$ scans). Middle lines in each set of three lines represent mean values, and *upper* and *lower* lines represent upper and lower 95 % confidence intervals. All curves differed significantly in height and shape with the exception of lateral ventricles, in which only height was different, and mid-sagittal area of the corpus callosum, in which neither height nor shape was different. (**a**) Total brain volume, (**b**) gray matter volume, (**c**) white matter volume, and (**d**) lateral ventricle volume (Adapted from Lenroot et al. 2007, figure in public domain)

an entirely different list of structures, varying by sample size and age distribution, is generated.

The challenge of how best to account for the male/female difference in sub-components of the brain in light of the total size difference is informed by allometry, the study of the relationship between size and shape. The size of neurons, vasculature, and other components of brain anatomy is constrained by metabolic and physical considerations, which creates the need for brains of different sizes to have variable enlargement of all parts (Finlay and Darlington 1995). For instance, in comparisons across and within species, white matter (WM) to gray matter (GM) ratios increase with enlargement of total brain volume following a 4/3 power law (Zhang and Sejnowski 2000). This phenomenon may account for why reported differences of greater GM/WM ratios in females (Allen et al. 2003; Gur et al. 2002) are not nearly as robust when comparing males and females with similar total brain volumes (Leonard et al. 2008).

Cerebellum

The cerebellum is relatively understudied in pediatric neuroimaging studies but has three features that make it of compelling interest. Of our large-scale measures, the cerebellum is (1) the most sexually dimorphic; (2) the latest to reach adult volume; and (3) the least heritable (i.e., most influenced by environment). Although only about 1/9 the volume of the cerebrum, the cerebellum contains more brain cells than the cerebrum. The cerebellum has traditionally been conceptualized as being related to motor control, but it is now commonly accepted that the cerebellum is also involved in emotional processing and other higher cognitive functions that mature throughout adolescence (Riva and Giorgi 2000; Schmahmann 2004).

Similar to the cerebrum, developmental curves of total cerebellum size follow an inverted, U-shaped developmental trajectory, with peak size occurring at 11.3 years in girls and 15.6 in boys (Tiemeier et al. 2010). The cerebellum is not a unitary structure but composed of functionally distinct subunits. In cross section, the anatomy of the cerebellum resembles a butterfly shape, with the central body part corresponding to the cerebellar vermis and the wings corresponding to the cerebellar hemispheric lobes. In contrast to the evolutionarily more recent cerebellar hemispheric lobes that followed the inverted, U-shaped developmental trajectory, cerebellar vermis size did not change across this age span.

Lateral Ventricles

The lateral ventricles are unlike the other structures reported here, as they are cerebrospinal fluid-filled compartments, not GM or WM. Increased lateral ventricle volume measures are usually interpreted as an indirect assessment of loss of the tissue from neighboring structures. Ventricular volume increases during typical child and adolescent development should be considered when interpreting the many reports of increased ventricular volumes in a broad range of neuropsychiatric conditions.

White Matter

Whether brain tissue is classified as GM or WM depends largely on the amount of myelinated axons. Myelination is the wrapping of oligodendrocytes around axons, which acts as an electrical insulator, increasing the speed of neuronal signal transmission. The insulating properties of myelin allow signals to travel at speeds up to 100× faster than in unmyelinated axons. Additionally, myelination allows ion pumps to reset the ion gradients only at the nodes between sections of myelin (called nodes of Ranvier), instead of along the entire expanse of the axons, resulting

in up to a 30-fold increase in the frequency with which a given neuron can transmit information. The combination of increased speed (100×) and quicker recovery time (30×) can yield a 3,000-fold increase in the amount of information transmitted per second. Myelination does not simply maximize speed of transmission but also modulates the timing and synchrony of neuronal firing patterns that convey meaning in the brain (Fields 2002). Signal processing plays a central role in developmental changes in the brain's ability to adapt in response to its environment by inhibiting axon sprouting and the creation of new synapses (Fields 2008). These non-subtle impacts of myelin on the brain's ability to process information may underlie many of the cognitive abilities associated with our species.

WM volumes increase steadily throughout childhood and adolescence (Fig. 1c), with similar rates across the major lobes (i.e., the frontal, temporal, and parietal lobes). However, for smaller regions, the growth rates can be quite dynamic, with as much as a 50 % change over a 2-year period (Thompson et al. 2000). The most prominent WM structure is the corpus callosum. It is comprised of approximately 200 million mostly myelinated axons connecting homologous areas of the left and right cerebral hemispheres. Its developmental trajectory reflects the general increases in total WM volume.

Increasing WM volumes on anatomic MRI and greater coherence on fMRI, EEG, and MEG converge to identify a pattern of increased connectivity among spatially disparate brain regions as a hallmark of adolescent development. Diffusion tensor imaging studies, which assess the directionality of WM, also demonstrate an increase in WM organization during adolescence. Changes in WM organization in specific regions correlate with improvements in language (Nagy et al. 2004), reading (Deutsch et al. 2005), response inhibition ability (Liston et al. 2006) and memory (Nagy et al. 2004). Characterizing developing neural circuitry and the changing relationships among disparate brain components is one of the most active areas of neuroimaging research, utilizing graph theory to quantify such things as the small world network properties of the brain (Hagmann et al. 2010).

Gray Matter

Cortical GM

GM is comprised predominantly of cell bodies and dendrites but may also include axons, glia, blood vessels, and extracellular space (Braitenberg 2001). Despite a lifelong reciprocal relationship and shared components of neural circuits, GM and WM have distinctly different developmental trajectories (Fig. 1b). In contrast to the roughly linear increase of WM, GM developmental trajectories follow an inverted, U-shaped curve with peak sizes occurring at different times and in different regions. For example, in the frontal lobes, peak cortical GM volume occurs at 9.5 years in

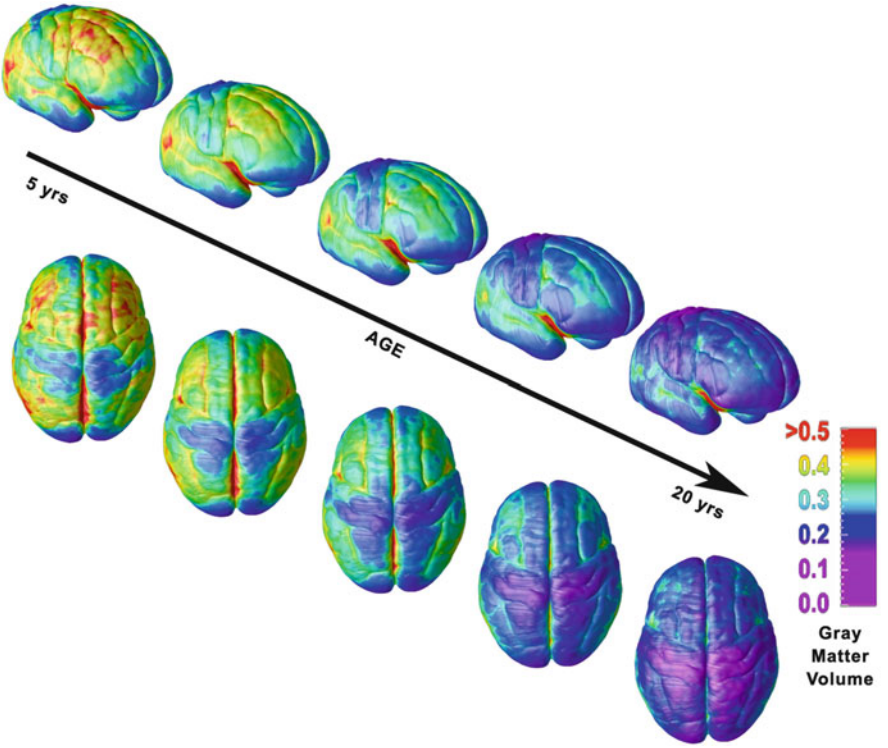

Fig. 2 Right lateral and top views of the dynamic sequence of GM maturation over the cortical surface. The *side bar* shows a color representation in units of GM volume. The initial frames depict regions of interest in the cortex (Adapted from Gogtay et al. 2004; copyright 2004 by the National Academy of Sciences)

girls and 10.5 in boys; at 10.0 in girls and 11.0 in boys in the temporal lobes; and at 7.5 in girls and 9 in boys in the parietal lobes.

Although lobar level GM volumes provide some functional relevance, the capacity to quantify GM thickness at each of approximately 40,000 voxels on the brain surface provides a better spatial resolution to discern functionally distinct regions. By analyzing scans acquired from the same individuals over the course of development, movies of cortical thickness change can be created. One such animation derived from scans of 13 subjects who had each undergone scanning four times at approximately 2-year intervals between the ages of 4 and 22 is available at http://www.nimh.nih.gov/videos/press/prbrainmaturing.mpeg. Still images of the movie at different ages are shown in Fig. 2 (Gogtay et al. 2004).

GM peaks earliest in primary sensorimotor areas and latest in higher order association areas that integrate those primary functions (i.e., dorsolateral prefrontal cortex, inferior parietal, and superior temporal gyrus). Postmortem studies suggest that part of the GM changes may be related to synaptic proliferation and pruning (Huttenlocher 1994). The connection between GM volume reductions, EEG

changes, and synaptic pruning was also supported by an MRI/quantified EEG study of 138 healthy subjects from ages 10 to 30 years. It was found that curvilinear reductions in frontal and parietal GM were matched by similar curvilinear reductions in the EEG power of the corresponding regions (Whitford et al. 2007). Because EEG power reflects synaptic activity, the temporally linked EEG power and GM changes suggest that the GM volume reductions are accompanied by reductions in the number of synapses. Another consideration is that myelination may change classification of voxels along the interior cortical border from GM to WM, resulting in ostensible cortical thinning as assessed by MRI volumetrics, but would not necessarily entail changes in the number of synapses (Sowell et al. 2001). Knowledge of the degree to which these and other phenomena may be driving the MRI changes has profound implications for interpreting the imaging results. Imaging of nonhuman primates with post-mortem validation may help discern the cellular phenomenon underlying the MRI changes.

Subcortical Gray Matter

Amygdala and Hippocampus

The temporal lobes, amygdala, and hippocampus are also involved in a myriad of cognitive functions particularly in the realms of emotion, language, and memory (Nolte 1993). These functions change markedly during adolescence (Diener et al. 1985; Jerslid 1963; Wechsler 1974), yet the relationship between the development of cognitive capacities and transformations in the size or shape of the temporal lobe structures subserving these functions is poorly understood.

Amygdala volume increases significantly during adolescence only in males, and hippocampal volume increases significantly only in females (Giedd et al. 1996). This pattern of gender-specific maturational volumetric changes is consistent with nonhuman primate studies indicating a relatively high number of androgen receptors in the amygdala (Clark et al. 1988) and a relatively higher number of estrogen receptors in the hippocampus (Morse et al. 1986).

Basal Ganglia

The basal ganglia are a collection of nuclei (the striatum, pallidum, and thalamus) lying beneath the cortical surface. They are components of neural circuits involved in mediating movement but are also critical to diverse, developmentally emergent higher cognitive functions, attention allocation, and affective control. Basal ganglia anomalies have been reported for almost all the neuropsychiatric disorders that have been investigated by neuroimaging (Giedd et al. 2006).

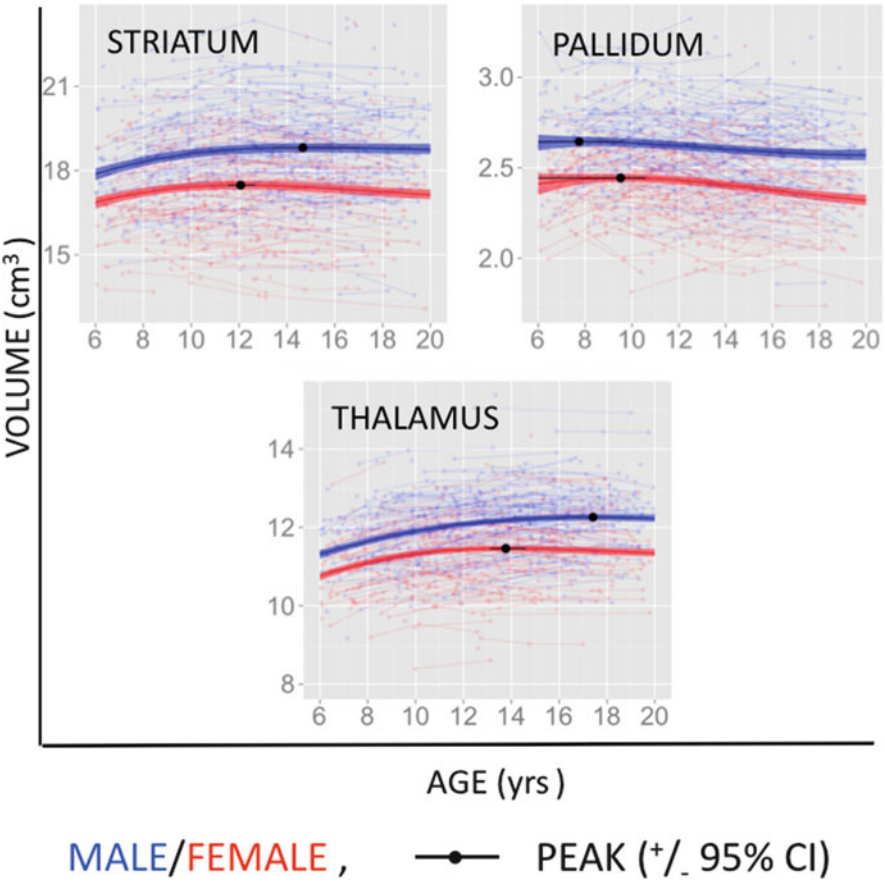

Fig. 3 Developmental trajectories for global volume. Plots showing individual-level anatomical data and best-fit group-level trajectories for bilateral striatal, pallidal, and thalamic volume. *Shaded ribbons* around each curve denote 95 % confidence intervals (Adapted from Raznahan et al. 2014; copyright 2014 by the National Academy of Sciences)

In a recent study of 1,171 longitudinally acquired MRI scans from 618 typically developing males and females aged 5–25 years, we showed that the striatum, pallidum, and thalamus each followed an inverted, U-shaped developmental trajectory (Raznahan et al. 2014).

The striatum and thalamus volumes peak later than cortical volumes and show a relative delay in males. Analyzing the changing shape of these subcortical structures over time reveals that striatal, pallidal, and thalamic domains linked to specific fronto-parietal association cortices contract with age whereas other subcortical territories expand. Furthermore, each structure has areas of sexual dimorphism that change dynamically during adolescence (Fig. 3). These differences may have relevance for sex-biased mental disorders emerging in youth.

Discussion

During adolescence the brain does not mature by becoming larger; it matures by becoming more inter-connected and more specialized. The increased connectivity is reflected in observed WM volume increases but also by electroencephalographic, functional MRI, postmortem, and neuropsychological studies. "Connectivity" characterizes several neuroscience concepts. In anatomic studies, connectivity can mean a physical link between areas of the brain that share common developmental trajectories. In studies of brain function, connectivity describes the relationship between different parts of the brain that activate together during a task. In genetics, it refers to different regions influenced by the same genetic or environmental factors. All of these types of connectivity increase during adolescence (Power et al. 2010). The greater communication among disparate brain regions underlies many of the cognitive advances that occur during the second decade of life. A tradeoff for the increased connectivity is that myelin releases molecules such as Nogo-A (Chen et al. 2000; GrandPr et al. 2000), MAG (GrandPr et al. 2000) and OMgp (McKerracher et al. 1994; Wang et al. 2002) that impede arborization of new connections (Huang et al. 2005; Schwab and Thoenen 1985) and thus decrease plasticity (Fields 2008).

The increasing specialization of the brain is reflected by the inverted, U-shaped GM developmental trajectories of childhood peaks followed by adolescent declines. This pattern is found not only for GM volumes but also for the number of synapses (Huttenlocher and Dabholkar 1997; Huttenlocher and de Courten 1987; Huttenlocher et al. 1982), glucose utilization (Chugani et al. 1987), EEG power (Thatcher 1991), and neurotransmitter receptor densities (Benes 2001). The powerful process of overproduction followed by selective/competitive elimination that shapes the developing nervous system in utero seems to continue to refine the brain throughout adolescent development.

The ability of the brain to change in response to the demands of the environment is often referred to as "plasticity," a highly adaptive feature of our species. Brain plasticity is a life-long process but tends to be most robust in early development. Compared to other species, humans have a very protracted period of life when they are dependent upon their parents or other adults for survival. A benefit of this protracted period of protection is that it allows our brains to stay flexible to changing demands, even more so than our close genetic kin, the Neanderthals, whose tool use changed remarkably little in over 1 million years. They were well suited to deal with the stable, albeit harsh, environment of the time but less facile at adapting to changing demands (Banks et al. 2008).

Plasticity enables humans to be remarkably adaptable to physical and social environments alike. In 10,000 years, we have gone from spending the majority of our time hunting for food and shelter to relying on words and symbols, skills that are approximately only 5,000-years-old. The adaptability of the adolescent brain is being put to the test in the modern world, where digital technologies have dramatically changed the way we learn, play, and interact (Giedd 2012).

The dynamic brain changes of adolescence do confer an increased risk of psychopathology (Paus et al. 2008), but they also provide an excellent basis for learning and resilience. We are faced with an important initiative to utilize our growing knowledge of developmental neurobiology to improve the lives of youth and their families.

References

Allen JS, Damasio H, Grabowski TJ, Bruss J, Zhang W (2003) Sexual dimorphism and asymmetries in the gray-white composition of the human cerebrum. Neuroimage 18:880–894

Banks WE, d'Errico F, Peterson AT, Kageyama M, Sima A, Sánchez-Goñi MF (2008) Neanderthal extinction by competitive exclusion. PLoS One 3(12):e3972. doi:10.1371/journal.pone.0003972

Benes FM (2001) The development of prefrontal cortex: the maturation of neurotransmitter systems and their interactions. In: Nelson CA, Luciana M (eds) Handbook of developmental cognitive neuroscience, vol 2. MIT Press, Cambridge, MA, pp 79–92

Braitenberg V (2001) Brain size and number of neurons: an exercise in synthetic neuroanatomy. J Comput Neurosci 10:71–77

Chen MS, Huber AB, van der Haar ME, Frank M, Schnell L, Spillmann AA, Schwab ME (2000) Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature 403:434–439

Chugani HT, Phelps ME, Mazziotta JC (1987) Positron emission tomography study of human brain functional development. Ann Neurol 22:487–497

Clark AS, MacLusky NJ, Goldman-Rakic PS (1988) Androgen binding and metabolism in the cerebral cortex of the developing rhesus monkey. Endocrinology 123:932–940

Deutsch GK, Dougherty RF, Bammer R, Siok WT, Gabrieli JD, Wandell B (2005) Children's reading performance is correlated with white matter structure measured by diffusion tensor imaging. Cortex 41:354–363

Diener E, Sandvik E, Larsen RF (1985) Age and sex effects for affect intensity. Dev Psychol 21:542–546

Fields RD (2008) White matter in learning, cognition and psychiatric disorders. Trends Neurosci 31:361–370. doi:10.1016/j.tins.2008.04.001, S0166-2236(08)00132-X [pii]

Fields RD, Stevens-Graham B (2002) New insights into neuron-glia communication. Science 298:556–562

Finlay BL, Darlington RB (1995) Linked regularities in the development and evolution of mammalian brains. Science 268:1578–1584

Giedd J (2012) The digital revolution and adolescent brain evolution. J Adolesc Health 51:101–105

Giedd JN, Vaituzis AC, Hamburger SD, Lange N, Rajapakse JC, Kaysen D, Rapoport JL (1996) Quantitative MRI of the temporal lobe, amygdala, and hippocampus in normal human development: ages 4–18 years. J Comp Neurol 366:223–230

Giedd JN, Shaw P, Wallace G, Gogtay N, Lenroot RK (2006) Anatomic brain imaging studies of normal and abnormal brain development in children and adolescents. In: Cicchetti D, Cohen DJ (eds) Developmental psychopathology, vol 2, 2nd edn. Wiley, Hoboken, pp 127–194

Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC, Thompson PM (2004) Dynamic mapping of human cortical development during childhood through early adulthood. Proc Natl Acad Sci U S A 101:8174–8179

GrandPr T, Nakamura F, Vartanian T, Strittmatter SM (2000) Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. Nature 403:439–444

Gur RC, Gunning-Dixon F, Bilker WB, Gur RE (2002) Sex differences in temporo-limbic and frontal brain volumes of healthy adults. Cereb Cortex 12: 998–1003. doi: Cited By (since 1996) 112 Export Date 23 December 2011

Hagmann P, Sporns O, Madan N, Cammoun L, Pienaar R, Wedeen VJ, Meuli R, Thiran JP, Grant PE (2010) White matter maturation reshapes structural connectivity in the late developing human brain. Proc Natl Acad Sci U S A 107:19067–19072

Huang J, Phillips G, Roth A, Pedraza L, Shan W, Belkaid W, Colman D (2005) Glial membranes at the node of Ranvier prevent neurite outgrowth. Science 310:1813–1817

Huttenlocher PR (1994) Synaptogenesis in human cerebral cortex. In: Dawson G, Fischer K (eds) Human behavior and the developing brain. Guilford Press, New York, pp 137–152

Huttenlocher PR, Dabholkar AS (1997) Regional differences in synaptogenesis in human cerebral cortex. J Comp Neurol 387:167–178

Huttenlocher PR, de Courten C (1987) The development of synapses in striate cortex of man. Hum Neurobiol 6:1–9

Huttenlocher PR, de Courten C, Garey LJ, Van der Loos H (1982) Synaptic development in human cerebral cortex. Int J Neurol 16–17:144–154

Jerslid AT (1963) The psychology of adolescence, 2nd edn. Macmillan, New York

Lenroot RK, Gogtay N, Greenstein DK, Wells EM, Wallace GL, Clasen LS, Giedd JN (2007) Sexual dimorphism of brain developmental trajectories during childhood and adolescence. Neuroimage 36:1065–1073. doi:10.1016/J.Neuroimage.2007.03.053

Leonard CM, Towler S, Welcome S, Halderman LK, Otto R, Eckert MA, Chiarello C (2008) Size matters: cerebral volume influences sex differences in neuroanatomy. Cereb Cortex 18:2920–2931

Liston C, Watts R, Tottenham N, Davidson MC, Niogi S, Ulug AM, Casey BJ (2006) Frontostriatal microstructure modulates efficient recruitment of cognitive control. Cereb Cortex 16:553–560

McKerracher L, David S, Jackson DL, Kottis V, Dunn RJ, Braun PE (1994) Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth. Neuron 13:805–811

Morse JK, Scheff SW, DeKosky ST (1986) Gonadal steroids influence axonal sprouting in the hippocampal dentate gyrus: a sexually dimorphic response. Exp Neurol 94:649–658

Nagy Z, Westerberg H, Klingberg T (2004) Maturation of white matter is associated with the development of cognitive functions during childhood. J Cogn Neurosci 16:1227–1233

Nolte J (1993) Olfactory and limbic systems. In: Farrell R (ed) The human brain. An introduction to its functional anatomy, 3rd edn. Mosby, St Louis, pp 397–413

Paus T, Keshavan M, Giedd JN (2008) Why do many psychiatric disorders emerge during adolescence? Nat Rev Neurosci 9:947–957

Power JD, Fair DA, Schlaggar BL, Petersen SE (2010) The development of human functional brain networks. Neuron 67:735–748

Raznahan A, Shaw PW, Lerch JP, Clasen LS, Greenstein D, Berman R, Giedd JN (2014) Longitudinal four-dimensional mapping of subcortical anatomy in human development. Proc Natl Acad Sci U S A 111:1592–1597. doi:10.1073/pnas.1316911111

Riva D, Giorgi C (2000) The cerebellum contributes to higher functions during development: evidence from a series of children surgically treated for posterior fossa tumours. Brain 123 (Pt 5):1051–1061

Schmahmann JD (2004) Disorders of the cerebellum: ataxia, dysmetria of thought, and the cerebellar cognitive affective syndrome. J Neuropsychiatry Clin Neurosci 16(3):367–378. doi:10.1176/appi.neuropsych.16.3.36716/3/367 [pii]

Schwab ME, Thoenen H (1985) Dissociated neurons regenerate into sciatic but not optic nerve explants in culture irrespective of neurotrophic factors. J Neurosci 5:2415–2423

Sowell ER, Thompson PM, Tessner KD, Toga AW (2001) Mapping continued brain growth and gray matter density reduction in dorsal frontal cortex: Inverse relationships during postadolescent brain maturation. J Neurosci 21:8819–8829

Thatcher RW (1991) Are rhythms of human cerebral development “traveling waves”? Behav Brain Sci 14(4):575

Thompson PM, Giedd JN, Woods RP, MacDonald D, Evans AC, Toga AW (2000) Growth patterns in the developing brain detected by using continuum mechanical tensor maps. Nature 404:190–193. doi:10.1038/35004593

Tiemeier H, Lenroot RK, Greenstein DK, Tran L, Pierson R, Giedd JN (2010) Cerebellum development during childhood and adolescence: a longitudinal morphometric MRI study. Neuroimage 49:63–70

Wang K, Koprivica V, Kim J, Sivasankaran R, Guo Y, Neve R, He Z (2002) Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. Nature 417:941–944

Wechsler D (1974) Wechsler intelligence scale for children – revised. The Psychological Corporation, New York

Whitford TJ, Rennie CJ, Grieve SM, Clark CR, Gordon E, Williams LM (2007) Brain maturation in adolescence: concurrent changes in neuroanatomy and neurophysiology. Hum Brain Mapp 28:228–237

Zhang K, Sejnowski TJ (2000) A universal scaling law between gray matter and white matter of cerebral cortex. Proc Natl Acad Sci USA 97:5621–5626. doi: Cited By (since 1996) 141

Gene Networks, Epigenetics and the Control of Female Puberty

Alejandro Lomniczi, Juan Manuel Castellano, Hollis Wright, Basak Selcuk, Kemal Sonmez, and Sergio R. Ojeda

Abstract Puberty is a major developmental milestone set in motion by the interaction of genetic factors and environmental cues. The pubertal process is initiated by an increased pulsatile release of gonadotropin releasing hormone (GnRH) from neurosecretory neurons of the hypothalamus. Although single genes have been identified that are essential for puberty to occur, it appears clear now that many genes controlling diverse cellular functions contribute to the process. The polygenic nature of the neuroendocrine complex controlling puberty has prompted two important questions: are these genes functionally connected and, if they are, is their activity subject to a dynamic level of control independent of changes in DNA sequence? In this article we will discuss emerging evidence suggesting that the onset of puberty is controlled at the transcriptional level by interactive gene networks subjected to epigenetic regulation. At least two modes of epigenetic regulation provide coordination and transcriptional plasticity to these networks: changes in DNA methylation and differential association of histone modifications to genomic regions controlling gene activity. Architecturally, puberty-controlling networks are endowed with "activators," which move the process along by setting in motion key developmental events, and "repressors," which play a central role in preventing the untimely unfolding of these events.

Introduction

Many years of research have been devoted to characterizing the hormonal changes associated with the initiation of puberty and identifying the neurotransmitter systems involved in controlling the gonadotropin-releasing hormone (GnRH) neuronal network during the pubertal transition. Thanks to these efforts, we now have a much better understanding of the basic biological underpinnings of the pubertal process.

A. Lomniczi • J.M. Castellano • H. Wright • B. Selcuk • K. Sonmez • S.R. Ojeda (✉)
Division of Neuroscience, Oregon National Primate Research Center/Oregon Health and Science University, Beaverton, OR 97006, USA
e-mail: lomniczi@ohsu.edu; ojedas@ohsu.edu

J.-P. Bourguignon et al. (eds.), *Brain Crosstalk in Puberty and Adolescence*,
Research and Perspectives in Endocrine Interactions 13,
DOI 10.1007/978-3-319-09168-6_8

It was more than 40 years ago that Boyar et al. (1972) demonstrated that the advent of human puberty is signaled by a nocturnal increase in pulsatile luteinizing hormone (LH) release. Consistent with this finding, subsequent work established the concept that an increase in pulsatile release of GnRH is ultimately responsible for setting in motion the endocrine manifestations of puberty. It also became clear that GnRH neurons, though essential for puberty to occur, are neither the *primum movens* of puberty nor do they temporarily limit its initiation. Instead, GnRH neurons are controlled by upstream cellular systems consisting of both neurons and glial cells. While a variety of neurotransmitter/neuromodulatory systems have been shown to act trans-synaptically to advance or delay the advent of puberty, the overarching concept emerging from these findings is that puberty is set in motion by a coordinated reduction in inhibitory influences and an increase in excitatory inputs to GnRH neurons (Plant and Witchel 2006; Terasawa and Fernandez 2001; Ojeda and Terasawa 2002). It is also clear that, in addition to a dual neuronal input (Kordon et al. 1994; Ojeda and Terasawa 2002), GnRH neurons are subjected to excitatory glial inputs (Ojeda et al. 2008, 2010), provided by growth factors and small molecules acting in a paracrine fashion within the glial-GnRH neuron interface (Ojeda and Skinner 2006; Lomniczi and Ojeda 2009).

With the availability of new tools to explore the mammalian genome, attention has shifted over the last decade towards the identification of the genes involved in controlling puberty-related changes in trans-synaptic and cell-cell communication (Ojeda et al. 2006). Importantly, researchers have also taken advantage of examples of pubertal failure and sexual precocity provided by nature to begin the identification of an elite group of genes required for human puberty to occur (Sykiotis et al. 2010). While several of these genes are now known, it has also become apparent that puberty is not set in motion by a single gene but instead requires the contribution of several, functionally related gene sets (Lomniczi et al. 2013b). In this article we will provide a brief description of the neurobiology of puberty, review the contribution of genetic networks to the timing of puberty, and discuss the emerging concept that a central component of the neuroendocrine machinery by which the brain controls puberty is gene silencing, imposed by transcriptional repressors and coordinated by changes in epigenetic information.

The Trans-synaptic Regulation of Puberty

Although it has been known for many years that a significant fraction of the excitatory control of puberty is provided by glutamatergic neurons (Plant and Witchel 2006; Ojeda and Skinner 2006), more recently it has become clear that an even stronger stimulatory input derives from neurons producing kisspeptins (Oakley et al. 2009; d'Anglemont et al. 2010). These powerful stimulators of

GnRH release (Kauffman et al. 2007; Oakley et al. 2009; Shahab et al. 2005) are encoded by the *KISS1*/*Kiss1* gene (Ohtaki et al. 2001; Kotani et al. 2001). Loss of function of either *KISS1* or *GPR54*/*KISS1R*, the gene encoding the kisspeptin receptor, results in pubertal failure (Seminara et al. 2003; de Roux et al. 2003; Lapatto et al. 2007; Topaloglu et al. 2012). In humans and nonhuman primates, a population of kisspeptin neurons is located in the arcuate nucleus (ARC) of the medial basal hypothalamus (Shahab et al. 2005; Hrabovszky et al. 2010) and another, less well-recognized group in the rostral periventricular area (Hrabovszky et al. 2010). In rodents, this second population of kisspeptin neurons is located in the periventricular region of the anteroventral periventricular nucleus (AVPV; Clarkson et al. 2009; Gottsch et al. 2004). Current evidence indicates that ARC kisspeptin neurons are required for pulsatile GnRH release (Navarro et al. 2011; Wakabayashi et al. 2010), and that AVPV kisspeptin neurons are needed for the pre-ovulatory surge of gonadotropins (Khan and Kauffman 2011; Smith et al. 2006).

No less than three neuronal subsets are responsible for the inhibitory transsynaptic circuitry controlling GnRH release. They include GABAergic and opiatergic neurons (reviewed in Terasawa and Fernandez 2001) and some more recently recognized neurons that employ RFamide-related peptide (RFRP) to inhibit GnRH neuronal activity (Ducret et al. 2009; Gibson et al. 2008; Tsutsui et al. 2010). RFRP is the mammalian ortholog of the peptide gonadotropin-inhibiting hormone (GnIH), originally described in birds (reviewed in Ebling and Luckman 2008). RFRP neurons use two peptides for transsynaptic communication (RFRP1 and RFRP3); they are recognized by a high-affinity receptor termed GPR147 or NPFFR1 (Hinuma et al. 2000; Tsutsui et al. 2010) and a low-affinity receptor termed GPR74 or NPFFR2 (Fukusumi et al. 2006). Because GPR147 is expressed in GnRH neurons (Ducret et al. 2009; Poling et al. 2012), it is thought that RFRP neurons can directly repress GnRH secretion.

GABAergic neurons can inhibit GnRH secretion by acting on neurons connected to the GnRH neuronal network (Ojeda and Skinner 2006; Terasawa and Fernandez 2001), but they can also excite GnRH neurons by activating $GABA_A$ receptors located on GnRH neurons themselves (DeFazio et al. 2002; Moenter and DeFazio 2005). Opiatergic neurons, on the other hand, only inhibit GnRH neuronal activity; they do so using different peptides that act on different receptors (Kordon et al. 1994) located either on GnRH neurons (Dudas and Merchenthaler 2006) or on neurons involved in the stimulatory control of GnRH. A prominent example of this latter synaptic pathway can be found in a subset of ARC neurons, termed KNDy neurons because they produced **K**isspeptins, **N**eurokinin B (NKB) and **D**ynorphin. The latter is an opioid peptide that inhibits GnRH secretion, at least in part, by repressing kisspeptin release from KNDy neurons (Navarro et al. 2009).

The Glial Contribution

Astrocytes and tanycytes (ependymoglial cells lining the ventro-lateral surface of the third ventricle) facilitate GnRH secretion via at least two mechanisms. One of them involves several growth factors, including transforming growth factor-beta (TGFβ), recognized by cell-membrane receptors with serine-threonine kinase activity, and growth factors recognized by receptors with tyrosine kinase activity: the epidermal growth factor (EGF) family, basic fibroblast growth factor (bFGF), and insulin-like growth factor 1 (IGF-I; reviewed in Prevot 2002; Mahesh et al. 2006). Of these, the EGF family is perhaps the most extensively studied, with substantial evidence showing that EGF-like peptides do not stimulate GnRH neurons directly but instead facilitate GnRH release by eliciting prostaglandin E2 (PGE_2) release from astroglial cells and tanycytes (Lomniczi and Ojeda 2009; Clasadonte et al. 2011).

In addition to this mechanism of glia-to-GnRH neuron communication, glial cells facilitate GnRH neuronal activity via plastic rearrangement in cell adhesiveness mediated by different adhesion molecules with signaling capabilities. These molecules include the sialylated form of the neural cell adhesion molecule NCAM (PSA-NCAM; Parkash and Kaur 2005; Perera et al. 1993), the synaptic adhesion molecule Synaptic Cell Adhesion Molecule 1 (SynCAM1); (Sandau et al. 2011a, b), and the recognition molecule Receptor-like Protein Tyrosine Phosphatase-β (RPTPβ) which, expressed in astrocytes, signals to GnRH neurons by associating with contactin, a cell-surface protein expressed in GnRH neurons (Parent et al. 2007). Because the adhesive proteins used by each of these systems contain intracellular signaling domains, it is apparent that glial cells regulate GnRH neuronal activity not only by releasing cell-cell signaling molecules (such as growth factors and PGE_2) but also by setting in motion intracellular signaling mechanisms via direct contact of cell adhesive molecules [reviewed in (Lomniczi and Ojeda 2009)].

Identifying Gene Networks Controlling Puberty

Several monogenic mutations have been shown to cause pubertal failure by disrupting the function of genes such as *GNRHR* (Bedecarrats and Kaiser 2007) encoding the GnRH receptor, *KISS1R* (Seminara et al. 2003; de Roux et al. 2003) encoding the kisspeptin receptor, *KiSS1* (Lapatto et al. 2007; Topaloglu et al. 2012) encoding kisspeptins, *TAC3* encoding NKB and *TAC3R* (Topaloglu et al. 2008) encoding an NKB receptor. Although less information exists about the genetic mutations underlying familial precocious puberty or idiopathic central precocity, two mutations with very different underlying biological underpinnings have been described. One consists of activating mutations of *KISS1R* (Teles et al. 2008) and the other – reported very recently – involves inactivating mutations of *MKRN3*,

a transcriptional repressor (Abreu et al. 2013). Though very important, the mutations thus far described account for only a small percentage of individuals affected by pubertal disorders, suggesting the existence of several additional genes not yet identified. Indeed, genome-wide analyses have revealed that sequence variations in more than 40 genes are associated with an early age at menarche (Ong et al. 2009; Perry et al. 2009; Sulem et al. 2009; He et al. 2009; Elks et al. 2010), indicating that, as predicted by the aforementioned clinical findings, the initiation and completion of puberty involve the participation of a multiplicity of genes.

This is not in itself all that surprising, because reproductive function – essential for the continuity of the species – would be expected to be governed by a robust regulatory system endowed not only with a high degree of redundancy but also with compensatory mechanisms geared to ensure the functional integrity of the system in the event of loss of a component. The re-establishment of normal reproductive function following either early death of kisspeptin neurons (Mayer and Boehm 2011) or loss of Tacr3, the NKB receptor (Yang et al. 2012) is a prominent example of this safeguard mechanism.

If several genes are involved in the central control of puberty, a certain degree of functional interconnectivity among them would also be expected. Using bioinformatics and systems biology tools, coupled with high-throughput approaches, our group identified a group of genes predicted to be organized into a functional network that appears to contribute to the systems-wide control of puberty (Ojeda et al. 2006; Roth et al. 2007). A main characteristic of this gene set is the composition of the predicted network, which mostly contains genes (referred to as either tumor suppressor or tumor related genes, TSGs/TRGs) with diverse cellular functions but sharing a common involvement in tumor suppression/tumor formation. Structural characterization of the network predicted the existence of well-connected central nodes providing control at a *transcriptional* level and many peripheral, subordinate genes acting as portals for network output and perhaps as conduits of externally derived regulatory inputs (Ojeda et al. 2006; Roth et al. 2007). Subsequent studies supported the original model of the network by showing that, of the five putative central nodes of the network (*CDP/CUTL1/CUX1, MAF, p53, YY1, and USF2*), YY1 and an isoform of CDP/CUTL1/CUX1 known as CUX1p120 repress *KISS1* transcription, whereas another CUX1 isoform, CUX1p200, acts as a trans-activator (Mueller et al. 2011).

These and earlier studies provided the bases for the view that mammalian puberty is controlled by genetic networks that, operating within different cell contexts in the neuroendocrine brain, coordinate the activity of GnRH neurons at puberty. A more detailed account of this subject can be found in Lomniczi et al. (2013b).

Transcriptional Repression: A Key Regulatory Mechanism of Prepubertal Development

A much-discussed view proposed to explain the cell-cell communication events leading to the initiation of puberty states that GnRH neurons are under a tonic inhibitory trans-synaptic control, which is lifted at the end of juvenile development, unleashing the pubertal process [reviewed in (Terasawa 1999)]. It has been argued, however, that lifting an inhibitory tone cannot by itself trigger puberty and that concurrent activation of excitatory inputs is a sine qua non feature of pubertal development (Ojeda 1991). The demonstration that activation of kisspeptin neurons, the bona fide prototype of excitatory system controlling GnRH secretion, is essential for puberty to occur (reviewed in Pinilla et al. 2012) provided strong support for this latter concept. On the surface, it also appeared to dismiss the idea of a pubertal "brake," as it showed that all that is required for puberty to occur is the activation of a stimulatory system.

New evidence now indicates that an important inhibitory condition does indeed exist, but it resides at a transcriptional, rather than a trans-synaptic, level. The first inkling that a transcriptionally repressive regulatory system may be biologically significant was provided by the realization that at least three central nodes of the TRG network (*YY1, EAP1, CUX1*) repress the transcriptional activity of puberty-related genes (Heger et al. 2007; Mueller et al. 2011, 2012). Very recently, we identified the Polycomb Group (PcG) as a silencing complex that keeps puberty in check by repressing the *Kiss1* gene in kisspeptin neurons of the ARC (Lomniczi et al. 2013a). The PcG complex plays a major role in imposing gene silencing at various developmental stages (Schwartz and Pirrotta 2007; Simon and Kingston 2009; Kohler and Villar 2008). Our findings suggest that a mechanism of transcriptional repression operating within excitatory neurons themselves is an important component of the so-called "central restraint" that prevents puberty from starting prematurely.

New studies in our laboratory suggest that the PcG complex is not the only repressor involved. To search for factors that may inhibit hypothalamic PcG expression at puberty, we again use a combination of gene expression arrays and qPCR, in addition to in silico analysis of transcription factor binding sites in PcG promoter regions. This search identified a subfamily of the BTB POZ-ZF (for Broad Complex, Tramtrack, Bric-à-brac, poxvirus and Zinc finger) group of transcriptional repressors – known as the POK family (for POZ and Krüppel; Kelly and Daniel 2006; Maeda et al. 2005) – as potential regulators of PcG gene activity (Lomniczi et al., unpublished results). POK genes contribute to controlling a variety of developmental processes, with alterations of their function resulting in tumorigenesis and developmental disorders (Dhordain et al. 1997; Lin et al. 1998; David et al. 1998). We observed that hypothalamic expression of three of these genes (*Zbtb7a*/*LRF, Bcl6* and Zbtb16/*Plzf*) increases – instead of decreasing –during prepubertal development of the female rat. Promoter assays demonstrated that all

three POK proteins are potent inhibitors of PcG gene expression, suggesting that they may function as "repressors of repressors."

Several members of the ZNF superfamily of transcriptional repressors (Urrutia 2003; Vogel et al. 2006; Shannon et al. 1996; Filion et al. 2006) may also contribute to the transcriptional silencing of puberty, as expression of some of these genes declines in the primate hypothalamus at the initiation of puberty (Lomniczi et al. 2013b). The idea that a reduction in ZNF inhibitory output at puberty may lift an inhibitory influence from puberty-activating genes (i.e., genes involved in the stimulatory control of puberty) was recently given credence by the finding that a ZNF gene known as MKRN3/ZNF127 plays a repressive role in human puberty (Abreu et al. 2013). This report showed that inactivating mutations in the coding region of MKRN3 are responsible for the sexual precocity observed in several members of a cohort of 15 families with familial central precocious puberty. MKRN3 is an intronless, maternally imprinted gene encoding a protein with a RING finger domain and several zinc finger motifs (Jong et al. 1999) with predicted ubiquitin ligase-like activity.

MKRN3 may represent a class of genes encoding RING and zinc finger containing proteins that contribute to the repressive transcriptional control of puberty, as the structurally similar gene *EAP1* (*IRF-2BPL*) has also been implicated in the control of puberty and reproductive function (Heger et al. 2007; Dissen et al. 2012; Lomniczi et al. 2012). Hypothalamic expression of *MKRN3* declines during juvenile development in mice (Abreu et al. 2013), rats and nonhuman primates (unpublished observations), implying a prepubertal decline in a MKRN3-dependent inhibitory influence and suggesting that MKRN3 controls puberty by repressing downstream target genes, instead of activating the GnRH neuronal network (Lomniczi et al. 2013b). Interestingly, hypothalamic expression of the POK genes *Bcl6*, *Zbtb7a*/*LRF* and *Zbtb16*/*PLZF* mirrors that of MKRN3 in female rats (unpublished observations), suggesting a causal relationship.

An additional mechanism of repression may be provided by LIN28 proteins acting post-transcriptionally. This involvement was suggested by the finding that a single nucleotide polymorphism near the *LIN28B* gene in human chromosome 6 (q21) is associated with earlier puberty and shorter stature in girls (Perry et al. 2009; Sulem et al. 2009; Ong et al. 2009). *LIN28B* encodes a cytoplasmic protein that controls gene expression post-transcriptionally by binding to miRNAs and diminishing miRNA-mediated mRNA degradation (Moss et al. 1997; Viswanathan et al. 2009). Its main target appears to be members of the *let-7* family of miRNAs. LIN28 proteins bind to the terminal loops of *let-7* precursors and block their processing into mature miRNAs (Viswanathan et al. 2008). The hypothalamic expression of *Lin28* and *Lin28b* decreases substantially during prepubertal development in rats and non-human primates with *let-7a* and *let-7b* RNA levels showing an opposite pattern of expression (Sangiao-Alvarellos et al. 2013). These findings suggest that LIN28 may contribute to controlling the pubertal process by down regulating miRNAs potentially involved in silencing inhibitors of puberty, i.e., acting as a repressor of repressors but at a post-transcriptional level.

Altogether, the aforementioned observations suggest that the timing of mammalian puberty is controlled by overlapping layers of repressive regulation (Lomniczi et al. 2013b) (Fig. 1). They are also consistent with the concept recently put forward by Lomniczi et al. (2013a) that transcriptional repression is a core component of theneuroendocrine circuitry regulating puberty.

Epigenetic Information: An Integrating Mechanism of Reproductive Neuroendocrine Development

The discovery of gene networks controlling puberty raises the question as to the existence of mechanisms able to regulate gene expression in a plastic, dynamic, and coordinated fashion during pre- and peripubertal development. These mechanisms are likely epigenetic, because epigenetic information can not only provide gene-specific gatekeeper functions (Garcia-Bassets et al. 2007) but also has a degree of plasticity able to transiently change gene expression within hours (Miller and Sweatt 2007) and even minutes (Kangaspeska et al. 2008; Metivier et al. 2008). It is now clear that epigenetic information is also essential for a variety of neural functions, including memory formation (Miller and Sweatt 2007), recovery of learning and memory (Fischer et al. 2007), dendritic development (Wu et al. 2007), neuronal and behavioral plasticity (Kumar et al. 2005), estrogen-induced gene expression (Perillo et al. 2008; Subramanian et al. 2008), glial-neuronal interactions (Shen et al. 2008), circadian rhythms (Nakahata et al. 2008), and sexual differentiation of the brain (McCarthy et al. 2009).

Mechanisms of Epigenetic Control

The basic mechanisms of epigenetic information are: (1) DNA methylation and hydroxymethylation, two covalent modifications found in cytosine residues of the dinucleotide sequence CpG (Jaenisch and Bird 2003; Bjornsson et al. 2004), and (2) Chromatin modifications, consisting of post-translational modifications that alter the N-terminus tails of core histones (H2A, H2B, H3, and H4) in nucleosomes (Kouzarides 2007; Khorasanizadeh 2004).

While basal DNA methylation (i.e., the addition of a methyl group to position 5 in the cytosine ring to yield 5-methycytosine, 5-mC) is maintained by DNA methyltransferase1 (DNMT1), de novo methylation is a function of DNMT3a and DNMT3b, which target unmethylated and hemimethylated DNA with similar efficiencies (Jaenisch and Bird 2003). Increased DNA methylation is associated with gene repression; conversely, hypomethylation is associated with transcriptional activation. Consistent with the concept that loss of 5-mC is accompanied by corresponding increases in 5-hydroxymethycytosine (5-hmC), DNA

hydroxymethylation is associated with activation of transcription, as evidenced by the abundance of 5-hmC in euchromatin (i.e., chromatin in the open state) and promoter regions of active genes (Ficz et al. 2011).

In contrast to this relative simplicity, core histones are subjected to several post-translational modifications, including acetylation, methylation, phosphorylation, ubiquitination, and sumoylation (Kouzarides 2007; Jenuwein and Allis 2001). Among these, acetylation by acetyltransferases (HATs), deacetylation by histone deacetylases (HDACs), and methylation by methyltransferases (HMTs) are some of the histone modifications most intimately involved in gene regulation. In general, acetylation is associated with activation of transcription, whereas deacetylation is a hallmark of gene silencing (Kouzarides 2007; Dillon 2006). In addition to histone acetylation, promoter activity is regulated by histones methylated at specific lysine residues. The most common histone modification associated with active transcription is trimethylation of H3 at lysine 4 (H3K4me3; Wang et al. 2008; Berger 2007; Guttman et al. 2009). In contrast, H3 methylation of lysine 9 and 27 (H3K9me and H3K27me) is a feature of silenced genes (Wang et al. 2008; Guttman et al. 2009; Ruthenburg et al. 2007). Adding complexity to this scenario, DNA methylation and histone modifications work in concert to regulate gene expression (Cameron et al. 1999; Cedar and Bergman 2009), with H3K4me2/3 preventing DNA methylation (Ooi et al. 2007; Cedar and Bergman 2009), and H3K9me facilitating DNMT3a and DNMTb recruitment to target genes (Cedar and Bergman 2009). Once recruited, DNMTs associate with HDACs to bring about gene silencing (Rountree et al. 2000; Robertson et al. 2000; Fuks et al. 2000; Burgers et al. 2002). The dynamic nature of these mechanisms make epigenetic information ideally suited to provide temporal and spatial cues, not furnished by DNA sequence, to complex biological processes. The neuroendocrine regulation of puberty is one such process.

Most, if not all, genes presumed to play a role in the hypothalamic control of puberty are decorated with epigenetic marks (http://www.epigenome.org/, http://epigenome.usc.edu/ and http://epigenomegateway.wustl.edu/). Changes in DNA methylation of the GnRH promoter accompany the increase in GnRH expression that occur during embryonic development of nonhuman primates (Kurian et al. 2010), and estrogen-positive feedback results in increases acetylation of histone 3 in the *kiss1* promoter of AVPV kisspeptin neurons (Tomikawa et al. 2012). Nevertheless, it is only recently that the identification of the PcG group as a major epigenetic regulator of puberty (Lomniczi et al. 2013a) has shed light into the potential contribution of epigenetic cues to the neuroendocrine control of the pubertal process. The PcG system is composed of three types of Pc repressive complexes (PRCs) working together to bring about gene silencing. They are known as PRC1, PRC2 and PhoRC (Simon and Kingston 2009; Schwartz and Pirrotta 2007). The PRC1 complex includes a group termed Cbx, because its members contain a highly conserved chromodomain (Cbx) at their amino terminus (Ren et al. 2008; Dietrich et al. 2007; Gil et al. 2004; Scott et al. 2007). The mammalian homologs of Drosophila polycomb proteins are Cbx2, 4, 6, 7, and 8 (Schwartz and Pirrotta 2007). In different cells, the PRC1 complex may contain different *Cbx*

genes (Otte and Kwaks 2003). In mammals, the PRC2 complex includes four core subunits: enhancer of zeste 1 or 2 (EZH1, EZH2), suppressor of zeste (Suz12), and the WD40 domain proteins EED and P55 (Schwartz and Pirrotta 2007; Di and Helin 2013). In Droshophila, the PhoRC complex contains two proteins, Pho and its homologue Phol, which bind directly to DNA (Schwartz and Pirrotta 2007). In mammals, these proteins are replaced by YY1, which has both repressive and activating functions (Shrivastava and Calame 1994; Wilkinson et al. 2006; Thomas and Seto 1999). It is now clear that PcG-mediated gene silencing requires H3K27me3; while PRC2 initiates silencing by methylating H3 at K27, the Cbx components of PRC1 maintain the silenced state by binding to H3K27me3 via their chromodomain (Schwartz and Pirrotta 2007; Otte and Kwaks 2003; Simon and Kingston 2009) to form a repressive complex (Simon and Kingston 2009; Schwartz and Pirrotta 2007). Yy1 recruits PRC2 and PRC1 proteins, in addition to H3K27me3 to gene promoters to silence transcription (Wilkinson et al. 2006; Woo et al. 2010). A greater complexity of the PcG complex was recently revealed by studies showing the existence of six different non-canonical PRC1 complexes that do not contain CBX proteins (Gao et al. 2012). One of them, containing the protein RYBP (RING1/YY1 binding protein), appears to be critical for PRC1-dependent monoubiquitination of histone 2A at lysine 119 (Tavares et al. 2012). H2AK119ub1 plays a central role in suppressing RNA polymerase activity in bivalent promoters and also appears to prevent H3K4 methylation (Di and Helin 2013), a hallmark of activated genes (Wang et al. 2008; Berger 2007; Guttman et al. 2009).

Lomniczi and colleagues (2013a) examined the hypothalamus at three developmental phases encompassing the juvenile and peripubertal stages of female rat reproductive maturation: early juvenile (day 21), late juvenile (day 28), and the day of the first preovulatory surge of gonadotropins (days 30–36). These ages were selected because day 21 marks the initiation of the juvenile period (Ojeda and Urbanski 1994), day 28 signals the beginning of puberty, and the first preovulatory surge of gonadotropins represents the completion of puberty. In the female rat, the first neuroendocrine manifestation of puberty can be detected in the form of a diurnal change in pulsatile LH release at the end of juvenile development (Urbanski and Ojeda 1985).

Pulsatile GnRH secretion is thought to be driven by KNDy neurons (Wakabayashi et al. 2010; Navarro et al. 2011) of the ARC (Navarro et al. 2011; Lehman et al. 2010). KNDy neurons release NKB, which acts on other KNDy neurons via specific receptors to stimulate kisspeptin release (Wakabayashi et al. 2010; Navarro et al. 2011). NKB and kisspeptin are released periodically, and this oscillatory behavior is determined by a phase-delayed inhibitory feedback of dynorphin on NKB release (Wakabayashi et al. 2010; Navarro et al. 2011). Because of these characteristics, we consider *Kiss1* and *Tac2* to be unique members of a class of "puberty-activating" genes. Using the Kiss1 as the prototype, Lomniczi et al. (2013a) showed that two core components of the PcG complex (*Eed* and *Cbx7)* are expressed in kisspeptin neurons of the ARC, and their encoded proteins are associated with the 5′ flanking region of the *Kiss1* gene. At the end of juvenile

development, the expression of both *Eed* and *Cbx7* decreases coinciding with increased methylation of their promoters. As these changes take place, the association of EED and CBX proteins with the *Kiss1* promoter is reduced. The chromatin status of the *Kiss1* promoter also changes at this time, displaying increased levels of two histone marks (H3K9, 14ac, and H3K4me3) associated with gene activation. The net outcome of these alterations is activation of *Kiss1* transcription, as evidenced by the increase in *Kiss1* expression that occurs at this time. Surprisingly, the content of H3K27me3, a PcG-dependent repressive histone modification (Wang et al. 2008; Ruthenburg et al. 2007) did not decrease significantly at the initiation of puberty (i.e., at the end of juvenile development), but it did so later, on the day of the first preovulatory surge of gonadotropins. This developmental profile suggests that the association of H3K27me3 and H3K4me3 with the *Kiss1* promoter has the features of bivalent domains (Bernstein et al. 2006), that is, the co-existence of repressive and activating histone modifications (Young et al. 2011; Bernstein et al. 2006) in the regulatory region of genes "poised" for activation in response to incoming inputs (Bernstein et al. 2007).

Importantly, inhibition of DNA methylation with 5-azacytidine prevented not only the peripubertal decline in *Cbx7* and *Eed* mRNA expression but also the eviction of CBX7 and EED from the *Kiss1* promoter, the association of activating histone marks with the promoter, and the increase in *Kiss1* expression that occurs at this time of development. It is thus plausible that, when an increase in DNA methylation of PcG promoters fails to occur, EED/CBX7 occupancy of the *Kiss1* promoter persists resulting in diminished accessibility of activating histone marks to the promoter. The increase in H3K4me3 content at the *Kiss1* promoter raises the possibility that an activating complex is recruited as loss of PcG inhibition unfolds. Trithorax proteins appear to be particularly well suited to play this role as they are known to be chromatin-activating factors that antagonize PcG silencing by inducing H3K4 trimethylation and H3 acetylation (Simon and Kingston 2009). As such, they may play an important role in counteracting the repressive effects of PcG on puberty-activating genes. The contribution of Trithorax genes to the epigenetic control of puberty may be significant, especially because mutations in *CHD7* have been associated with hypothalamic hypogonadism in humans (Bianco and Kaiser 2009; Kim et al. 2008). *CHD7* is a gene encoding an ATP-dependent chromatin-remodeling protein that forms part of Trithorax-containing complexes (Schuettengruber et al. 2011).

That PcG-mediated silencing is intimately involved in restraining puberty was made evident by experiments in which EED was overexpressed – via lentivirus-mediated delivery – in the ARC of early juvenile rats to prevent the pubertal decline in EED availability to kisspeptin neurons. These studies showed that the overexpressed protein became associated with the *Kiss1* promoter, resulting in a reduced number of immunoreactive kisspeptin neurons, blunted pulsatile GnRH release, delayed puberty, and disrupted estrous cyclicity. The number of pups delivered by these animals was severely reduced, indicating that, if the repressive influence of the PcG complex on kisspeptin neurons is not lifted, fertility is compromised (Lomniczi et al. 2013a).

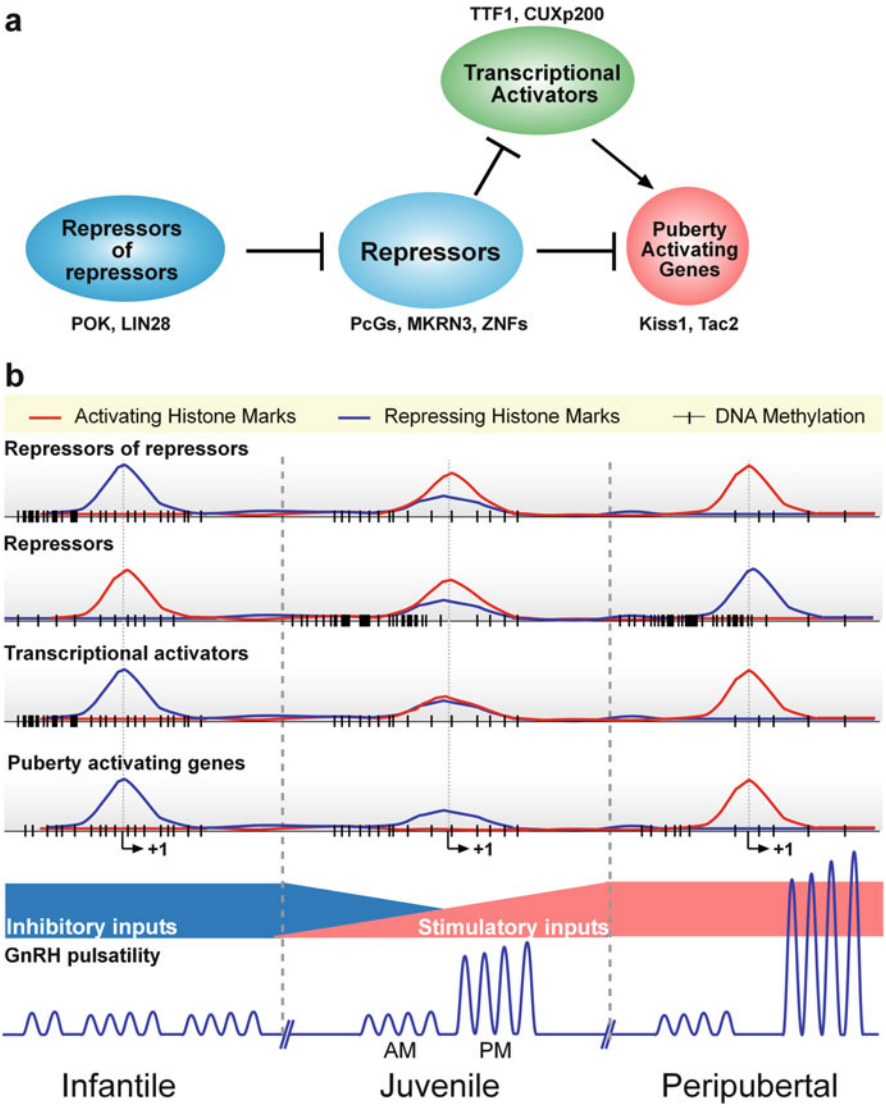

Fig. 1 Hypothetical transcriptional regulatory network controlling the initiation of puberty. (**a**) The diagram illustrates the emerging notion that transcriptional repression is a central component of the mechanism by which the hypothalamus controls the onset of female puberty. According to this view, puberty-activating genes (*Kiss1*, *Tac2*, etc.) are controlled by transcriptional activators (e.g., TTF1, CUXp200) and by a level of repression provided by at least two silencing systems: the PcG complex and members of the ZNF gene family, represented by MKRN3. We also envision that an upstream repressive layer provided by members of the POK family, and perhaps LIN28 (acting postranscriptionally), gives developmental dynamism to the system by repressing the proximal layer of repressors, i.e., acting as repressors of repressors. (**b**) Systems-wide coordination would be achieved by epigenetic cues provided by changes in DNA methylation and the composition of histone posttranslational modifications at each participating gene locus. The profiles depicted illustrate hypothetical changes in histone association (*blue* and *red* profiles) and DNA methylation (*short vertical lines*) around the promoter region of each class of genes. The

Finally, this study by Lomniczi et al. also showed that estradiol (E2) is not responsible for the decrease in *Eed* or *Cbx7* expression that antedates the onset of puberty, because this decrease occurs before the pubertal increase in circulating E2 levels. Although E2 may have activated the transcription of genes repressing PcG expression via estrogen receptor alpha (ERα)-mediated activation of enhancer RNA transcription (Li et al. 2013), there are no canonical estrogen responsive elements (EREs) in the *Eed* or *Cbx* promoter. Earlier findings demonstrated that E2 does not repress but instead induces demethylation of DNA and loss of H3K9me2/3 from E2-target promoters (Kangaspeska et al. 2008; Metivier et al. 2008; Perillo et al. 2008). It is likely, however, that some of the epigenetic modifications affecting puberty-related genes other than PcG genes are caused by E2. In fact, E2-mediated increases in gene transcription do involve epigenetic modifications, such as fluctuations in DNA methylation (Kangaspeska et al. 2008; Metivier et al. 2008), and formation of co-activating complexes containing histone acetyltransferases and histone methyltransferases (Metivier et al. 2003). Expression of ERα itself is regulated by DNA methylation (Issa et al. 1994; Westberry et al. 2008; Belinsky et al. 2002). A seemingly major epigenetic mechanism of E2 action is the ability of E2-ERα complexes to induce demethylation of the inhibitory histone mark H3K9me2/3, resulting in enhanced transcription (Perillo et al. 2008; Garcia-Bassets et al. 2007). Recently, Tomikawa and colleagues demonstrated that E2 increases acetylated H3 content of the *Kiss1* promoter in the AVPV but reduces acetylated H3 in the ARC (Tomikawa et al. 2012). Furthermore, E2 increased ERα binding to the *Kiss1* promoter in the AVPV, but not the ARC. In harmony with the results of Lomniczi et al. (2013a), Tomikawa et al. did not detect changes in *Kiss1* promoter DNA methylation in either the AVPV or ARC, suggesting that DNA methylation may not be involved in regulating *Kiss1* promoter activity. A finding with potentially important implications for the understanding of how puberty-activating genes may be regulated by E2 is the presence of an estrogen-responsive enhancer in the 3′-region of the *Kiss1* gene (Tomikawa et al. 2012). This interaction occurs in the AVPV but not the ARC, consistent with the lack of ERα recruitment to the *Kiss1* locus in the ARC. While these findings provide a blueprint for the epigentic contribution to E2-positive feeback, they leave unresolved the critical questions as to the identity and nature of the mechanism (s) underlying the inhibitory effect of estrogen on ARC *Kiss1* expression.

Notwithstanding the biological significance of these observations, it is likely that changes in epigenetic information affect simultaneously several hierarchically different components of the gene networks controlling puberty. For instance, POK proteins, which we believe to be located at a hierarchical level higher than

Fig. 1 (continued) transcription start site is denoted by the bent arrow followed by +1. The net outcome of these changes would be a switch from inhibitory to stimulatory transcriptional control leading to increased pulsatile GnRH, increased LH secretion, and the initiation of puberty

the PcG complex, recruit HDACs to a repressor complex to silence gene expression (Dhordain et al. 1997; Lin et al. 1998; David et al. 1998), and some POZ-ZF proteins recognize methylated CpG dinucleotides for binding and repression of gene transcription (Daniel et al. 2002; Prokhortchouk et al. 2001).

Concluding Remarks and Perspectives

Two main concepts emerge from the aforementioned observations: (1) that the central control of mammalian puberty depends on the interaction of gene sets organized into functionally connected networks, and (2) that the neuroendocrine control of female puberty involves the participation of a repressive mechanism of epigenetic regulation (Fig. 1). We believe that this silencing influence is exerted at various hierarchical levels within the architecture of gene networks controlling the pubertal process. A proximal repressive layer is provided by members of the Polycomb group of transcriptional silencers. They use epigenetic mechanisms to control downstream "puberty-activating" genes epitomized by the *Kiss1* gene, and their own expression is controlled by changes in DNA methylation. Another set of transcriptional repressors, perhaps targeting the same downstream genes repressed by the PcG complex, is provided by members of the ZNF family of transcriptional silencers, typified by MKRN3, which encodes a RING and zinc-finger containing protein. Finally, the POK family of transcriptional repressors, which shows a preference for methylated regions controlling gene expression, may provide "hard-wiring" to the above networks by acting as both "repressors of repressors" and repressors of puberty-activating genes (Fig. 1).

While these observations lay out the initial scaffold upon which to build a more complete understanding of the epigenetic control of puberty, they also raise a myriad of questions. For instance, do the different neuronal and glial subsets involved in the control of puberty express a different composition of transcriptional repressors? Or do all cells, regardless of their position in the chain of command, express the same repressors – and is the ability of each repressor to interact with relevant DNA regulatory regions what determines the final outcome? If there are cell-specific developmental changes in transcriptional repressor expression, what factors are responsible for those changes? If different puberty-controlling cell populations in the hypothalamus have their own epigenetic landscape, what determines this cellular specificity? And how are the developmental changes in epigenetic landscape determined? No less important is the identity of the specific "inlets" conveying environmental inputs as diverse as nutrition, toxins, light, and social interactions to the hypothalamic epigenome. We envision that future research will not only provide answers to these and other questions but also lead to the identification of specific epigenetic defects underlying the human disorders of idiopathic precocious and delayed puberty.

Acknowledgments This research was supported by grant IOS IOS1121691 from the National Science Foundation (USA).

References

Abreu AP, Dauber A, Macedo DB, Noel SD, Brito VN, Gill JC, Cukier P, Thompson IR, Navarro VM, Gagliardi PC, Rodrigues T, Kochi C, Longui CA, Beckers D, de Zegher F, Montenegro LR, Mendonca BB, Carroll RS, Hirschhorn JN, Latronico AC, Kaiser UB (2013) Central precocious puberty caused by mutations in the imprinted gene MKRN3. N Engl J Med 368:2467–2475

Bedecarrats GY, Kaiser UB (2007) Mutations in the human gonadotropin-releasing hormone receptor: insights into receptor biology and function. Semin Reprod Med 25:368–378

Belinsky SA, Snow SS, Nikula KJ, Finch GL, Tellez CS, Palmisano WA (2002) Aberrant CpG island methylation of the p16(INK4a) and estrogen receptor genes in rat lung tumors induced by particulate carcinogens. Carcinogenesis 23:335–339

Berger SL (2007) The complex language of chromatin regulation during transcription. Nature 447:407–412

Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 125:315–326

Bernstein BE, Meissner A, Lander ES (2007) The mammalian epigenome. Cell 128:669–681

Bianco SD, Kaiser UB (2009) The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. Nat Rev Endocrinol 5:569–576

Bjornsson HT, Fallin MD, Feinberg AP (2004) An integrated epigenetic and genetic approach to common human disease. Trends Genet 20:350–358

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

Burgers WA, Fuks F, Kouzarides T (2002) DNA methyltransferases get connected to chromatin. Trends Genet 18:275–277

Cameron EE, Bachman KE, Myohanen S, Herman JG, Baylin SB (1999) Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. Nat Genet 21:103–107

Cedar H, Bergman Y (2009) Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet 10:295–304

Clarkson J, d'Anglemont de Tassigny X, Colledge WH, Caraty A, Herbison AE (2009) Distribution of kisspeptin neurones in the adult female mouse brain. J Neuroendocrinol 21:673–682

Clasadonte J, Poulain P, Hanchate NK, Corfas G, Ojeda SR, Prevot V (2011) Prostaglandin E2 release from astrocytes triggers gonadotropin-releasing hormone (GnRH) neuron firing via EP2 receptor activation. Proc Natl Acad Sci U S A 108:16104–16109

d'Anglemont de Tassigny X, Colledge WH (2010) The role of kisspeptin signaling in reproduction. Physiology (Bethesda) 25:207–217

Daniel JM, Spring CM, Crawford HC, Reynolds AB, Baig A (2002) The p120(ctn)-binding partner Kaiso is a bi-modal DNA-binding protein that recognizes both a sequence-specific consensus and methylated CpG dinucleotides. Nucleic Acids Res 30:2911–2919

David G, Alland L, Hong SH, Wong CW, Depinho RA, Dejean A (1998) Histone deacetylase associated with mSin3A mediates repression by the acute promyelocytic leukemia-associated PLZF protein. Oncogene 16:2549–2556

de Roux N, Genin E, Carel J-C, Matsuda F, Chaussain J-L, Milgrom E (2003) Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci U S A 100:10972–10976

DeFazio RA, Heger S, Ojeda SR, Moenter SM (2002) Activation of A-type g-aminobutyric acid receptors excites gonadotropin-releasing hormone neurons. Mol Endocrinol 16:2872–2891

Dhordain P, Albagli O, Lin RJ, Ansieau S, Quief S, Leutz A, Kerckaert JP, Evans RM, Leprince D (1997) Corepressor SMRT binds the BTB/POZ repressing domain of the LAZ3/BCL6 oncoprotein. Proc Natl Acad Sci U S A 94:10762–10767

Di CL, Helin K (2013) Transcriptional regulation by Polycomb group proteins. Nat Struct Mol Biol 20:1147–1155

Dietrich N, Bracken AP, Trinh E, Schjerling CK, Koseki H, Rappsilber J, Helin K, Hansen KH (2007) Bypass of senescence by the polycomb group protein CBX8 through direct binding to the INK4A-ARF locus. EMBO J 26:1637–1648

Dillon N (2006) Gene regulation and large-scale chromatin organization in the nucleus. Chromosome Res 14:117–126

Dissen GA, Lomniczi A, Heger S, Neff TL, Ojeda SR (2012) Hypothalamic enhanced at puberty 1 (EAP1) is required for menstrual cyclicity in non-human primates. Endocrinology 153: 350–361

Ducret E, Anderson GM, Herbison AE (2009) RFamide-related peptide-3, a mammalian gonadotropin-inhibitory hormone ortholog, regulates gonadotropin-releasing hormone neuron firing in the mouse. Endocrinology 150:2799–2804

Dudas B, Merchenthaler I (2006) Three-dimensional representation of the neurotransmitter systems of the human hypothalamus: inputs of the gonadotrophin hormone-releasing hormone neuronal system. J Neuroendocrinol 18:79–95

Ebling FJ, Luckman SM (2008) RFAmide-related peptide: another sexy peptide? Endocrinology 149:899–901

Elks CE, Perry JR, Sulem P, Chasman DI, Franceschini N, He C, Lunetta KL, Visser JA, Byrne EM, Cousminer DL, Gudbjartsson DF, Esko T, Feenstra B, Hottenga JJ, Koller DL, Kutalik Z, Lin P, Mangino M, Marongiu M, McArdle PF, Smith AV, Stolk L, van Wingerden SH, Zhao JH, Albrecht E, Corre T, Ingelsson E, Hayward C, Magnusson PK, Smith EN, Ulivi S, Warrington NM, Zgaga L, Alavere H, Amin N, Aspelund T, Bandinelli S, Barroso I, Berenson GS, Bergmann S, Blackburn H, Boerwinkle E, Buring JE, Busonero F, Campbell H, Chanock SJ, Chen W, Cornelis MC, Couper D, Coviello AD, d'Adamo P, de Faire U, de Geus EJ, Deloukas P, Döring A, Smith GD, Easton DF, Eiriksdottir G, Emilsson V, Eriksson J, Ferrucci L, Folsom AR, Foroud T, Garcia M, Gasparini P, Geller F, Gieger C, Giant Consortium, Gudnason V, Hall P, Hankinson SE, Ferreli L, Heath AC, Hernandez DG, Hofman A, Hu FB, Illig T, Järvelin MR, Johnson AD, Karasik D, Khaw KT, Kiel DP, Kilpeläinen TO, Kolcic I, Kraft P, Launer LJ, Laven JS, Li S, Liu J, Levy D, Martin NG, McArdle WL, Melbye M, Mooser V, Murray JC, Murray SS, Nalls MA, Navarro P, Nelis M, Ness AR, Northstone K, Oostra BA, Peacock M, Palmer LJ, Palotie A, Paré G, Parker AN, Pedersen NL, Peltonen L, Pennell CE, Pharoah P, Polasek O, Plump AS, Pouta A, Porcu E, Rafnar T, Rice JP, Ring SM, Rivadeneira F, Rudan I, Sala C, Salomaa V, Sanna S, Schlessinger D, Schork NJ, Scuteri A, Segrè AV, Shuldiner AR, Soranzo N, Sovio U, Srinivasan SR, Strachan DP, Tammesoo ML, Tikkanen E, Toniolo D, Tsui K, Tryggvadottir L, Tyrer J, Uda M, van Dam RM, van Meurs JB, Vollenweider P, Waeber G, Wareham NJ, Waterworth DM, Weedon MN, Wichmann HE, Willemsen G, Wilson JF, Wright AF, Young L, Zhai G, Zhuang WV, Bierut LJ, Boomsma DI, Boyd HA, Crisponi L, Demerath EW, van Duijn CM, Econs MJ, Harris TB, Hunter DJ, Loos RJ, Metspalu A, Montgomery GW, Ridker PM, Spector TD, Streeten EA, Stefansson K, Thorsteinsdottir U, Uitterlinden AG, Widen E, Murabito JM, Ong KK, Murray A (2010) Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. Nat Genet 42:1077–1085

Ficz G, Branco MR, Seisenberger S, Santos F, Krueger F, Hore TA, Marques CJ, Andrews S, Reik W (2011) Dynamic regulation of 5-hydroxymethylcytosine in mouse ES cells and during differentiation. Nature 473:398–402

Filion GJ, Zhenilo S, Salozhin S, Yamada D, Prokhortchouk E, Defossez PA (2006) A family of human zinc finger proteins that bind methylated DNA and repress transcription. Mol Cell Biol 26:169–181

Fischer A, Sananbenesi F, Wang X, Dobbin M, Tsai LH (2007) Recovery of learning and memory is associated with chromatin remodelling. Nature 447:178–182

Fuks F, Burgers WA, Brehm A, Hughes-Davies L, Kouzarides T (2000) DNA methyltransferase Dnmt1 associates with histone deacetylase activity. Nat Genet 24:88–91

Fukusumi S, Fujii R, Hinuma S (2006) Recent advances in mammalian RFamide peptides: the discovery and functional analyses of PrRP, RFRPs and QRFP. Peptides 27:1073–1086

Gao Z, Zhang J, Bonasio R, Strino F, Sawai A, Parisi F, Kluger Y, Reinberg D (2012) PCGF homologs, CBX proteins, and RYBP define functionally distinct PRC1 family complexes. Mol Cell 45:344–356

Garcia-Bassets I, Kwon YS, Telese F, Prefontaine GG, Hutt KR, Cheng CS, Ju BG, Ohgi KA, Wang J, Escoubet-Lozach L, Rose DW, Glass CK, Fu XD, Rosenfeld MG (2007) Histone methylation-dependent mechanisms impose ligand dependency for gene activation by nuclear receptors. Cell 128:505–518

Gibson EM, Humber SA, Jain S, Williams WP III, Zhao S, Bentley GE, Tsutsui K, Kriegsfeld LJ (2008) Alterations in RFamide-related peptide expression are coordinated with the preovulatory luteinizing hormone surge. Endocrinology 149:4958–4969

Gil J, Bernard D, Martinez D, Beach D (2004) Polycomb CBX7 has a unifying role in cellular lifespan. Nat Cell Biol 6:67–72

Gottsch ML, Cunningham MJ, Smith JT, Popa SM, Acohido BV, Crowley WF, Seminara S, Clifton DK, Steiner RA (2004) A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. Endocrinology 145:4073–4077

Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP, Cabili MN, Jaenisch R, Mikkelsen TS, Jacks T, Hacohen N, Bernstein BE, Kellis M, Regev A, Rinn JL, Lander ES (2009) Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 458:223–227

He C, Kraft P, Chen C, Buring JE, Pare G, Hankinson SE, Chanock SJ, Ridker PM, Hunter DJ, Chasman DI (2009) Genome-wide association studies identify loci associated with age at menarche and age at natural menopause. Nat Genet 41:724–728

Heger S, Mastronardi C, Dissen GA, Lomniczi A, Cabrera R, Roth CL, Jung H, Galimi F, Sippell W, Ojeda SR (2007) Enhanced at puberty 1 (EAP1) is a new transcriptional regulator of the female neuroendocrine reproductive axis. J Clin Invest 117:2145–2154

Hinuma S, Shintani Y, Fukusumi S, Iijima N, Matsumoto Y, Hosoya M, Fujii R, Watanabe T, Kikuchi K, Terao Y, Yano T, Yamamoto T, Kawamata Y, Habata Y, Asada M, Kitada C, Kurokawa T, Onda H, Nishimura O, Tanaka M, Ibata Y, Fujino M (2000) New neuropeptides containing carboxy-terminal RFamide and their receptor in mammals. Nat Cell Biol 2:703–708

Hrabovszky E, Ciofi P, Vida B, Horvath MC, Keller E, Caraty A, Bloom SR, Ghatei MA, Dhillo WS, Liposits Z, Kallo I (2010) The kisspeptin system of the human hypothalamus: sexual dimorphism and relationship with gonadotropin-releasing hormone and neurokinin B neurons. Eur J Neurosci 31:1984–1998

Issa JP, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, Baylin SB (1994) Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. Nat Genet 7: 536–540

Jaenisch R, Bird A (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 33(Suppl):245–254

Jenuwein T, Allis CD (2001) Translating the histone code. Science 293:1074–1080

Jong MT, Gray TA, Ji Y, Glenn CC, Saitoh S, Driscoll DJ, Nicholls RD (1999) A novel imprinted gene, encoding a RING zinc-finger protein, and overlapping antisense transcript in the Prader-Willi syndrome critical region. Hum Mol Genet 8:783–793

Kangaspeska S, Stride B, Metivier R, Polycarpou-Schwarz M, Ibberson D, Carmouche RP, Benes V, Gannon F, Reid G (2008) Transient cyclical methylation of promoter DNA. Nature 452:112–115

Kauffman AS, Clifton DK, Steiner RA (2007) Emerging ideas about kisspeptin- GPR54 signaling in the neuroendocrine regulation of reproduction. Trends Neurosci 30:504–511

Kelly KF, Daniel JM (2006) POZ for effect–POZ-ZF transcription factors in cancer and development. Trends Cell Biol 16:578–587

Khan AR, Kauffman AS (2011) The role of Kisspeptin and RFRP-3 neurons in the circadian-timed preovulatory luteinizing hormone surge. J Neuroendocrinol 24:131–143

Khorasanizadeh S (2004) The nucleosome: from genomic organization to genomic regulation. Cell 116:259–272

Kim HG, Kurth I, Lan F, Meliciani I, Wenzel W, Eom SH, Kang GB, Rosenberger G, Tekin M, Ozata M, Bick DP, Sherins RJ, Walker SL, Shi Y, Gusella JF, Layman LC (2008) Mutations in CHD7, encoding a chromatin-remodeling protein, cause idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. Am J Hum Genet 83:511–519

Kohler C, Villar CB (2008) Programming of gene expression by Polycomb group proteins. Trends Cell Biol 18:236–243

Kordon C, Drouva SV, Martínez de la Escalera G, Weiner RI (1994) Role of classic and peptide neuromediators in the neuroendocrine regulation of luteinizing hormone and prolactin. In: Knobil E, Neill JD (eds) The physiology of reproduction, vol 1, 2nd edn. Raven Press, New York, pp 1621–1681

Kotani M, Detheux M, Vandenbogaerde A, Communi D, Vanderwinden JM, Le Poul E, Brezillon S, Tyldesley R, Suarez-Huerta N, Vandeput F, Blanpain C, Schiffmann SN, Vassart G, Parmentier M (2001) The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. J Biol Chem 276: 34631–34636

Kouzarides T (2007) Chromatin modifications and their function. Cell 128:693–705

Kumar A, Choi KH, Renthal W, Tsankova NM, Theobald DE, Truong HT, Russo SJ, Laplant Q, Sasaki TS, Whistler KN, Neve RL, Self DW, Nestler EJ (2005) Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. Neuron 48:303–314

Kurian JR, Keen KL, Terasawa E (2010) Epigenetic changes coincide with in vitro primate GnRH neuronal maturation. Endocrinology 151:5359–5368

Lapatto R, Pallais JC, Zhang D, Chan YM, Mahan A, Cerrato F, Le WW, Hoffman GE, Seminara SB (2007) Kiss1−/− mice exhibit more variable hypogonadism than Gpr54−/− mice. Endocrinology 148:4927–4936

Lehman MN, Coolen LM, Goodman RL (2010) Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. Endocrinology 151:3479–3489

Li W, Notani D, Ma Q, Tanasa B, Nunez E, Chen AY, Merkurjev D, Zhang J, Ohgi K, Song X, Oh S, Kim HS, Glass CK, Rosenfeld MG (2013) Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. Nature 498:516–520

Lin RJ, Nagy L, Inoue S, Shao W, Miller WH Jr, Evans RM (1998) Role of the histone deacetylase complex in acute promyelocytic leukaemia. Nature 391:811–814

Lomniczi A, Ojeda SR (2009) A role for glial cells of the neuroendocrine brain in the central control of female sexual development. In: Parpura V, Haydon P (eds) Astrocytes in (patho) physiology of the nervous system. Springer, New York, pp 487–511

Lomniczi A, Garcia-Rudaz C, Ramakrishnan R, Wilmont B, Khouangsathiene S, Ferguson B, Dissen GA, Ojeda SR (2012) A single nucleotide polymorphism in the *EAP1* gene is associated with amenorrhea/oligomenorrhea in nonhuman primates. Endocrinology 153:339–349

Lomniczi A, Loche A, Castellano JM, Ronnekleiv OK, Bosh M, Kaidar G, Knoll JG, Wright H, Pfeifer GP, Ojeda SR (2013a) Epigenetic control of female puberty. Nat Neurosci 16:281–289

Lomniczi A, Wright H, Castellano JM, Sonmez K, Ojeda SR (2013b) A system biology approach to identify regulatory pathways underlying the neuroendocrine control of female puberty in rats and nonhuman primates. Horm Behav 64:175–186

Maeda T, Hobbs RM, Merghoub T, Guernah I, Zelent A, Cordon-Cardo C, Teruya-Feldstein J, Pandolfi PP (2005) Role of the proto-oncogene Pokemon in cellular transformation and ARF repression. Nature 433:278–285

Mahesh VB, Dhandapani KM, Brann DW (2006) Role of astrocytes in reproduction and neuroprotection. Mol Cell Endocrinol 246:1–9

Mayer C, Boehm U (2011) Female reproductive maturation in the absence of kisspeptin/GPR54 signaling. Nat Neurosci 14:704–710

McCarthy MM, Auger AP, Bale TL, De Vries GJ, Dunn GA, Forger NG, Murray EK, Nugent BM, Schwarz JM, Wilson ME (2009) The epigenetics of sex differences in the brain. J Neurosci 29:12815–12823

Metivier R, Penot G, Hubner MR, Reid G, Brand H, Kos M, Gannon F (2003) Estrogen receptor-alpha directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. Cell 115:751–763

Metivier R, Gallais R, Tiffoche C, Le PC, Jurkowska RZ, Carmouche RP, Ibberson D, Barath P, Demay F, Reid G, Benes V, Jeltsch A, Gannon F, Salbert G (2008) Cyclical DNA methylation of a transcriptionally active promoter. Nature 452:45–50

Miller CA, Sweatt JD (2007) Covalent modification of DNA regulates memory formation. Neuron 53:857–869

Moenter SM, DeFazio RA (2005) Endogenous gamma-aminobutyric acid can excite gonadotropin-releasing hormone neurons. Endocrinology 146:5374–5379

Moss EG, Lee RC, Ambros V (1997) The cold shock domain protein LIN-28 controls developmental timing in C. elegans and is regulated by the lin-4 RNA. Cell 88:637–646

Mueller JK, Dietzel A, Lomniczi A, Loche A, Tefs K, Kiess W, Danne T, Ojeda SR, Heger S (2011) Transcriptional regulation of the human KiSS1 gene. Mol Cell Endocrinol 342:8–19

Mueller JK, Koch I, Lomniczi A, Loche A, Rulfs T, Castellano JM, Kiess W, Ojeda S, Heger S (2012) Transcription of the human EAP1 gene is regulated by upstream components of a puberty-controlling Tumor Suppressor Gene network. Mol Cell Endocrinol 351:184–198

Nakahata Y, Kaluzova M, Grimaldi B, Sahar S, Hirayama J, Chen D, Guarente LP, Sassone-Corsi P (2008) The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. Cell 134:329–340

Navarro VM, Gottsch ML, Chavkin C, Okamura H, Clifton DK, Steiner RA (2009) Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. J Neurosci 29:11859–11866

Navarro VM, Gottsch ML, Wu M, Garcia-Galiano D, Hobbs SJ, Bosch MA, Pinilla L, Clifton DK, Dearth A, Ronnekleiv OK, Braun RE, Palmiter RD, Tena-Sempere M, Alreja M, Steiner RA (2011) Regulation of NKB pathways and their roles in the control of Kiss1 neurons in the arcuate nucleus of the male mouse. Endocrinology 152:4265–4275

Oakley AE, Clifton DK, Steiner RA (2009) Kisspeptin signaling in the brain. Endocr Rev 30: 713–743

Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, Terao Y, Kumano S, Takatsu Y, Masuda Y, Ishibashi Y, Watanabe T, Asada M, Yamada T, Suenaga M, Kitada C, Usuki S, Kurokawa T, Onda H, Nishimura O, Fujino M (2001) Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. Nature 411:613–617

Ojeda SR (1991) The mystery of mammalian puberty: how much more do we know? Perspect Biol Med 34:365–383

Ojeda SR, Skinner MK (2006) Puberty in the rat. In: Neill JD (ed) The physiology of reproduction, 3rd edn. Academic/Elsevier, San Diego, pp 2061–2126

Ojeda SR, Terasawa E (2002) Neuroendocrine regulation of puberty. In: Pfaff D, Arnold A, Etgen A, Fahrbach S, Moss R, Rubin R (eds) Hormones, brain and behavior, vol 4. Elsevier, New York, pp 589–659

Ojeda SR, Urbanski HF (1994) Puberty in the rat. In: Knobil E, Neill JD (eds) The physiology of reproduction, vol 2, 2nd edn. Raven Press, New York, pp 363–409

Ojeda SR, Lomniczi A, Mastronardi C, Heger S, Roth C, Parent AS, Matagne V, Mungenast AE (2006) Minireview: the neuroendocrine regulation of puberty: is the time ripe for a systems biology approach? Endocrinology 147:1166–1174

Ojeda SR, Lomniczi A, Sandau US (2008) Glial-gonadotrophin hormone (GnRH) neurone interactions in the median eminence and the control of GnRH secretion. J Neuroendocrinol 20: 732–742

Ojeda SR, Lomniczi A, Sandau U (2010) Contribution of glial-neuronal interactions to the neuroendocrine control of female puberty. Eur J Neurosci 32:2003–2010

Ong KK, Elks CE, Li S, Zhao JH, Luan J, Andersen LB, Bingham SA, Brage S, Smith GD, Ekelund U, Gillson CJ, Glaser B, Golding J, Hardy R, Khaw KT, Kuh D, Luben R, Marcus M, McGeehin MA, Ness AR, Northstone K, Ring SM, Rubin C, Sims MA, Song K, Strachan DP, Vollenweider P, Waeber G, Waterworth DM, Wong A, Deloukas P, Barroso I, Mooser V, Loos RJ, Wareham NJ (2009) Genetic variation in LIN28B is associated with the timing of puberty. Nat Genet 41:729–733

Ooi SK, Qiu C, Bernstein E, Li K, Jia D, Yang Z, Erdjument-Bromage H, Tempst P, Lin SP, Allis CD, Cheng X, Bestor TH (2007) DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. Nature 448:714–717

Otte AP, Kwaks TH (2003) Gene repression by Polycomb group protein complexes: a distinct complex for every occasion? Curr Opin Genet Dev 13:448–454

Parent AS, Mungenast AE, Lomniczi A, Sandau US, Peles E, Bosch MA, Ronnekleiv OK, Ojeda SR (2007) A contactin-receptor-like protein tyrosine phosphatase beta complex mediates adhesive communication between astroglial cells and gonadotrophin-releasing hormone neurones. J Neuroendocrinol 19:847–859

Parkash J, Kaur G (2005) Neuronal-glial plasticity in gonadotropin-releasing hormone release in adult female rats: role of the polysialylated form of the neural cell adhesion molecule. J Endocrinol 186:397–409

Perera AD, Lagenaur CF, Plant TM (1993) Postnatal expression of polysialic acid-neural cell adhesion molecule in the hypothalamus of the male rhesus monkey (Macaca mulatta). Endocrinology 133:2729–2735

Perillo B, Ombra MN, Bertoni A, Cuozzo C, Sacchetti S, Sasso A, Chiariotti L, Malorni A, Abbondanza C, Avvedimento EV (2008) DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression. Science 319:202–206

Perry JR, Stolk L, Franceschini N, Lunetta KL, Zhai G, McArdle PF, Smith AV, Aspelund T, Bandinelli S, Boerwinkle E, Cherkas L, Eiriksdottir G, Estrada K, Ferrucci L, Folsom AR, Garcia M, Gudnason V, Hofman A, Karasik D, Kiel DP, Launer LJ, van Meurs J, Nalls MA, Rivadeneira F, Shuldiner AR, Singleton A, Soranzo N, Tanaka T, Visser JA, Weedon MN, Wilson SG, Zhuang V, Streeten EA, Harris TB, Murray A, Spector TD, Demerath EW, Uitterlinden AG, Murabito JM (2009) Meta-analysis of genome-wide association data identifies two loci influencing age at menarche. Nat Genet 41:648–650

Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M (2012) Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol Rev 92:1235–1316

Plant TM, Witchel SF (2006) Puberty in nonhuman primates and humans. In: Neill JD (ed) The physiology of reproduction, 3rd edn. Academic/Elsevier, San Diego, pp 2177–2230

Poling MC, Kim J, Dhamija S, Kauffman AS (2012) Development, sex steroid regulation, and phenotypic characterization of RFamide-related peptide (Rfrp) gene expression and RFamide receptors in the mouse hypothalamus. Endocrinology 153:1827–1840

Prevot V (2002) Glial-neuronal-endothelial interactions are involved in the control of GnRH secretion. J Neuroendocrinol 14:247–255

Prokhortchouk A, Hendrich B, Jorgensen H, Ruzov A, Wilm M, Georgiev G, Bird A, Prokhortchouk E (2001) The p120 catenin partner Kaiso is a DNA methylation-dependent transcriptional repressor. Genes Dev 15:1613–1618

Ren X, Vincenz C, Kerppola TK (2008) Changes in the distributions and dynamics of polycomb repressive complexes during embryonic stem cell differentiation. Mol Cell Biol 28:2884–2895

Robertson KD, Ait-Si-Ali S, Yokochi T, Wade PA, Jones PL, Wolffe AP (2000) DNMT1 forms a complex with Rb, E2F1 and HDAC1 and represses transcription from E2F-responsive promoters. Nat Genet 25:338–342

Roth CL, Mastronardi C, Lomniczi A, Wright H, Cabrera R, Mungenast AE, Heger S, Jung H, Dubay C, Ojeda SR (2007) Expression of a tumor-related gene network increases in the mammalian hypothalamus at the time of female puberty. Endocrinology 148:5147–5161

Rountree MR, Bachman KE, Baylin SB (2000) DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. Nat Genet 25:269–277

Ruthenburg AJ, Li H, Patel DJ, Allis CD (2007) Multivalent engagement of chromatin modifications by linked binding modules. Nat Rev Mol Cell Biol 8:983–994

Sandau US, Mungenast AE, Alderman Z, Sardi SP, Fogel AI, Taylor B, Parent AS, Biederer T, Corfas G, Ojeda SR (2011a) SynCAM1, a synaptic adhesion molecule, is expressed in astrocytes and contributes to erbB4 receptor-mediated control of female sexual development. Endocrinology 152:2364–2376

Sandau US, Mungenast AE, McCarthy J, Biederer T, Corfas G, Ojeda SR (2011b) The synaptic cell adhesion molecule, SynCAM1, mediates astrocyte-to-astrocyte and astrocyte-to-GnRH neuron adhesiveness in the mouse hypothalamus. Endocrinology 152:2353–2363

Sangiao-Alvarellos S, Manfredi-Lozano M, Ruiz-Pino F, Navarro V, Sanchez-Garrido MA, Leon S, Dieguez C, Cordido F, Matagne V, Dissen GA, Ojeda SR, Pinilla L, Tena-Sempere M (2013) Changes in hypothalamic expression of the Lin28/let-7 system and related microRNAs during postnatal maturation and after experimental manipulations of puberty. Endocrinology 154: 942–955

Schuettengruber B, Martinez AM, Iovino N, Cavalli G (2011) Trithorax group proteins: switching genes on and keeping them active. Nat Rev Mol Cell Biol 12:799–814

Schwartz YB, Pirrotta V (2007) Polycomb silencing mechanisms and the management of genomic programmes. Nat Rev Genet 8:9–22

Scott CL, Gil J, Hernando E, Teruya-Feldstein J, Narita M, Martinez D, Visakorpi T, Mu D, Cordon-Cardo C, Peters G, Beach D, Lowe SW (2007) Role of the chromobox protein CBX7 in lymphomagenesis. Proc Natl Acad Sci U S A 104:5389–5394

Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA, Gusella JF, O'Rahilly S, Carlton MB, Crowley WF Jr, Aparicio SA, Colledge WH (2003) The GPR54 gene as a regulator of puberty. N Engl J Med 349:1614–1627

Shahab M, Mastronardi C, Seminara SB, Crowley WF, Ojeda SR, Plant TM (2005) Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates. Proc Natl Acad Sci U S A 102:2129–2134

Shannon M, Ashworth LK, Mucenski ML, Lamerdin JE, Branscomb E, Stubbs L (1996) Comparative analysis of a conserved zinc finger gene cluster on human chromosome 19q and mouse chromosome 7. Genomics 33:112–120

Shen S, Sandoval J, Swiss VA, Li J, Dupree J, Franklin RJM, Casaccia-Bonnefil P (2008) Age-dependent epigenetic control of differentiation inhibitors is critical for remyelination efficiency. Nat Neurosci 11:1024–1034

Shrivastava A, Calame K (1994) An analysis of genes regulated by the multi-functional transcriptional regulator Yin Yang-1. Nucleic Acids Res 22:5151–5155

Simon JA, Kingston RE (2009) Mechanisms of polycomb gene silencing: knowns and unknowns. Nat Rev Mol Cell Biol 10:697–708

Smith JT, Popa SM, Clifton DK, Hoffman GE, Steiner RA (2006) Kiss1 neurons in the forebrain as central processors for generating the preovulatory luteinizing hormone surge. J Neurosci 26: 6687–6694

Subramanian K, Jia D, Kapoor-Vazirani P, Powell DR, Collins RE, Sharma D, Peng J, Cheng X, Vertino PM (2008) Regulation of estrogen receptor alpha by the SET7 lysine methyltransferase. Mol Cell 30:336–347

Sulem P, Gudbjartsson DF, Rafnar T, Holm H, Olafsdottir EJ, Olafsdottir GH, Jonsson T, Alexandersen P, Feenstra B, Boyd HA, Aben KK, Verbeek AL, Roeleveld N, Jonasdottir A, Styrkarsdottir U, Steinthorsdottir V, Karason A, Stacey SN, Gudmundsson J, Jakobsdottir M, Thorleifsson G, Hardarson G, Gulcher J, Kong A, Kiemeney LA, Melbye M, Christiansen C, Tryggvadottir L, Thorsteinsdottir U, Stefansson K (2009) Genome-wide association study identifies sequence variants on 6q21 associated with age at menarche. Nat Genet 41:734–738

Sykiotis GP, Pitteloud N, Seminara SB, Kaiser UB, Crowley WF Jr (2010) Deciphering genetic disease in the genomic era: the model of GnRH deficiency. Sci Transl Med 2:32rv2

Tavares L, Dimitrova E, Oxley D, Webster J, Poot R, Demmers J, Bezstarosti K, Taylor S, Ura H, Koide H, Wutz A, Vidal M, Elderkin S, Brockdorff N (2012) RYBP-PRC1 complexes mediate H2A ubiquitylation at polycomb target sites independently of PRC2 and H3K27me3. Cell 148:664–678

Teles MG, Bianco SD, Brito VN, Trarbach EB, Kuohung W, Xu S, Seminara SB, Mendonca BB, Kaiser UB, Latronico AC (2008) A GPR54-activating mutation in a patient with central precocious puberty. N Engl J Med 358:709–715

Terasawa E (1999) Hypothalamic control of the onset of puberty. Curr Opin Endocrinol Diabetes 6:44–49

Terasawa E, Fernandez DL (2001) Neurobiological mechanisms of the onset of puberty in primates. Endocr Rev 22:111–151

Thomas MJ, Seto E (1999) Unlocking the mechanisms of transcription factor YY1: are chromatin modifying enzymes the key? Gene 236:197–208

Tomikawa J, Uenoyama Y, Ozawa M, Fukanuma T, Takase K, Goto T, Abe H, Ieda N, Minabe S, Deura C, Inoue N, Sanbo M, Tomita K, Hirabayashi M, Tanaka S, Imamura T, Okamura H, Maeda K, Tsukamura H (2012) Epigenetic regulation of Kiss1 gene expression mediating estrogen-positive feedback action in the mouse brain. Proc Natl Acad Sci U S A 109: E1294–E1301

Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Ozbek MN, Imamoglu S, Akalin NS, Yuksel B, O'Rahilly S, Semple RK (2008) TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. Nat Genet 41:354–358

Topaloglu AK, Tello JA, Kotan LD, Ozbek MN, Yilmaz MB, Erdogan S, Gurbuz F, Temiz F, Millar RP, Yuksel B (2012) Inactivating KISS1 mutation and hypogonadotropic hypogonadism. N Engl J Med 366:629–635

Tsutsui K, Bentley GE, Bedecarrats G, Osugi T, Ubuka T, Kriegsfeld LJ (2010) Gonadotropin-inhibitory hormone (GnIH) and its control of central and peripheral reproductive function. Front Neuroendocrinol 31:284–295

Urbanski HF, Ojeda SR (1985) The juvenile-peripubertal transition period in the female rat: establishment of a diurnal pattern of pulsatile luteinizing hormone secretion. Endocrinology 117:644–649

Urrutia R (2003) KRAB-containing zinc-finger repressor proteins. Genome Biol 4:231

Viswanathan SR, Daley GQ, Gregory RI (2008) Selective blockade of microRNA processing by Lin28. Science 320:97–100

Viswanathan SR, Powers JT, Einhorn W, Hoshida Y, Ng TL, Toffanin S, O'Sullivan M, Lu J, Phillips LA, Lockhart VL, Shah SP, Tanwar PS, Mermel CH, Beroukhim R, Azam M, Teixeira J, Meyerson M, Hughes TP, Llovet JM, Radich J, Mullighan CG, Golub TR, Sorensen PH, Daley GQ (2009) Lin28 promotes transformation and is associated with advanced human malignancies. Nat Genet 41:843–848

Vogel MJ, Guelen L, de Wit E, Peric-Hupkes D, Loden M, Talhout W, Feenstra M, Abbas B, Classen AK, van Steensel B (2006) Human heterochromatin proteins form large domains containing KRAB-ZNF genes. Genome Res 16:1493–1504

Wakabayashi Y, Nakada T, Murata K, Ohkura S, Mogi K, Navarro VM, Clifton DK, Mori Y, Tsukamura H, Maeda K, Steiner RA, Okamura H (2010) Neurokinin B and dynorphin A in kisspeptin neurons of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat. J Neurosci 30:3124–3132

Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S, Cui K, Roh TY, Peng W, Zhang MQ, Zhao K (2008) Combinatorial patterns of histone acetylations and methylations in the human genome. Nat Genet 40:897–903

Westberry JM, Prewitt AK, Wilson ME (2008) Epigenetic regulation of the estrogen receptor alpha promoter in the cerebral cortex following ischemia in male and female rats. Neuroscience 152: 982–989

Wilkinson FH, Park K, Atchison ML (2006) Polycomb recruitment to DNA in vivo by the YY1 REPO domain. Proc Natl Acad Sci U S A 103:19296–19301

Woo CJ, Kharchenko PV, Daheron L, Park PJ, Kingston RE (2010) A region of the human HOXD cluster that confers polycomb-group responsiveness. Cell 140:99–110

Wu JI, Lessard J, Olave IA, Qiu Z, Ghosh A, Graef IA, Crabtree GR (2007) Regulation of dendritic development by neuron-specific chromatin remodeling complexes. Neuron 56:94–108

Yang JJ, Caligioni CS, Chan YM, Seminara SB (2012) Uncovering novel reproductive defects in neurokinin B receptor null mice: closing the gap between mice and men. Endocrinology 153: 1498–1508

Young MD, Willson TA, Wakefield MJ, Trounson E, Hilton DJ, Blewitt ME, Oshlack A, Majewski IJ (2011) ChIP-seq analysis reveals distinct H3K27me3 profiles that correlate with transcriptional activity. Nucleic Acids Res 39:7415–7427

Neuroendocrine and Molecular Mechanisms for the Metabolic Control of Puberty: Recent Developments

Manuel Tena-Sempere

Abstract Because reproduction is an energy-demanding function, acquisition of reproductive capacity at puberty, especially in the female, is metabolically gated, as a means of preventing fertility in conditions of energy insufficiency. On the other hand, obesity has been shown to also impact the timing of puberty and may be among the causes for the earlier trends of pubertal age reported in various countries, especially in girls but probably also in boys. The metabolic control of puberty is the result of the concerted action of different peripheral hormones and central transmitters that sense the metabolic state of the organism and transmit this information to the various elements of the reproductive brain, ultimately affecting GnRH neurons. Much has been learned in recent decades about the important roles of different neuropeptide pathways that are essential for the control of pulsatile GnRH secretion at puberty and its modulation by numerous physiological (and eventually pathological) signals, including metabolic cues. Remarkably, the essential roles of kisspeptins, the products of the Kiss1 gene that operate through the receptor, Gpr54 (also named Kiss1R), have been recognized in the last few years. In addition, the involvement of other central transmitters and molecular mediators, which may interplay with kisspeptins or operate in a kisspeptin-independent manner, has been unveiled recently by a combination of genetic, neuroanatomical, physiological and clinical studies. In this chapter, we will discuss some recent advances in our understanding of the neuroendocrine and molecular bases of the metabolic control of the onset of puberty. Special emphasis will be paid to summarizing the putative roles of the hypothalamic Kiss1 system in mediating the metabolic modulation of puberty, either via direct or (preferentially) indirect pathways, and to present some of our studies addressing the potential interplay of kisspeptins with other presumable metabolic regulators of puberty. In addition, recent progress in the identification of central molecular mediators, such as mTOR and AMPK, that are putatively involved in the metabolic gating of puberty will be reviewed here. We expect that

M. Tena-Sempere (✉)
Department of Cell Biology, Physiology and Immunology, University of Córdoba; CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Madrid, Spain

Instituto Maimónides de Investigaciones Biomédicas (IMIBIC), Hospital Universitario Reina Sofia, 14004 Córdoba, Spain
e-mail: fi1tesem@uco.es

J.-P. Bourguignon et al. (eds.), *Brain Crosstalk in Puberty and Adolescence*,
Research and Perspectives in Endocrine Interactions 13,
DOI 10.1007/978-3-319-09168-6_9

such information will help to better understand the physiological basis of normal puberty and its eventual perturbations in conditions of metabolic stress, ranging from anorexia to morbid obesity.

Introduction: Metabolic Regulation of Puberty: Physiological and Pathophysiological Implications

Reproduction is an energy-demanding function that, despite being essential for the perpetuation of the species, is dispensable at the individual level. Hence, in extreme conditions that challenge energy homeostasis, other body functions are prioritized while the reproductive axis is suppressed, therefore causing alterations in puberty and/or fertility problems. In this context, it is well known that the onset of puberty is influenced by the magnitude of body energy reserves and different nutritional and metabolic factors (Fernandez-Fernandez et al. 2006; Navarro et al. 2007). Indeed, critical thresholds of fat reserves are required to attain complete pubertal development (Frisch and Revelle 1970), a phenomenon that is especially important in the female because of the substantial energy demands of pregnancy and lactation (Casanueva and Dieguez 1999). However, such a tight association likely occurs in both sexes, and different reports suggest that conditions of metabolic stress can also perturb puberty onset in males (Tena-Sempere 2008). Furthermore, conditions of sustained energy excess, such as morbid obesity, are frequently associated with reproductive alterations, through as yet poorly characterized patho-physiological mechanisms. All in all, a better understanding of the signals and pathways responsible for the metabolic regulation of puberty will help to define the basis for perturbations of puberty in humans, whose incidence seems to be increasing and may have a deleterious impact in health later in life (Lakshman et al. 2009).

Compelling evidence suggests that a substantial component of the mechanisms for the metabolic regulation of puberty takes place at central levels, ultimately by the modulation of the secretory activity of GnRH neurons in the hypothalamus (Elias and Purohit 2012; Navarro and Tena-Sempere 2012; Xu et al. 2012). The central mode of action of various metabolic regulators of puberty has been illustrated by functional genomics analyses, which showed that neuronal elimination of the receptors for insulin or leptin impaired puberty onset (Bruning et al. 2000; Quennell et al. 2009). Likewise, different studies have documented the capacity of leptin, insulin and ghrelin to modulate GnRH secretion in different ex vivo and in vitro settings (Cunningham et al. 1999; Tena-Sempere 2007; Pralong 2010). However, the emerging consensus is that the actions of most of the metabolic hormones influencing puberty are not conducted directly on GnRH neurons but rather take place through intermediary neuronal pathways.

Thus, GnRH neuron-specific knockout of the leptin receptor did not alter the timing of puberty in female mice, which displayed preserved fertility as adults

(Quennell et al. 2009). Similarly, it was recently documented that selective elimination of insulin receptors from GnRH neurons did not cause detectable alterations in the timing of puberty or adult reproductive function in male and female mice (Divall et al. 2010). Admittedly, results from congenital knockout mice should be interpreted with caution because of the possibility of developmental compensatory mechanisms. Yet, the above findings, together with data from expression and functional analyses, strongly suggest that leptin or insulin signaling directly in GnRH neurons is dispensable for pubertal onset, at least in rodents, therefore pointing out the existence of indirect pathways that would operate as conduits for the transmission of key metabolic information to GnRH neurons.

Endocrine Control of Puberty and Its Modulation by Metabolic Cues: Role of Leptin

Since the critical fat mass hypothesis was initially postulated in the 1970s by Frisch and co-workers (Frisch and Revelle 1970), the mechanisms whereby metabolic signals and body energy status influence puberty onset and later fertility have been analyzed thoroughly. Research in this area underwent a complete revolution with the identification, in 1994, and the subsequent characterization of the major biological actions, of the adipose hormone, leptin. Indeed, in the last two decades, leptin has been unanimously recognized not only as a key element in the hormonal control of body weight and energy homeostasis but also as an essential neuroendocrine integrator, linking the magnitude of body fat stores to different neuroendocrine axes, including the reproductive system. While detailed recapitulation of the biological features of leptin is clearly beyond the scope of this chapter, it is important to stress that, given that leptin is secreted in proportion to the amount of white adipose tissue and hence fuel reserves, it operates as a signal for the size of body fat stores to different body systems; thus it is indispensable for proper adjustment of the functioning of numerous physiological systems to changes in metabolic conditions.

Particularly in the context of the reproductive axis, leptin plays a key role in the metabolic control of puberty and fertility (Casanueva and Dieguez 1999; Ahima and Flier 2000; Tena-Sempere 2007). Thus, conditions of leptin insufficiency, as observed in humans with low or null leptin levels as well as in different rodent models (Fernandez-Fernandez et al. 2006; Tena-Sempere 2007), are often linked to a delay in or absence of puberty and perturbed fertility (Roa et al. 2010). Concerning the effects of leptin on puberty onset, there was an initial debate on whether leptin was a trigger or, instead, a permissive signal for puberty to proceed. The consensus derived from different observations in humans and model species indicated that, while threshold leptin levels are required to achieve normal pubertal development (and to retain reproductive function in adulthood), leptin alone is not sufficient per se to trigger complete puberty onset. This finding is compatible with a predominantly permissive function of leptin on puberty, which has been better

characterized in females. Of note, however, leptin deficiency, such as in ob/ob mice, has been linked to reproductive defects in males also (Elias and Purohit 2012), whereas leptin replacement can rescue low testosterone and gonadotropin secretion during short-term fasting in men (Chan et al. 2003). Therefore, it is tenable that leptin plays a key role in the control of puberty in both sexes, albeit with some differences in terms of relative importance vs. other metabolic signals. In any event, recent studies have raised some doubts about the actual roles of leptin in some aspects of the metabolic control of the gonadotropic axis, e.g., the rescue of the hypogonadotropic state following the termination of short-term food deprivation can apparently take place in the absence of detectable changes of circulating leptin levels in rodents and sheep (Szymanski et al. 2007; True et al. 2011). Nevertheless, to our knowledge, no study has challenged the consensus view that proper leptin levels are mandatory for puberty to proceed.

Neuroendocrine Control of Puberty and Its Metabolic Modulation: Role of Kisspeptins

Because of the predominant, if not exclusive, indirect mode of action of metabolic signals in the regulation of GnRH neurons, much attention have been devoted to elucidating the mechanisms whereby different hormones, including leptin, regulate GnRH neurosecretion. Admittedly, different factors are likely to operate via different routes and mechanisms. For the sake of concision, much of the discussion below will focus on the putative pathways whereby leptin, as the key factor for the integral control of energy balance and puberty, transmits its regulatory effects to the elements of the reproductive brain, and how neuronal pathways using kisspeptins as transmitters may participate in this function.

The identification of kisspeptins as gatekeepers of puberty and fertility, back in 2003, was a major breakthrough that has revolutionized our understanding of the neuroendocrine mechanisms that control the reproductive axis. A recapitulation of the major features of kisspeptins, the product of the *Kiss1* gene that operate via the surface receptor, Gpr54 (also termed Kiss1R), is clearly beyond the aims of this chapter; recent extensive reviews of this topic can be found elsewhere (Pinilla et al. 2012). In any event, as a means of introduction to the later contents of this review, some brief account of the major features of kisspeptins as major regulators of puberty and its modulation by metabolic signals is provided below.

The initial observation that patients or mice with null mutations in *Gpr54* or *Kiss1* genes do not undergo puberty already suggested a prominent, indispensable role of kisspeptin signaling in the control of puberty. This function has now been substantiated by a wealth of experimental data indicating that, during puberty, the hypothalamic Kiss1 system undergoes an extensive and complex activational program that seems to be essential for proper pubertal timing (Roa and Tena-Sempere 2010; Tena-Sempere 2010). Key aspects of such developmental activation

during pubertal maturation are (1) a rise in the hypothalamic expression of the *Kiss1* gene and kisspeptin content in key hypothalamic areas; (2) an increase in the sensitivity to the stimulatory effects of kisspeptin in terms of GnRH/LH responses; (3) an increase in the number of GnRH neurons expressing Gpr54, as well as in Gpr54 signaling efficiency; and (4) a rise in the number of kisspeptin neurons and of their projections/appositions to GnRH neurons (Sanchez-Garrido and Tena-Sempere 2013).

In good agreement with the above evidence, pharmacological analyses demonstrated that repeated administration of kisspeptins was sufficient to advance the occurrence of phenotypic or hormonal indices of puberty in rodents and monkeys, whereas blockade of Gpr54 by means of a specific kisspeptin antagonist caused an overt delay in puberty onset in female rats (Pineda et al. 2010). Admittedly, however, recent data have suggested that, under some circumstances, kisspeptin signaling might be dispensable for puberty onset in rodents. It must be stressed, however, that the latter experimental data involved the extensive (albeit probably not complete) congenital ablation of Kiss1 neurons, a condition that is likely to bring about important developmental compensation (Mayer and Boehm 2011). In fact, when similar ablation approaches were applied to early juvenile mice, puberty was overtly altered following selective elimination of Kiss1 cells, confirming that puberty does indeed require the activational contribution of preserved kisspeptin signaling to proceed normally.

Because of their paramount importance in the control of the reproductive axis in general, and of puberty in particular, the possibility that Kiss1 neurons may participate in the metabolic control of puberty has received quite some attention in recent years. Expression and functional data do suggest that Kiss1 neurons are influenced by metabolic cues, a contention that has been mainly documented in conditions of severe negative energy balance, such as acute fasting, chronic subnutrition, uncontrolled diabetes and inflammatory challenge (Castellano et al. 2005, 2006, 2010; Roa et al. 2009). Furthermore, in those conditions, replacement with pharmacological doses of kisspeptins was sufficient to rescue many of the reproductive deficits associated with energy insufficiency. Altogether, these data indirectly support the view that Kiss1 neurons are key sensors of the metabolic state of the organisms and play a key role in transmitting this information, ultimately, to GnRH neurons (Navarro and Tena-Sempere 2012). These observations also raised the obvious question of which metabolic signals are responsible for such control of Kiss 1 neurons; due to its essential roles in the metabolic control of reproduction, leptin was considered to be a tenable candidate (Sanchez-Garrido and Tena-Sempere 2013).

The possibility that leptin might operate as a regulator of Kiss1 neurons was initially supported by reports showing the expression of the mRNA encoding the functional leptin receptor in Kiss1 neurons of the arcuate nucleus (ARC) in the mouse and sheep (Smith et al. 2006; Backholer et al. 2010); however, only a subset of Kiss1 neurons, whose abundance varied from <10 % to ~40 depending on the studies, apparently expressed leptin receptors (LepR) in the mouse (Cravo et al. 2011). In addition, conditions of defective leptin levels are linked to blunted

Kiss1 mRNA expression, especially in the ARC (Smith et al. 2006; Quennell et al. 2011); reduced numbers of kisspeptin neurons in the rostral hypothalamic area have been also reported in situations of hypoleptinemia (Quennell et al. 2011). In turn, leptin administration caused an increase in hypothalamic *Kiss1* gene expression in rodent models of leptin deficiency (Castellano et al. 2006; Smith et al. 2006; Luque et al. 2007; Backholer et al. 2010). In line with these observations, leptin enhanced *Kiss1* mRNA expression in mouse and human neuronal cell lines (Luque et al. 2007; Morelli et al. 2008), and leptin injections in a sheep model of low leptin levels and central hypogonadism due to leanness significantly increased hypothalamic *Kiss1* mRNA expression (Backholer et al. 2010). Furthermore, it has recently been shown that leptin can directly activate ARC Kiss1 neurons in the guinea pig (Qiu et al. 2011). Altogether, these data are fully compatible with (and highly suggestive of) a direct mode of action of leptin in the control of Kiss1 neurons.

Notwithstanding the above evidence, recent data have questioned the hypothesis that direct actions of leptin on Kiss1 neurons are indispensable for its role as a metabolic regulator of puberty and fertility. Thus, congenital elimination of leptin receptors selectively from *Kiss1* cells apparently did not cause alterations in the timing of puberty or adult fertility (Donato et al. 2011). Caution should be exercised when interpreting these data, however, as congenital elimination of leptin receptors from Kiss1 cells might have caused compensatory changes that could mitigate the phenotypic impact of selective elimination of leptin signalling. Yet, additional studies in sheep and rodents did support the possibility of a predominantly indirect mode of action of leptin on Kiss1 neurons. These studies did not find evidence of the presence of functional leptin receptors in Kiss1 (or GnRH) neurons, except for a small population of Kiss1 cells in the ARC (Louis et al. 2011). Intriguingly, however, that work identified a previously unknown neuronal population expressing leptin receptor, located in the ARC and periventricular areas of the rostral hypothalamus (globally termed RP3V); they were found in close proximity to Kiss1 neurons and might contribute to conveying the biological effects of leptin to Kiss1 (and GnRH) neurons (Louis et al. 2011).

All in all, the latter results suggest that a significant fraction of the effects of leptin on the hypothalamic Kiss1 system is conducted indirectly and transmitted via as yet uncharacterized pathways (Louis et al. 2011). These may involve circuits originating from (or projecting to) the ventral pre-mammillary nucleus (PMV), as recent evidence has conclusively documented the indispensable role of PMV pathways in conveying the permissive effects of leptin on puberty onset (Donato et al. 2009, 2011). In fact, recent data have demonstrated alterations of the Kiss1 system following PMV lesions (Donato et al. 2013), suggesting that leptin-sensitive pathways originating from the PMV may impinge on Kiss1 circuits for conveying at least part of the effects of leptin in terms of metabolic gating of puberty. To add further complexity to this phenomenon, it has been recently suggested that leptin can carry out direct effects on Kiss1 neurons but only after pubertal maturation (Cravo et al. 2013); the physiological relevance of this phenomenon in the control of puberty and later fertility awaits further investigation.

Neuropeptide Partners of Kisspeptins and Metabolic Regulation of Puberty: The Role of Neurokinin B

In the last few years, it has been recognized that a group of Kiss1 neurons in the ARC co-express at least two other relevant transmitters involved in the control of the gonadotropic axis, namely, neurokinin B (NKB) and dynorphin (Dyn; Lehman et al. 2010; Pinilla et al. 2012). This subpopulation of ARC Kiss1 neurons has been termed KNDy to recognize such neuropeptide diversity (Lehman et al. 2010). It must be stressed, however, that the percentage of ARC Kiss1 neurons that co-express NKB and/or Dyn seems to vary depending on the species and sex (Hrabovszky et al. 2012); hence, Kiss1-only (and NKB-only) neurons, which are not KNDy neurons, likely exist in the ARC. Nonetheless, the importance of NKB and Dyn as central regulators of the reproductive axis is illustrated not only by their conserved co-expression with kisspeptins in discrete neuronal populations of the ARC in several species, including rodents and primates, but also by the fact that inactivating mutations of the genes encoding NKB (*TAC3*) or its receptor, NK3R (*TAC3R*), in humans are associated with a state of hypogonadotropic hypogonadism (Topaloglu et al. 2009; Gianetti et al. 2010; Young et al. 2010), similar to that caused by defects in kisspeptin signaling.

In addition, compelling pharmacological data support the roles of NKB and Dyn in the central regulation of gonadotropin secretion. Thus, stimulatory actions of the NKB agonist, senktide, on luteinizing hormone (LH) secretion have been reported in different species (Billings et al. 2010; Ramaswamy et al. 2010; Navarro et al. 2011; Garcia-Galiano et al. 2012). In turn, different studies have documented the effects of Dyn as an inhibitor of GnRH/gonadotropin secretion. These functional data, together with the neuroanatomical features of KNDy neurons, which are profusely interconnected by numerous collaterals, provided the grounds for the hypothesis that this neuronal population in the ARC is essential for the generation of GnRH pulses, due to its capacity to produce a very potent stimulatory output signal (kisspeptin) to GnRH neurons. The release of kisspeptins, in turn, would be subjected to the reciprocal control of NKB and Dyn, acting as predominantly stimulatory and inhibitory modulators, respectively (Navarro et al. 2009; Navarro and Tena-Sempere 2012). While this model has been widely accepted and explains the integration of kisspeptins, NKB and Dyn in the dynamic regulation of GnRH/gonadotropin secretion, important functional aspects of the KNDy neuronal network are yet to be fully elucidated, and species differences in the physiological relevance of these neurons in the control of GnRH neurons may exist.

Regarding the metabolic modulation of the reproductive axis, recent studies in models of metabolic challenge, conducted mostly in pubertal rodents, have documented that, just as previously demonstrated for *Kiss1*, the NKB system is also influenced by conditions of negative energy balance. Thus, complete food deprivation, as a means to induce a profound state of energy deficit, caused a concomitant suppression of *Kiss1* mRNA levels, in the ARC and RP3V, and of the hypothalamic expression of the genes encoding NKB and its receptor in

pubertal female rats (Navarro et al. 2012). Of note, in another model of metabolic stress, such as chronic under-nutrition during the pubertal transition, the delay of puberty caused by this state of negative energy balance was partially reversed by chronic injections of the NKB agonist, senktide. Altogether, these findings support the concept that NKB likely cooperates with kisspeptins in the metabolic control of puberty (Navarro et al. 2012). This possibility is fully compatible with the proposed KNDy paradigm, in which NKB would participate in the central regulation of GnRH secretion by increasing the release of kisspeptins (Navarro and Tena-Sempere 2012). Also in keeping with a putative role for NKB signaling in the metabolic regulation of puberty, it was recently shown that high fat diet administered to immature female rats from weaning onwards caused a significant advancement of puberty onset and enhanced the expression not only of *Kiss1* but also of the gene encoding NKB in the ARC (Feng Li et al. 2012). To our knowledge, the physiological roles of the inhibitory signal, Dyn, in the metabolic control of puberty and fertility remain largely unexplored.

Other Neuropeptide Pathways for the Metabolic Control of Puberty: The Case of Nesfatin-1

In addition to kisspeptins and NKB, (many) other central transmitters are involved in the joint control of energy homeostasis, metabolism and the reproductive axis. One prototypical example is nesfatin-1, one of the peptide products encoded by the gene Nucleobinding-2 (*Nucb2*). Nesfatin-1 has been shown to act as an anorectic signal in the hypothalamus (Oh et al. 2006; Garcia-Galiano et al. 2010a). In fact, due to its function as a satiety signal in various species and its expression in hypothalamic areas with key roles in the control of food intake, such as the ARC, the paraventricular nucleus (PVN) and the LHA, nesfatin-1 has been proposed to play a role in the regulation of energy homeostasis (Garcia-Galiano et al. 2010a). In addition, nesfatin-1 has been recently shown to participate in the control of female puberty in the rat and, therefore, might participate in the central networks responsible for the metabolic control of pubertal maturation.

The experimental evidence supporting such a role in puberty derives from expression and functional studies. Thus, hypothalamic NUCB2/nesfatin-1 expression has been shown to increase during pubertal maturation. In contrast, conditions of negative energy balance that suppress or delay puberty onset, e.g., chronic sub-nutrition or short-term fasting, are associated with decreased hypothalamic *NUCB2* mRNA and protein levels in pubertal females (Garcia-Galiano et al. 2010b). In addition, central injections of low (pmol) doses of nesfatin-1 have been reported to induce significant LH secretory responses in peripubertal female rats (Garcia-Galiano et al. 2010b), whereas suppression of the endogenous tone of nesfatin-1 in the hypothalamus during the pubertal transition, by means of

the central infusion of an antisense morfolino oligonucleotide (as-MON) against NUCB2, delayed the timing of puberty and lowered LH levels and ovarian weights.

Interestingly, studies in adult female rats did not detect gonadotropin responses to central injection of nesfatin-1 at doses that were effective in peripubertal rats; neither did infusion of as-MON against NUCB2 affect spontaneous pre-ovulatory surges of LH and FSH (Garcia-Galiano et al. 2010b). However, recent preliminary data indicate that, at higher doses, nesfatin-1 can stimulate LH secretion in adult male rats (Tadross et al. 2010), as well as in adult mice (Navarro et al., unpublished observations). Altogether, these features strongly suggest that nesfatin-1 might operate as a putative effector for the metabolic regulation of puberty and gonadotropic function (Garcia-Galiano et al. 2010a). In keeping with this view, preliminary data from my laboratory suggest that blockade of endogenous nesfatin-1 tone, by the use of as-MON against NUCB2, induces a suppression of *Kiss1* gene expression in the ARC and RP3V in pubertal female rats, whereas absence of kisspeptin signaling (i.e., in Kiss1r null mice) blocks LH responses to nesfatin-1 (Navarro et al., unpublished observations). The latter finding suggests a putative interplay between nesfatin-1 and kisspeptin pathways in the central control of the HPG axis, which may be relevant for its modulation by metabolic cues.

Molecular Mediators for the Metabolic Control of Puberty

Efforts have also been devoted recently to identifying putative molecular mediators that, acting at central levels, may participate in the metabolic control of puberty. Admittedly, progress in the field is still limited and is mostly restricted to the characterization of potential mediators of leptin actions. Just as illustrative examples, the cases of the energy sensors, mTOR and AMPK, will be briefly summarized below.

The mammalian target of rapamycin (mTOR) and the downstream elements of its signaling cascade are known to play a key role as a metabolic gauge of the cell, linking external (nutrient and hormonal) signals and basic cellular processes (Schmelzle and Hall 2000; Martin and Hall 2005; Wullschleger et al. 2006; Chiang and Abraham 2007; Tsang et al. 2007). In addition, mTOR signaling in the hypothalamus, and specifically the rapamycin-sensitive mTORC1 pathway, has been shown to participate in the control of energy homeostasis at the whole body level (Cota et al. 2006; Woods et al. 2008). Thus, leptin has been reported to modulate the mTOR pathway in the ARC, as an effector mechanism for its anorectic actions (Cota et al. 2006). Admittedly, the roles of hypothalamic mTOR signaling in the control of energy balance are more complex and involve nuclei other than the ARC (Villanueva et al. 2009), where mTOR mediates the metabolic effects of other key hormones, such as ghrelin (Martins et al. 2012). In fact, nutritional and metabolic signals seem to modulate the mTORC1 pathway within the medial basal hypothalamus in a nucleus- and cell-specific manner, with

different (if not opposite) responses depending on the prevailing metabolic status (Villanueva et al. 2009).

Besides its role in energy homeostasis and the transduction of metabolic effects of key factors, such as leptin, hypothalamic mTORC1 signaling seems to play an important role in the control of puberty onset and in transmitting the positive/permissive effects of leptin on the reproductive axis (Roa et al. 2009). Thus, sustained blockade of central mTOR signaling, by means of repeated intracerebral administration of rapamycin, delayed the timing of puberty in female rats, as monitored by vaginal opening, reduced ovarian and uterus weights, perturbed ovarian follicular development and suppressed ovulation. Moreover, central inhibition of mTOR was sufficient to totally block the permissive effects of leptin on puberty onset in female rats subjected to chronic subnutrition, as a model of low endogenous leptin levels. In fact, while leptin was able to rescue the ovulatory failure induced by subnutrition in pubertal females, this effect was completely prevented by simultaneous administration of the mTOR inhibitor, rapamycin, into the brain. These observations strongly suggest a positive role for mTOR in the central control of puberty, which seems to be indispensable for leptin effects on brain centers driving the onset of puberty. Indeed, activation of central mTOR signaling, by means of central administration of L-leucine, stimulated LH secretory responses and partially rescued the state of low gonadotropin levels caused by chronic subnutrition in pubertal female rats (Roa et al. 2009). The available evidence strongly suggests that the positive action of mTOR on the HPG axis stems, at least partially, from its capacity to activate Kiss1 neurons, since persistent inhibition of central mTOR signaling significantly reduced *Kiss1* mRNA expression levels in the ARC and, to a lesser extent, the RP3V. The molecular and neuroanatomical basis for this putative leptin-mTOR-Kiss1 pathway is yet to be fully clarified, and it remains possible that as yet unknown mediators might be involved in linking mTOR signaling and *Kiss1* expression.

Another brain fuel-sensing mechanism that might participate in the central control of the HPG axis is the one involving the AMP-activated protein kinase (AMPK), a member of the metabolite-sensing protein kinase family (Naimi et al. 2009; Canto and Auwerx 2009). AMPK detects changes in the AMP/ATP ratio and hence in the cellular metabolic state. In conditions of energy deficit, when ATP is consumed and excess AMP accumulates in the cell, AMPK becomes activated, thus causing the phosphorylation and inactivation of diverse ATP-consuming metabolic cascades. As described for the mTOR pathway, brain AMPK signaling may be a pivotal regulator of energy balance and food intake. Activation of AMPK stimulates appetite (Kahn et al. 2005; Cota et al. 2007), and leptin has been shown to suppress hypothalamic AMPK activity, whereas ghrelin stimulates it (Andersson et al. 2004). Interestingly, AMPK and mTOR are mutually regulated, e.g., AMPK inactivates mTOR in different cell systems (Inoki et al. 2003; Tsang et al. 2007), and hence these two metabolic cell sensors have been proposed to reciprocally cooperate in the central control of energy homeostasis (Cota et al. 2006).

Whether the above interaction applies also to the metabolic regulation of the HPG axis is an appealing possibility but yet to be experimentally proven. Nonetheless, indirect evidence would support this possibility, as AMPK activation has been reported to suppress GnRH secretion in murine GT1-7 cells in vitro and to perturb the estrous cyclicity, as a proxy marker of ovarian cyclic function, in female rats (Coyral-Castel et al. 2008; Wen et al. 2008). In good agreement, preliminary data from studies conducted in our group, targeting specifically the roles of central AMPK in puberty, strongly suggest that activation of central AMPK signaling delays puberty onset and partially inhibits hypothalamic *Kiss1* expression in the ARC (Roa et al., unpublished observations). Altogether, these observations are compatible with a predominant inhibitory role for AMPK pathways in the central control of reproduction, which is in line with its function as a sensor of energy insufficiency and functional antagonist of mTOR.

Concluding Remarks

Puberty, a major life-changing developmental event with a major impact on growth, reproduction and metabolic health later in life, has been the subject of active clinical and experimental investigation over the last decades. Numerous studies have helped to unveil some of the neuro-hormonal and molecular bases of the close connection between metabolism and body energy stores on the one hand and pubertal maturation on the other. Indeed, in recent years, we have learned much about the reciprocal metabolic and pubertal actions of several peripheral hormones and central transmitters, and we have begun to elucidate the pathways whereby these reciprocal connections take place. In this chapter, we aimed to provide a brief recapitulation of recent developments in the field, with special attention to the identification and functional characterization of novel neuropeptides and molecular effectors for the metabolic regulation of puberty. While this review does not include all of the major recent findings in this area, we believe that the information summarized here will provide a flavor of some of the most active research lines in this area and will endow the reader with a deeper understanding of the central mechanisms that link puberty to the metabolic state of the organism, not only in health but also in disease conditions ranging from anorexia to early-onset morbid obesity, whose prevalence is steadily increasing worldwide.

References

Ahima RS, Flier JS (2000) Leptin. Annu Rev Physiol 62:413–437

Andersson U, Filipsson K, Abbott CR, Woods A, Smith K, Bloom SR, Carling D, Small CJ (2004) AMP-activated protein kinase plays a role in the control of food intake. J Biol Chem 279:12005–12008

Backholer K, Smith JT, Rao A, Pereira A, Iqbal J, Ogawa S, Li Q, Clarke IJ (2010) Kisspeptin cells in the ewe brain respond to leptin and communicate with neuropeptide Y and proopiomelanocortin cells. Endocrinology 151:2233–2243

Billings HJ, Connors JM, Altman SN, Hileman SM, Holaskova I, Lehman MN, McManus CJ, Nestor CC, Jacobs BH, Goodman RL (2010) Neurokinin B acts via the neurokinin-3 receptor in the retrochiasmatic area to stimulate luteinizing hormone secretion in sheep. Endocrinology 151:3836–3846

Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Muller-Wieland D, Kahn CR (2000) Role of brain insulin receptor in control of body weight and reproduction. Science 289:2122–2125

Canto C, Auwerx J (2009) PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. Curr Opin Lipidol 20:98–105

Casanueva FF, Dieguez C (1999) Neuroendocrine regulation and actions of leptin. Front Neuroendocrinol 20:317–363

Castellano JM, Navarro VM, Fernandez-Fernandez R, Nogueiras R, Tovar S, Roa J, Vazquez MJ, Vigo E, Casanueva FF, Aguilar E, Pinilla L, Dieguez C, Tena-Sempere M (2005) Changes in hypothalamic KiSS-1 system and restoration of pubertal activation of the reproductive axis by kisspeptin in undernutrition. Endocrinology 146:3917–3925

Castellano JM, Navarro VM, Fernandez-Fernandez R, Roa J, Vigo E, Pineda R, Dieguez C, Aguilar E, Pinilla L, Tena-Sempere M (2006) Expression of hypothalamic KiSS-1 system and rescue of defective gonadotropic responses by kisspeptin in streptozotocin-induced diabetic male rats. Diabetes 55:2602–2610

Castellano JM, Bentsen AH, Romero M, Pineda R, Ruiz-Pino F, Garcia-Galiano D, Sanchez-Garrido MA, Pinilla L, Mikkelsen JD, Tena-Sempere M (2010) Acute inflammation reduces kisspeptin immunoreactivity at the arcuate nucleus and decreases responsiveness to kisspeptin independently of its anorectic effects. Am J Physiol Endocrinol Metab 299:E54–E61

Chan JL, Heist K, DePaoli AM, Veldhuis JD, Mantzoros CS (2003) The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. J Clin Invest 111:1409–1421

Chiang GG, Abraham RT (2007) Targeting the mTOR signaling network in cancer. Trends Mol Med 13:433–442

Cota D, Proulx K, Smith KA, Kozma SC, Thomas G, Woods SC, Seeley RJ (2006) Hypothalamic mTOR signaling regulates food intake. Science 312:927–930

Cota D, Proulx K, Seeley RJ (2007) The role of CNS fuel sensing in energy and glucose regulation. Gastroenterology 132:2158–2168

Coyral-Castel S, Tosca L, Ferreira G, Jeanpierre E, Rame C, Lomet D, Caraty A, Monget P, Chabrolle C, Dupont J (2008) The effect of AMP-activated kinase activation on gonadotrophin-releasing hormone secretion in GT1-7 cells and its potential role in hypothalamic regulation of the oestrous cyclicity in rats. J Neuroendocrinol 20:335–346

Cravo RM, Margatho LO, Osborne-Lawrence S, Donato J Jr, Atkin S, Bookout AL, Rovinsky S, Frazao R, Lee CE, Gautron L, Zigman JM, Elias CF (2011) Characterization of Kiss1 neurons using transgenic mouse models. Neuroscience 173:37–56

Cravo RM, Frazao R, Perello M, Osborne-Lawrence S, Williams KW, Zigman JM, Vianna C, Elias CF (2013) Leptin signaling in kiss1 neurons arises after pubertal development. PLoS One 8:e58698

Cunningham MJ, Clifton DK, Steiner RA (1999) Leptin's actions on the reproductive axis: perspectives and mechanisms. Biol Reprod 60:216–222

Divall SA, Williams TR, Carver SE, Koch L, Bruning JC, Kahn CR, Wondisford F, Radovick S, Wolfe A (2010) Divergent roles of growth factors in the GnRH regulation of puberty in mice. J Clin Invest 120:2900–2909

Donato J Jr, Silva RJ, Sita LV, Lee S, Lee C, Lacchini S, Bittencourt JC, Franci CR, Canteras NS, Elias CF (2009) The ventral premammillary nucleus links fasting-induced changes in leptin levels and coordinated luteinizing hormone secretion. J Neurosci 29:5240–5250

Donato J Jr, Cravo RM, Frazao R, Gautron L, Scott MM, Lachey J, Castro IA, Margatho LO, Lee S, Lee C, Richardson JA, Friedman J, Chua S Jr, Coppari R, Zigman JM, Elmquist JK, Elias CF (2011) Leptin's effect on puberty in mice is relayed by the ventral premammillary nucleus and does not require signaling in Kiss1 neurons. J Clin Invest 121:355–368

Donato J Jr, Lee C, Ratra D, Franci CR, Canteras NS, Elias CF (2013) Lesions of the ventral premammillary nucleus disrupt the dynamic changes in Kiss1 and GnRH expression characteristic of the proestrus-estrus transition. Neuroscience 241:67–79

Elias CF, Purohit D (2012) Leptin signaling and circuits in puberty and fertility. Cell Mol Life Sci 70:841–862

Feng Li X, Lin YS, Kinsey-Jones JS, O'Byrne KT (2012) High-fat diet increases LH pulse frequency and Kisspeptin-Neurokinin B expression in puberty-advanced female rats. Endocrinology 153:4422–4431

Fernandez-Fernandez R, Martini AC, Navarro VM, Castellano JM, Dieguez C, Aguilar E, Pinilla L, Tena-Sempere M (2006) Novel signals for the integration of energy balance and reproduction. Mol Cell Endocrinol 254–255:127–132

Frisch E, Revelle R (1970) Height and weight at menarche and a hypothesis of critical body weights and adolescent events. Science 169:397–399

Garcia-Galiano D, Navarro VM, Gaytan F, Tena-Sempere M (2010a) Expanding roles of NUCB2/nesfatin-1 in neuroendocrine regulation. J Mol Endocrinol 45:281–290

Garcia-Galiano D, Navarro VM, Roa J, Ruiz-Pino F, Sanchez-Garrido MA, Pineda R, Castellano JM, Romero M, Aguilar E, Gaytan F, Dieguez C, Pinilla L, Tena-Sempere M (2010b) The anorexigenic neuropeptide, nesfatin-1, is indispensable for normal puberty onset in the female rat. J Neurosci 30:7783–7792

Garcia-Galiano D, van Ingen Schenau D, Leon S, Krajnc-Franken MA, Manfredi-Lozano M, Romero-Ruiz A, Navarro VM, Gaytan F, van Noort PI, Pinilla L, Blomenrohr M, Tena-Sempere M (2012) Kisspeptin signaling is indispensable for neurokinin B, but not glutamate, stimulation of gonadotropin secretion in mice. Endocrinology 153:316–328

Gianetti E et al (2010) TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood. J Clin Endocrinol Metab 95:2857–2867

Hrabovszky E, Sipos MT, Molnar CS, Ciofi P, Borsay BA, Gergely P, Herczeg L, Bloom SR, Ghatei MA, Dhillo WS, Liposits Z (2012) Low degree of overlap between kisspeptin, neurokinin B, and dynorphin immunoreactivities in the infundibular nucleus of young male human subjects challenges the KNDy neuron concept. Endocrinology 153:4978–4989

Inoki K, Zhu T, Guan KL (2003) TSC2 mediates cellular energy response to control cell growth and survival. Cell 115:577–590

Kahn BB, Alquier T, Carling D, Hardie DG (2005) AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab 1:15–25

Lakshman R, Forouhi NG, Sharp SJ, Luben R, Bingham SA, Khaw KT, Wareham NJ, Ong KK (2009) Early age at menarche associated with cardiovascular disease and mortality. J Clin Endocrinol Metab 94:4953–4960

Lehman MN, Coolen LM, Goodman RL (2010) Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. Endocrinology 151:3479–3489

Louis GW, Greenwald-Yarnell M, Phillips R, Coolen LM, Lehman MN, Myers MG Jr (2011) Molecular mapping of the neural pathways linking leptin to the neuroendocrine reproductive axis. Endocrinology 152:2302–2310

Luque RM, Kineman RD, Tena-Sempere M (2007) Regulation of hypothalamic expression of KiSS-1 and GPR54 genes by metabolic factors: analyses using mouse models and a cell line. Endocrinology 148:4601–4611

Martin DE, Hall MN (2005) The expanding TOR signaling network. Curr Opin Cell Biol 17:158–166

Martins L, Fernandez-Mallo D, Novelle MG, Vazquez MJ, Tena-Sempere M, Nogueiras R, Lopez M, Dieguez C (2012) Hypothalamic mTOR signaling mediates the orexigenic action of ghrelin. PLoS One 7:e46923

Mayer C, Boehm U (2011) Female reproductive maturation in the absence of kisspeptin/GPR54 signaling. Nat Neurosci 14:704–710

Morelli A, Marini M, Mancina R, Luconi M, Vignozzi L, Fibbi B, Filippi S, Pezzatini A, Forti G, Vannelli GB, Maggi M (2008) Sex steroids and leptin regulate the "first Kiss" (KiSS 1/G-protein-coupled receptor 54 system) in human gonadotropin-releasing-hormone-secreting neuroblasts. J Sex Med 5:1097–1113

Naimi M, Arous C, Van Obberghen E (2009) Energetic cell sensors: a key to metabolic homeostasis. Trends Endocrinol Metab 21:75–82

Navarro VM, Tena-Sempere M (2012) Neuroendocrine control by kisspeptins: role in metabolic regulation of fertility. Nat Rev Endocrinol 8:40–53

Navarro VM, Castellano JM, Garcia-Galiano D, Tena-Sempere M (2007) Neuroendocrine factors in the initiation of puberty: the emergent role of kisspeptin. Rev Endocr Metab Disord 8:11–20

Navarro VM, Gottsch ML, Chavkin C, Okamura H, Clifton DK, Steiner RA (2009) Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. J Neurosci 29:11859–11866

Navarro VM, Castellano JM, McConkey SM, Pineda R, Ruiz-Pino F, Pinilla L, Clifton DK, Tena-Sempere M, Steiner RA (2011) Interactions between kisspeptin and neurokinin B in the control of GnRH secretion in the female rat. Am J Physiol Endocrinol Metab 300:E202–E210

Navarro VM, Ruiz-Pino F, Sanchez-Garrido MA, Garcia-Galiano D, Hobbs SJ, Manfredi-Lozano M, Leon S, Sangiao-Alvarello S, Castellano JM, Clifton DK, Pinilla L, Steiner RA, Tena-Sempere M (2012) Role of neurokinin B in the control of female puberty and its modulation by metabolic status. J Neurosci 32:2388–2397

Oh IS, Shimizu H, Satoh T, Okada S, Adachi S, Inoue K, Eguchi H, Yamamoto M, Imaki T, Hashimoto K, Tsuchiya T, Monden T, Horiguchi K, Yamada M, Mori M (2006) Identification of nesfatin-1 as a satiety molecule in the hypothalamus. Nature 443:709–712

Pineda R, Garcia-Galiano D, Roseweir A, Romero M, Sanchez-Garrido MA, Ruiz-Pino F, Morgan K, Pinilla L, Millar RP, Tena-Sempere M (2010) Critical roles of kisspeptins in female puberty and preovulatory gonadotropin surges as revealed by a novel antagonist. Endocrinology 151:722–730

Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M (2012) Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol Rev 92:1235–1316

Pralong FP (2010) Insulin and NPY pathways and the control of GnRH function and puberty onset. Mol Cell Endocrinol 324:82–86

Qiu J, Fang Y, Bosch MA, Ronnekleiv OK, Kelly MJ (2011) Guinea pig kisspeptin neurons are depolarized by leptin via activation of TRPC channels. Endocrinology 152:1503–1514

Quennell JH, Mulligan AC, Tups A, Liu X, Phipps SJ, Kemp CJ, Herbison AE, Grattan DR, Anderson GM (2009) Leptin indirectly regulates gonadotropin-releasing hormone neuronal function. Endocrinology 150:2805–2812

Quennell JH, Howell CS, Roa J, Augustine RA, Grattan DR, Anderson GM (2011) Leptin deficiency and diet-induced obesity reduce hypothalamic kisspeptin expression in mice. Endocrinology 152:1541–1550

Ramaswamy S, Seminara SB, Ali B, Ciofi P, Amin NA, Plant TM (2010) Neurokinin B stimulates GnRH release in the male monkey (Macaca mulatta) and is colocalized with kisspeptin in the arcuate nucleus. Endocrinology 151:4494–4503

Roa J, Tena-Sempere M (2010) Energy balance and puberty onset: emerging role of central mTOR signaling. Trends Endocrinol Metab 21:519–528

Roa J, Garcia-Galiano D, Varela L, Sanchez-Garrido MA, Pineda R, Castellano JM, Ruiz-Pino F, Romero M, Aguilar E, Lopez M, Gaytan F, Dieguez C, Pinilla L, Tena-Sempere M (2009) The mammalian target of rapamycin as novel central regulator of puberty onset via modulation of hypothalamic Kiss1 system. Endocrinology 150:5016–5026

Roa J, Garcia-Galiano D, Castellano JM, Gaytan F, Pinilla L, Tena-Sempere M (2010) Metabolic control of puberty onset: new players, new mechanisms. Mol Cell Endocrinol 324:87–94

Sanchez-Garrido MA, Tena-Sempere M (2013) Metabolic control of puberty: roles of leptin and kisspeptins. Horm Behav 64:187–194

Schmelzle T, Hall MN (2000) TOR, a central controller of cell growth. Cell 103:253–262

Smith JT, Acohido BV, Clifton DK, Steiner RA (2006) KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. J Neuroendocrinol 18:298–303

Szymanski LA, Schneider JE, Friedman MI, Ji H, Kurose Y, Blache D, Rao A, Dunshea FR, Clarke IJ (2007) Changes in insulin, glucose and ketone bodies, but not leptin or body fat content precede restoration of luteinising hormone secretion in ewes. J Neuroendocrinol 19:449–460

Tadross JA, Patterson M, Wynne KJ, Patel S, Suzuki K, Ghatei MA, Bloom SR (2010) Nesfatin suppresses feeding and stimulates the hypothalamo-pituitary-gonadal axis. In: 14th international congress of endocrinology, p S442. Kyoto: Endocrine J.

Tena-Sempere M (2007) Roles of ghrelin and leptin in the control of reproductive function. Neuroendocrinology 86:229–241

Tena-Sempere M (2008) Ghrelin as a pleotrophic modulator of gonadal function and reproduction. Nat Clin Pract Endocrinol Metab 4:666–674

Tena-Sempere M (2010) Roles of Kisspeptins in the control of hypothalamic-gonadotropic function: focus on sexual differentiation and puberty onset. Endocr Dev 17:52–62

Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Ozbek MN, Imamoglu S, Akalin NS, Yuksel B, O'Rahilly S, Semple RK (2009) TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. Nat Genet 41:354–358

True C, Kirigiti MA, Kievit P, Grove KL, Susan Smith M (2011) Leptin is not the critical signal for kisspeptin or luteinising hormone restoration during exit from negative energy balance. J Neuroendocrinol 23:1099–1112

Tsang CK, Qi H, Liu LF, Zheng XF (2007) Targeting mammalian target of rapamycin (mTOR) for health and diseases. Drug Discov Today 12:112–124

Villanueva EC, Munzberg H, Cota D, Leshan RL, Kopp K, Ishida-Takahashi R, Jones JC, Fingar DC, Seeley RJ, Myers MG Jr (2009) Complex regulation of mammalian target of rapamycin complex 1 in the basomedial hypothalamus by leptin and nutritional status. Endocrinology 150:4541–4551

Wen JP, Lv WS, Yang J, Nie AF, Cheng XB, Yang Y, Ge Y, Li XY, Ning G (2008) Globular adiponectin inhibits GnRH secretion from GT1-7 hypothalamic GnRH neurons by induction of hyperpolarization of membrane potential. Biochem Biophys Res Commun 371:756–761

Woods SC, Seeley RJ, Cota D (2008) Regulation of food intake through hypothalamic signaling networks involving mTOR. Annu Rev Nutr 28:295–311

Wullschleger S, Loewith R, Hall MN (2006) TOR signaling in growth and metabolism. Cell 124:471–484

Xu Y, Faulkner LD, Hill JW (2012) Cross-talk between metabolism and reproduction: the role of POMC and SF1 neurons. Front Endocrinol (Lausanne) 2:98

Young J, Bouligand J, Francou B, Raffin-Sanson ML, Gaillez S, Jeanpierre M, Grynberg M, Kamenicky P, Chanson P, Brailly-Tabard S, Guiochon-Mantel A (2010) TAC3 and TACR3 defects cause hypothalamic congenital hypogonadotropic hypogonadism in humans. J Clin Endocrinol Metab 95:2287–2295

Prostaglandin E_2, Gliotransmission and the Onset of Puberty

Vincent Prevot and Jerome Clasadonte

Abstract Over the past four decades it has become clear that prostaglandin E_2 (PGE_2), a phospholipid-derived signaling molecule, plays a fundamental role in modulating the gonadotropin-releasing hormone (GnRH) neuroendocrine system and in shaping the hypothalamus. In this chapter, after a brief historical overview, we highlight studies revealing that PGE_2 released by astrocytes is intimately involved in the active control of GnRH neuronal activity and the acquisition of reproductive competence.

Introduction

Sexual development, puberty and adult fertility are achieved by events that are initiated within the central nervous system and require the maturation and function of a neural network that transmits both homeostatic and external cues to the discrete hypothalamic neuronal population that releases gonadotropin-releasing hormone (GnRH) from neuroendocrine terminals within the median eminence into the pituitary portal vessels to control gonadotropin [luteinizing hormone (LH) and follicle stimulating hormone (FSH)] secretion (Donato et al. 2011; Herbison and Neill 2006; Malpaux 2006; Ojeda and Skinner 2006; Plant 2006; Terasawa and Fernandez 2001). In turn, these gonadotropins act on the ovaries and testis to regulate the secretion of sex steroids and the production of eggs and sperm.

Accumulating evidence over the past two decades has indicated that, in addition to neurons, glial cells, and in particular astrocytes, contribute to the neural network that converges onto GnRH neurons to control reproduction. Both the neuronal and glial elements of this GnRH neural network are subject to the direct modulatory influence of gonadal steroids (Bellefontaine et al. 2011; Christian and Moenter

V. Prevot (✉) • J. Clasadonte
Jean-Pierre Aubert Research Center, U837, Development and Plasticity of the Postnatal Brain, Inserm, Lille, France

School of Medicine, University of Lille, F-59000 Lille, France
e-mail: vincent.prevot@inserm.fr

J.-P. Bourguignon et al. (eds.), *Brain Crosstalk in Puberty and Adolescence*, Research and Perspectives in Endocrine Interactions 13,
DOI 10.1007/978-3-319-09168-6_10

2010; Garcia-Segura and McCarthy 2004; Mong and Blutstein 2006; Prevot et al. 2010a; Ronnekleiv and Kelly 2005; Wintermantel et al. 2006). Although neuronal elements regulate the activity of GnRH neurons through a complex array of excitatory and inhibitory synaptic inputs, glial cells communicate with GnRH neurons via the activation of specific growth factor-dependent signaling pathways (reviewed in Sharif et al. 2013).

The main glial population in the brain consists of astrocytes that ensheathe synapses and are in contact with blood vessels. They regulate blood flow, provide much-needed energy to neurons, and supply the building blocks for neurotransmitters at the synapses, in addition to dynamically contributing to information processing within the central nervous system (Di Castro et al. 2011; Eroglu and Barres 2010; Halassa and Haydon 2010; Haydon and Carmignoto 2006; Iadecola and Nedergaard 2007; Martineau et al. 2006; Panatier et al. 2011; Pfrieger 2010), including the hypothalamus (Gordon et al. 2009; Hatton and Wang 2008; Oliet and Bonfardin 2010; Panatier 2009; Theodosis et al. 2008). As integrative hubs, astrocytes likely play a fundamental role in shaping and regulating the GnRH system.

Here, we will review recent findings that illustrate the remarkable interplay between glia and neurons within the hypothalamo-hypophyseal-gonadal axis. We will mainly restrict our focus to the roles of hypothalamic astrocytes subserved by the release of prostaglandin E_2 (PGE_2), a molecule that has long been known to regulate GnRH neuronal function and has recently been identified as a gliotransmitter.

PGE_2 Is Involved in the Hypothalamic Control of Reproduction

PGE_2 is one of a number of prostanoids synthesized from arachidonic acid, which is produced from membrane phospholipids by a phospholipase A_2. Arachidonic acid is converted to bioactive prostanoids by the cyclooxygenases (COX-1 and COX-2) and a class of terminal synthases (see for review Bosetti 2007). Several studies suggest that PGE_2 is mainly derived from the COX-2 pathway (Brock et al. 1999; Sang et al. 2005; Vidensky et al. 2003). PGE_2 signaling is propagated by four G-protein-coupled receptors, EP1-EP4 (see for review Coleman et al. 1994; Fig. 1).

For more than 35 years, PGE_2 has been known to play a role in the central control of reproduction. The first indication that PGE_2 was involved in the process of GnRH secretion was provided by experiments showing that, when PGE_2 was injected into the third ventricle of the rat brain, it induced the release of LH into the general circulation (Harms et al. 1973) and the release of GnRH into the pituitary portal blood vessels (Eskay et al. 1975; Ojeda et al. 1975b). A similar stimulatory effect of PGE2 on GnRH release has been documented using push-pull perfusion in conscious monkeys (Gearing and Terasawa 1991). To bring about the activation of the GnRH axis, PGE_2 acts at two main hypothalamic sites: the preoptic-anterior

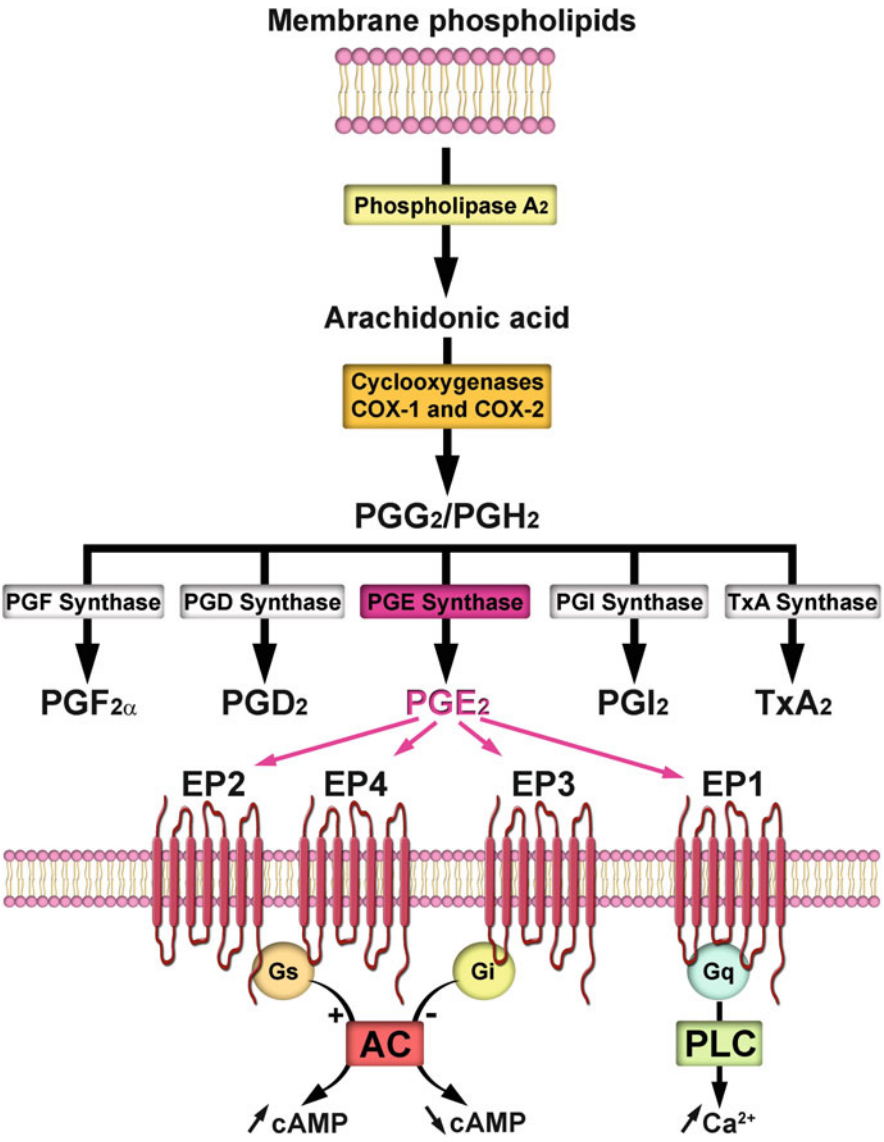

Fig. 1 Prostaglandin E2 (*PGE2*) biosynthesis and signaling. Upon its release from plasma membrane phospholipids by phospholipase A2, arachidonic acid is converted to the unstable endoperoxide intermediates, prostaglandin G2 (*PGG2*) and prostaglandin H2 (*PGH2*), by the cyclooxygenases (COX-1 and COX-2, encoded by separate genes). Both COX isoforms catalyze the same reactions, but while COX-1 is constitutively expressed, COX-2 is rapidly and transiently upregulated by cytokines and growth factors. Terminal synthases convert both PGG2 and PGH2 into prostaglandins [PGE2, PGD2, PGF2α, prostacyclin (PGI2)], and thromboxane (TxA2). Once synthesized, PGE2 immediately diffuses away and activates its specific E-prostanoid receptors (EP1-4), which belong to the family of 7-transmembrane G-protein-coupled receptors. EP2 and EP4 are coupled to Gs and stimulate the adenylyl cyclase (*AC*)-cyclic adenosine monophosphate (*cAMP*)-protein kinase A (*PKA*) pathway. In contrast, EP3 is coupled to Gi and inhibits AC activation, resulting in decreased cAMP concentrations. EP1 is thought to be coupled to the

hypothalamic region in which GnRH cell bodies reside and the tuberal region of the hypothalamus, which contains the median eminence and GnRH-releasing neuroendocrine terminals (Ojeda et al. 1977). The use of COX inhibitors such as indomethacin has provided further support for a physiological role of the prostaglandins in the control of GnRH release. Indomethacin administration suppresses the LH surge induced by estradiol during anestrus in ewes (Carlson et al. 1974) and during the early follicular phase in rhesus monkeys (Carlson et al. 1977). In rats, the intraventricular or intrahypothalamic administration of indomethacin inhibits both pulsatile LH release and the LH discharge induced by ovarian steroids (Ojeda et al. 1975a). Other studies have demonstrated that the microinjection of either aspirin, a non-steroidal COX inhibitor, or N-0164, a prostaglandin and thromboxane antagonist, into the tuberal region of the rat hypothalamus results in the suppression of ovulation (Botting et al. 1977; Labhsetwar and Zolovick 1973). Finally, experiments conducted using hypothalamic explants in vitro have revealed that PGE_2 is an effective stimulator of GnRH release from median eminence nerve terminals (Gallardo and Ramirez 1977; Ojeda et al. 1979, 1986b).

Evidence implicating PGE_2 as a physiological component of the GnRH system during postnatal development arises from findings showing that PGE_2 can induce the release of GnRH long before puberty in both mice and rats (Ojeda et al. 1986a; Prevot et al. 2003). As puberty approaches, the increasing output of estradiol from the developing ovaries induces a preovulatory surge of GnRH/LH. Biochemical analyses at this last phase of sexual maturation have demonstrated that the capacity of the reproductive hypothalamus to metabolize arachidonic acid through the COX pathway leads to a specific increase in PGE_2 synthesis (Fig. 1), particularly during the first proestrus (Ojeda and Campbell 1982). This effect appears to be estrogen-dependent since it is mimicked by the treatment of juvenile animals (early post-weaning period) with estradiol at doses capable of inducing a preovulatory surge of LH (Ojeda and Campbell 1982). More recent studies have shown that an estradiol-induced increase in hypothalamic PGE_2 levels can be seen even in newborn rats (Amateau and McCarthy 2002). Intriguingly, experiments showing that estradiol treatment upregulates both COX-2 mRNA and protein synthesis in the hypothalamus of female rats during postnatal development (Amateau and McCarthy 2004) raise the possibility that estrogens may act on COX-2 expression to promote PGE_2 synthesis at puberty.

Fig. 1 (continued) Gq-phospholipase C (*PLC*) pathway, leading to an elevation of free cytosolic calcium concentrations (Milatovic et al. 2011). Notably, an examination of the capacity of the hypothalamus to metabolize arachidonic acid through the COX pathway has revealed a pubertal increase in the formation of PGE2, particularly during the first proestrus (Ojeda and Campbell 1982). Intriguingly, the increase in PGE2 synthesis is not associated with changes in the formation of PGF2α, PGI2, PGD2 or thromboxane A2 from exogenous arachidonic acid, suggesting that it is a specific event directly associated with the peripubertal activation of the reproductive hypothalamus (Ojeda and Campbell 1982). Such a selective synthesis of PGE2 has also been shown to be triggered by estrogens during early postnatal development (Amateau and McCarthy 2002)

Astrocytes Appear to Be the Main Source of PGE_2 in the GnRH Neuroendocrine System

Although PGE_2 was initially postulated to be an intracellular messenger produced by the binding of neurotransmitters to receptors located on GnRH neurons and acting within these neurons (Gearing and Terasawa 1991; Ojeda et al. 1982; Rettori et al. 1992), this concept has been revisited following studies showing that the actions of PGE_2 on GnRH release are initiated by its binding to specific membrane receptors (Coleman et al. 1994) expressed by GnRH neurons (Rage et al. 1997) and the recognition that astrocytes represent a major source of PGE_2 in the brain (Bezzi et al. 1998; Hirst et al. 1999; Ma et al. 1997). Two decades ago, seminal studies by Ojeda and colleagues revealed that the PGE_2-mediated activation of GnRH neuronal secretory activity triggered by estrogen at the time of puberty required the activation of growth factor-dependent glial signaling pathways involving receptor tyrosine kinases of the erbB family (Junier et al. 1991; Ma et al. 1992; Ojeda et al. 1990).

Of the four known members of the erbB family (Fig. 2), three – erbB1, erbB3 and erbB4 – bind and are activated by cognate ligands. In contrast, erbB2 has no known ligand, and functions primarily as a modulator of the other members of the family (Hynes and Lane 2005). While erbB receptors do not appear to be expressed

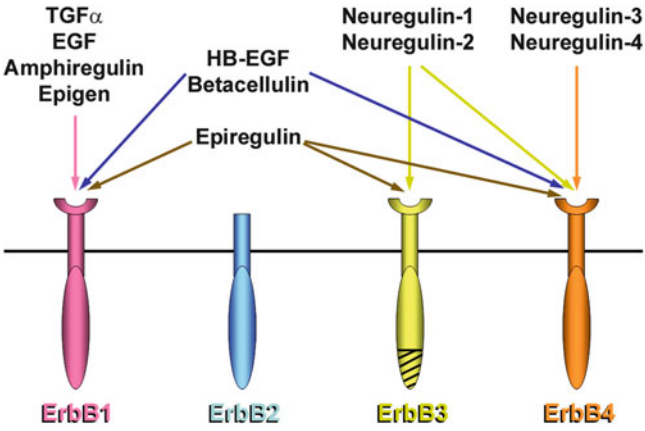

Fig. 2 The erbB family of receptors and their ligands. ErbB1 (or EGFR, epidermal growth factor receptor) and erbB4 are fully functional receptors that possess an extracellular ligand-binding domain and a cytoplasmic protein tyrosine kinase domain and can function as homo- or heterodimers. In contrast, erbB2 (or neu), which lacks a ligand-binding domain, and erbB3, which is defective in its intrinsic tyrosine kinase activity (*dashed lines*), must heterodimerize with another member of the erbB family for signal transduction. The different EGF-like growth factors exhibit different binding specificities for the erbB receptors. While TGFα, EGF, amphiregulin, epigen, neuregulin-3 and neuregulin-4 are specific for a single member of the receptor family, the five other EGF-like ligands can bind two or three receptors each. *EGF* epidermal growth factor, *HB-EGF* heparin binding-EGF, *TGFα* transforming growth factor α

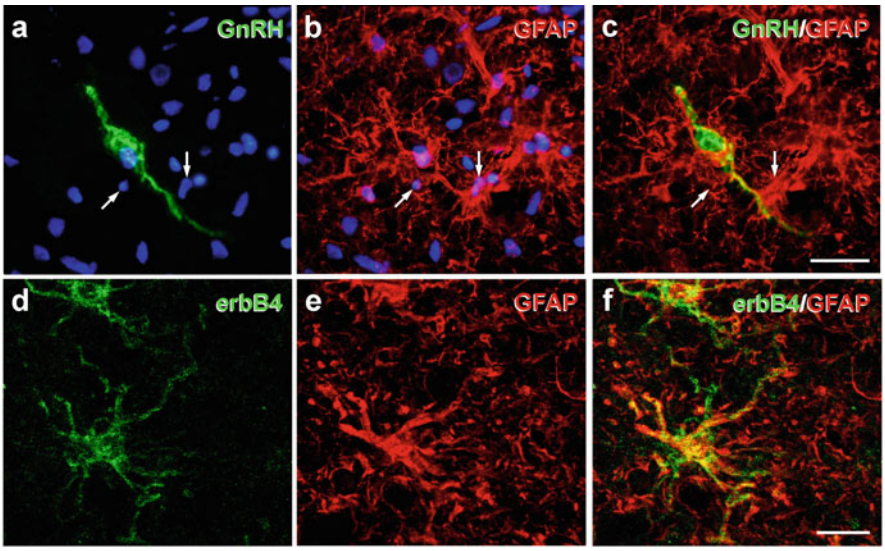

Fig. 3 Astrocytes morphologically interact with GnRH neurons and express erbB4 receptors in the tuberal region of the human hypothalamus. (**a**–**c**) Photomicrographs showing a GnRH neuronal cell body (*green*) to which the processes of glial fibrillary acidic protein (*GFAP*)-immunoreactive astrocytes (*red*, *arrows*) are abundantly apposed. Cell nuclei are stained with Hoechst (*blue*) (Adapted from Baroncini et al. 2007 with permission). (**d**–**f**) GFAP-immunoreactive astrocytes (*red*) of the tuberal region of the human hypothalamus express erbB4 receptors (*green*) (M. Baroncini, V. Prevot, unpublished data). Scale bars = 20 μm (**c**), 10 μm (**f**)

in GnRH neurons Ma et al. 1994b, 1999; Prevot et al. 2003; Voigt et al. 1996), erbB1, erbB2 and erbB4, but not erbB3, are expressed in hypothalamic astrocytes, known to morphologically and physically interact with GnRH cell bodies (Baroncini et al. 2007; Cashion et al. 2003; Sandau et al. 2011b; Witkin et al. 1995) both in rodents and humans (Figs 3 and 4; Ma et al. 1999; Prevot et al. 2003; Sharif et al. 2009). In addition, hypothalamic astrocytes express the erbB1 ligand, transforming growth factor alpha (TGFα; Fig. 4) and several forms of the erbB4 ligand, neuregulin (Ma et al. 1992, 1994a, 1999; Sharif et al. 2009). Importantly, gonadal steroids have been found to induce dramatic increases in the expression levels of the erbB receptors and their ligands within the hypothalamus at puberty; no such changes are seen in the cortex or other brain regions unrelated to reproductive control (Ma et al. 1992, 1994b, 1999).

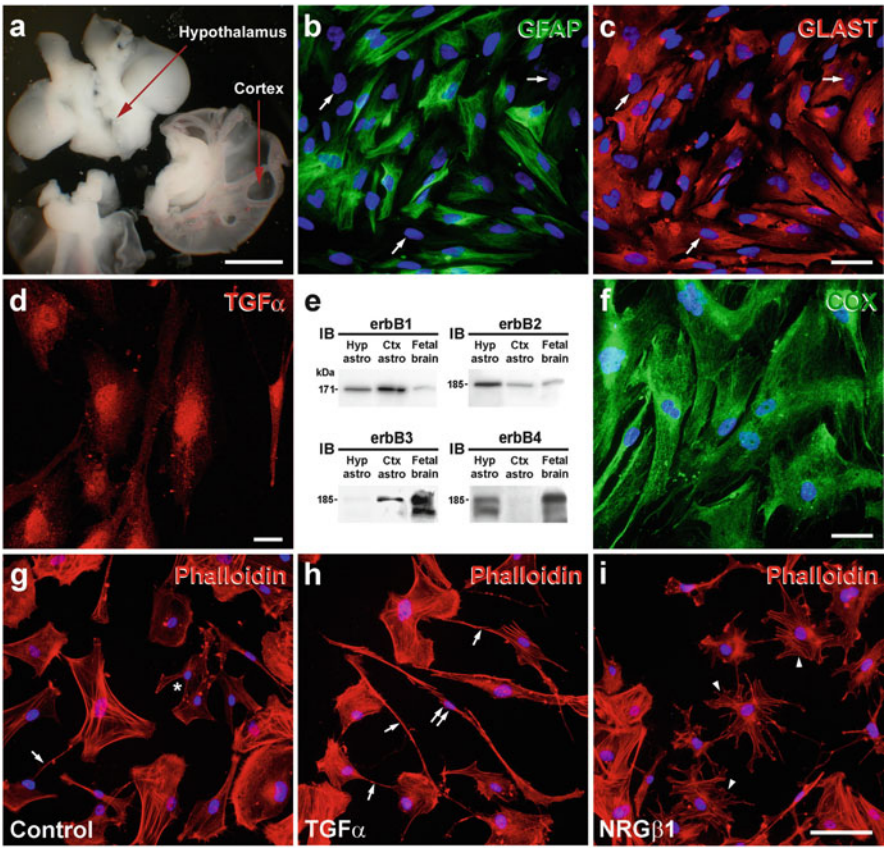

Fig. 4 Human hypothalamic astrocytes express the molecular components required for a glia-to-neuron communication through the erbB-prostaglandin signaling system. Primary cultures of human hypothalamic astrocytes prepared from 9- to 12-week-old human fetuses (**a**). The cultures are composed 98 % of cells immunopositive for the astrocytic markers GFAP (**b**, *green*) and the glutamate-aspartate transporter GLAST (**c**, *red*). Note that cells that express GFAP at low to undetectable levels are nevertheless strongly immunopositive for GLAST (*arrows*). (**d**) Human astrocytes in culture express TGFα protein (*red*). (**e**) Western blot analysis of erbB receptor expression in primary cultures of human cortical and hypothalamic astrocytes. While all four erbB receptors are expressed in the fetal brain, hypothalamic astrocytes (Hyp astro) express erbB1, erbB2, and erbB4, but not erbB3, and cortical astrocytes (Ctx astro) express erbB1, erbB2, and erbB3 but not erbB4 receptors. IB, immunoblot. (**f**) Human hypothalamic astrocytes in culture are immunopositive for COX (*green*). (**g–i**) EGF ligands induce profound morphological rearrangements of human hypothalamic astrocytes in vitro. Cell morphology was examined by visualization of the actin cytoskeleton using Alexafluor 568-conjugated phalloidin (*red*). Hypothalamic astrocytes exhibit heterogeneous shapes under control conditions, i.e., polygonal cells, cells with short and thick extensions (*asterisk*) or long and thin processes (*arrow*) (**g**). TGFα (50 ng/mL for 3 days) stimulates the extension of long and thin processes (*arrows*) and the apparition of bipolar cells (*double arrows*) (**h**) whereas treatment with the neuregulin-1 HRGβ1 (50 ng/mL for 3 days) increases the number of multipolar cells with thick processes (*arrowheads*). (**i**) Nuclei are counter-stained with Hoechst (**b**, **c**, **f**, **g**, **h**, **i**, *blue*). Scale bars = 3 mm (**a**), 50 μm (**b**, **c**, **f**), 20 μm (**d**), 100 μm (**g–i**) (Adapted from Sharif et al. 2009 with permission)

The pharmacological or genetic inhibition of erbB1, ebB2 and/or erbB4 receptors delays the onset of puberty (Apostolakis et al. 2000; Ma et al. 1992; Prevot et al. 2003, 2005) and alters adult reproductive function in rodents (Prevot et al. 2005). In vitro studies using either hypothalamic explants or primary cultures of hypothalamic astrocytes with a GnRH-producing neuronal cell line have shown that erbB receptor ligands can stimulate GnRH release from the explants or neuronal cells, but do so indirectly by inducing astrocytes to secrete PGE_2 (Ma et al. 1997, 1999; Ojeda et al. 1990; Prevot et al. 2003, 2005). In addition, ligand activation of erbB receptors has been shown to promote morphological rearrangements in hypothalamic astrocytes (Fig. 4g–i; Sharif et al. 2009) thus raising the possibility that erbB signaling may also influence the astrocytic coverage of GnRH neurons in vivo (see for review Prevot et al. 2010b).

In vitro experiments suggest that erbB signalling in hypothalamic astrocytes is functionally connected to the neuronal glutamatergic system, the primary mode of excitatory transsynaptic communication used by hypothalamic neurons (van den Pol and Trombley 1993), and one that is known to increase GnRH secretion (Claypool et al. 2000; Donoso et al. 1990) and accelerate the initiation of puberty in both rodents and primates (Plant et al. 1989; Urbanski and Ojeda 1987, 1990). In hypothalamic and non-hypothalamic astrocytes alike (Bezzi et al. 1998; Zonta et al. 2003a, b), transmitter spillover from nearby synaptic activity results in an elevation of PGE_2 release (Glanowska and Moenter 2011; McCarthy et al. 2008). For example, neuronally released glutamate can engage biochemical signaling in astrocytes through the co-activation of AMPA and metabotropic glutamate receptors to cause a ligand-dependent increase in astrocytic erbB signaling and PGE_2 release (Dziedzic et al. 2003), which, in turn, signals back to GnRH neurons (Fig. 5), facilitating neuroendocrine development and adult reproductive function (Prevot et al. 2003, 2005).

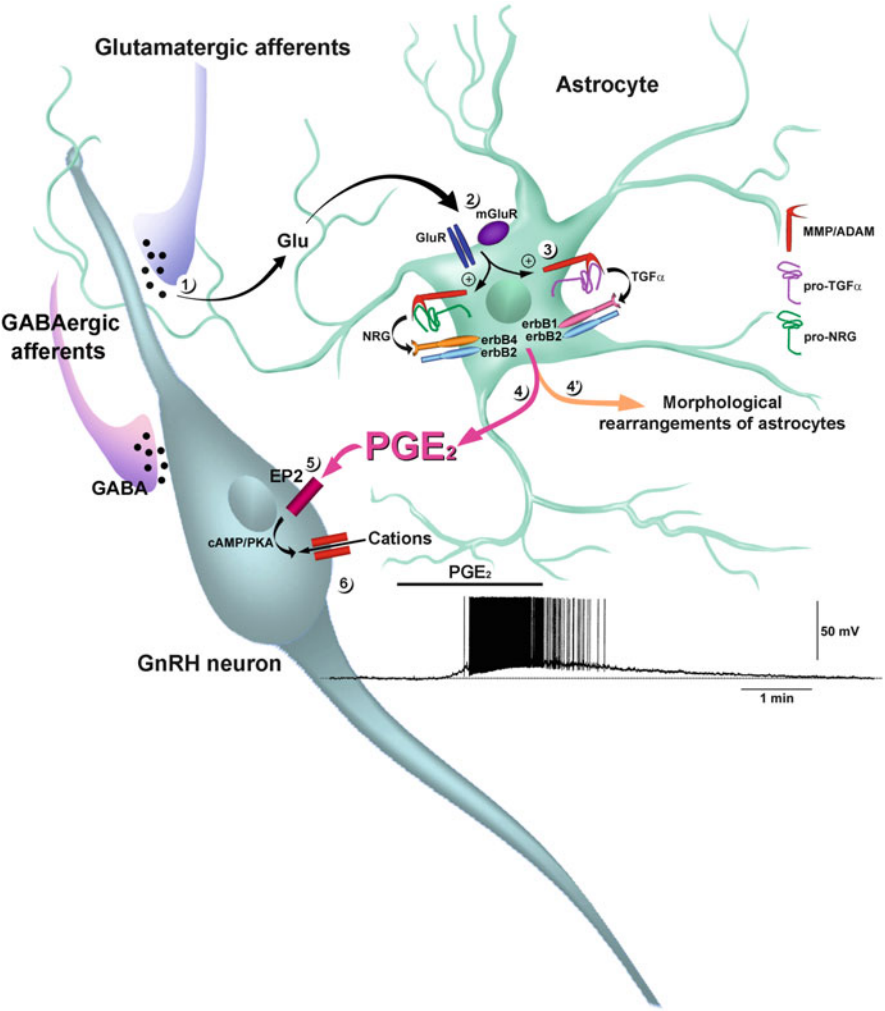

Fig. 5 PGE2 acts as a gliotransmitter to stimulate GnRH neuron electrical activity. Neuronally released glutamate (Glu) (*1*) co-activates metabotropic glutamatergic (*mGluR*) and AMPA glutamatergic receptors (*GluR*) in astrocytes (*2*), stimulating the activity of zinc-dependent matrix metalloproteinases (*MMPs*) of the ADAM (a disintegrin and metalloproteinase) family (*3*). The MMPs catalyze ectodomain shedding of the pro-EGF ligands pro-TGFα and pro-NRG (pro-neuregulin). In particular, the processing of pro-TGFα has been shown to involve the metalloproteinase ADAM17, also known as tumor necrosis factor α converting enzyme (TACE). The subsequently released mature TGFα and NRG activate erbB1/erbB2 and erbB4/erbB2 heterodimers, respectively (Dziedzic et al. 2003). The co-activation of glutamatergic receptors induces the recruitment of erbB1, erbB4 and their pro-ligands to the cell membrane, where multiprotein complexes form, as demonstrated by the direct physical association of glutamatergic and erbB receptors (not shown). The activation of erbB receptors in hypothalamic astrocytes promotes profound morphological changes, including the retraction of cytoplasm and the elongation and stellation of processes (see Fig. 4g–i) (*4'*). The activation of erbB receptors also promotes the release of PGE2 (Dziedzic et al. 2003; Ma et al. 1997, 1999) (*4*), which stimulates a cAMP/protein kinase A (PKA) pathway in GnRH neurons through the mobilization of EP2

Does Glial PGE_2 Control Dendritic Spine Plasticity in the GnRH Neural Network?

GnRH neurons exhibit a simple bipolar morphology with one or two very long dendritic processes that can extend up to 1 mm (Campbell et al. 2005, 2009). Intriguingly, recent studies have demonstrated that the density of spines along these dendrites is subject to robust increases during sexual development in immature animals (Cottrell et al. 2006). Although sexual maturation have been shown to require the neuronal expression of sex-steroid receptors (Mayer et al. 2010; Raskin et al. 2009; Wintermantel et al. 2006), studies suggesting that astrocytic mechanisms might control the stabilization of individual dendritic processes and their subsequent maturation into spines (Nishida and Okabe 2007), together with the demonstration that specific juxtacrine signaling pathways are involved in sculpting astrocyte-dendritic spine interactions (Murai et al. 2003), raise the possibility that astrocytes play a role in the physiological changes of synaptic structure underlying GnRH neuronal maturation and function. PGE_2 release by astrocytes could be central to this process, and PGE_2 has in fact been shown to mediate the dramatic neuronal spine plasticity induced by estrogens in the developing preoptic region (Amateau and McCarthy 2002, 2004; Wright and McCarthy 2009). This effect involves the activation of AMPA and metabotropic glutamate receptors (Amateau and McCarthy 2002; Wright and McCarthy 2009), which are known to promote erbB-dependent PGE_2 release in hypothalamic astrocytes (Dziedzic et al. 2003), as well as the EP2/PKA signaling pathway (Amateau and McCarthy 2002) recently found to be functional in native GnRH neurons (Clasadonte et al. 2011). Importantly, estrogens, which have long been known to regulate neuronal spine plasticity in the adult hippocampus (Woolley and McEwen 1992, 1994), have also been shown to promote comparable changes in the immature hippocampus (Amateau and McCarthy 2002). However, in the hippocampus, the underlying mechanisms do not appear to require PGE_2 synthesis (Amateau and McCarthy 2002), suggesting that increases in PGE_2 synthesis are selectively used by estrogens to promote dendritic spine plasticity in the developing preoptic region.

Fig. 5 (continued) receptors (EP2-R; Clasadonte et al. 2011) (*5*). Activation of this signaling pathway induces a reversible membrane depolarization of GnRH neurons, leading to the initiation of spike firing via a postsynaptic effect involving the activation of a nonselective cation current (Clasadonte et al. 2011) (*6*)

PGE_2 Acts as a Gliotransmitter in the GnRH Neuroendocrine System

Even though PGE_2 has been known to trigger GnRH release from the hypothalamic neurons controlling reproduction for almost 40 years, it is only very recently that it has been identified as a potent excitatory regulator of GnRH neuronal activity in both male and female mice (Clasadonte et al. 2011). Using patch-clamp recordings in brain slices from transgenic mice expressing green fluorescent protein (GFP) under the control of the GnRH promoter, we showed that PGE_2 induced a reversible membrane depolarization of GnRH neurons leading to the initiation of spike firing via the postsynaptic effect involving activation of a nonselective cation current (Fig. 5; Clasadonte et al. 2011) reminiscent of the ones recently described in GnRH neurons by other groups (Roland and Moenter 2011; Zhang et al. 2008). Although GnRH neurons are known to express both the EP1 and EP2 subtypes of prostaglandin receptors in vivo (Jasoni et al. 2005; Rage et al. 1997), the excitatory effect of PGE_2 on GnRH neuronal activity was selectively mimicked by the EP2 receptor agonist butaprost (Clasadonte et al. 2011), previously shown to promote GnRH release in the GnRH-producing neuronal cell line, GT1-7 (Rage et al. 1997). The PGE_2-mediated membrane depolarization of GnRH neurons was also shown to require the cAMP/protein kinase A (PKA) pathway (Clasadonte et al. 2011), which is known to be coupled to the EP2 receptor (Fig. 1; Coleman et al. 1994; Sang et al. 2005) and to underlie the stimulatory effect of PGE_2 on GnRH secretion (Fig. 5; Ojeda et al. 1985).

As alluded to above, the selective disruption of erbB4 signaling in astrocytes by the overexpression of a dominant-negative erbB4 receptor under the control of the human GFAP promoter leads to diminished PGE_2 release in response to ligand-dependent erbB4 activation; this in turn leads to reduced GnRH release, delayed puberty, and disrupted adult reproductive function (Prevot et al. 2003, 2005). Intriguingly, electrophysiological analyses have shown that the spontaneous activity of GnRH neurons in these animals is decreased and that this deficiency is mimicked by the bath application of either fluoroacetate, an inhibitor of astrocyte metabolism (Fonnum et al. 1997; Henneberger et al. 2010), or the COX blocker indomethacin, to slices of the preoptic region from wild-type animals (Clasadonte et al. 2011). The fact that GnRH neuronal activity in all these conditions can be rescued by exogenous PGE_2 (Clasadonte et al. 2011) strongly suggests that glial PGE_2 is an important component of the homeostatic mechanism controlling GnRH neuronal excitability. The role of glia in the control of GnRH neuronal activity is further supported by a recent study demonstrating that glial prostaglandins may regulate the efficacy of GABAergic inputs to GnRH neurons in ovariectomized mice (Glanowska and Moenter 2011). Using GnRH-GFP transgenic mice and patch-clamp recordings in brain slices, the authors demonstrated that the repeated action potential-like depolarization of a GnRH neuron caused a short-term reduction in the frequency of spontaneous GABAergic postsynaptic currents in the same neuron, suggesting the presence of local circuit interactions between GnRH neurons

and their GABAergic afferents (Chu and Moenter 2005; Glanowska and Moenter 2011). It is important to note that, in this local circuit, the activation of $GABA_A$ receptors exerts a depolarizing action that can trigger action potential firing due to the elevated chloride levels maintained in adult GnRH neurons (DeFazio et al. 2002; Han et al. 2002; Herbison and Moenter 2011). Consequently, this represents a negative feedback loop in which depolarized GnRH neurons reduce the activity of their own excitatory GABAergic afferents. In addition to being steroid-dependent and under the influence of both glutamatergic and endocannabinoid signaling mechanisms via the activation of presynaptic metabotropic glutamate receptors and cannabinoid CB1 receptors, respectively (Chu and Moenter 2005; Glanowska and Moenter 2011), this local negative feedback loop also requires the action of glia-derived prostanglandins (Glanowska and Moenter 2011). Indeed, the incubation of brain slices with indomethacin, the broad-spectrum prostaglandin receptor antagonist AH 6809, or fluorocitrate, which like fluoroacetate, is a specific blocker of astrocyte metabolism, prevents the depolarization-induced suppression of GABAergic transmission in GnRH neurons (Glanowska and Moenter 2011). Since GABA exerts a depolarizing action in this local circuit, we could envisage that glial prostaglandins, by suppressing excitatory drive, would reduce GnRH neuronal activity. Estradiol could also differentially influence this local inhibitory feedback to exert its positive or negative feedback effects (Glanowska and Moenter 2011). Thus, in addition to exerting a direct postsynaptic excitatory action on the cell body of GnRH neurons, prostaglandins released from astrocytes can participate in mechanisms that regulate the activity of their GABAergic presynaptic inputs (Fig. 5). Thus in the GnRH system, PGE_2 fulfils all the criteria that qualify a compound as a "gliotransmitter" (Parpura and Zorec 2010): (1) it is synthesized by astrocytes, (2) its regulated release is triggered by physiological stimuli, (3) it acutely activates the firing of GnRH neurons and modulates the activity of their GABAergic afferents, and (4) it plays a role in an important physiological function, i.e., the neuroendocrine control of reproduction, which is vital to species' survival.

Conclusions

Several observations made over the last two decades have demonstrated that PGE_2, which has been known for almost 40 years to play an important role in the regulation of the hypothalamic-pituitary-gonadal axis, is a transmitter released by astrocytes and intimately linked with GnRH neuronal function in the preoptic region, where the cell bodies of GnRH neurons in rodents are located. However, many mysteries regarding the underlying mechanisms remain unsolved. For example, even though recent studies suggest that GnRH neurons can directly communicate with neighboring astrocytes via juxtacrine signaling pathways (Sandau et al. 2011a, b), a true understanding of how these GnRH neurons interact with hypothalamic astrocytes to modulate PGE_2 gliotransmission is missing. Are these

communication processes involved in sculpting astrocyte-dendritic spine interactions and in promoting the physiological changes in synaptic structure that underlie GnRH neuronal maturation? How is PGE_2 released from hypothalamic astrocytes?

Now that a general strategy for the application of molecular genetics to the study of neuron-glia interactions and gliotransmission has been elucidated, the next several years should provide an opportunity to begin to address these questions.

Acknowledgments This research was supported by the Foundation pour la Recherche Médicale (Equipe FRM DEQ20130326524).

References

Amateau SK, McCarthy MM (2002) A novel mechanism of dendritic spine plasticity involving estradiol induction of prostaglandin-E2. J Neurosci 22:8586–8596

Amateau SK, McCarthy MM (2004) Induction of PGE2 by estradiol mediates developmental masculinization of sex behavior. Nat Neurosci 7:643–650

Apostolakis EM, Garai J, Lohmann JE, Clark JH, O'Malley BW (2000) Epidermal growth factor activates reproductive behavior independent of ovarian steroids in female rodents. Mol Endocrinol 14:1086–1098

Baroncini M, Allet C, Leroy D, Beauvillain JC, Francke JP, Prevot V (2007) Morphological evidence for direct interaction between gonadotrophin-releasing hormone neurones and astroglial cells in the human hypothalamus. J Neuroendocrinol 19:691–702

Bellefontaine N, Hanchate NK, Parkash J, Campagne C, de Seranno S, Clasadonte J, d'Anglemont de Tassigny X, Prevot V (2011) Nitric oxide as key mediator of neuron-to-neuron and endothelia-to-glia communication involved in the neuroendocrine control of reproduction. Neuroendocrinology 93:74–89

Bezzi P, Carmignoto G, Pasti L, Vesce S, Rossi D, Rizzini BL, Pozzan T, Volterra A (1998) Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. Nature 391:281–285

Bosetti F (2007) Arachidonic acid metabolism in brain physiology and pathology: lessons from genetically altered mouse models. J Neurochem 102:577–586

Botting JH, Linton EA, Whitehead SA (1977) Blockade of ovulation in the rat by a prostaglandin antogonist (N-0164). J Endocrinol 75:335–336

Brock TG, McNish RW, Peters-Golden M (1999) Arachidonic acid is preferentially metabolized by cyclooxygenase-2 to prostacyclin and prostaglandin E2. J Biol Chem 274:11660–11666

Campbell RE, Han SK, Herbison AE (2005) Biocytin filling of adult gonadotropin-releasing hormone neurons in situ reveals extensive, spiny, dendritic processes. Endocrinology 146:1163–1169

Campbell RE, Gaidamaka G, Han SK, Herbison AE (2009) Dendro-dendritic bundling and shared synapses between gonadotropin-releasing hormone neurons. Proc Natl Acad Sci U S A 106:10835–10840

Carlson JC, Barcikowski B, Cargill V, McCracken JA (1974) The blockade of LH release by indomethacin. J Clin Endocrinol Metab 39:399–402

Carlson JC, Wong AP, Perrin DG (1977) Luteinizing hormone secretion in the rhesus monkey and a possible role for prostaglandins. Biol Reprod 16:622–626

Cashion AB, Smith MJ, Wise PM (2003) The morphometry of astrocytes in the rostral preoptic area exhibits a diurnal rhythm on proestrus: relationship to the luteinizing hormone surge and effects of age. Endocrinology 144:274–280

Christian CA, Moenter SM (2010) The neurobiology of preovulatory and estradiol-induced gonadotropin-releasing hormone surges. Endocr Rev 31:544–577

Chu Z, Moenter SM (2005) Endogenous activation of metabotropic glutamate receptors modulates GABAergic transmission to gonadotropin-releasing hormone neurons and alters their firing rate: a possible local feedback circuit. J Neurosci 25:5740–5749

Clasadonte J, Poulain P, Hanchate NK, Corfas G, Ojeda SR, Prevot V (2011) Prostaglandin E2 release from astrocytes triggers gonadotropin-releasing hormone (GnRH) neuron firing via EP2 receptor activation. Proc Natl Acad Sci U S A 108:16104–16109

Claypool LE, Kasuya E, Saitoh Y, Marzban F, Terasawa E (2000) N-methyl D, L-aspartate induces the release of luteinizing hormone-releasing hormone in the prepubertal and pubertal female rhesus monkey as measured by in vivo push-pull perfusion in the stalk-median eminence. Endocrinology 141:219–228

Coleman RA, Smith WL, Narumiya S (1994) International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. Pharmacol Rev 46:205–229

Cottrell EC, Campbell RE, Han SK, Herbison AE (2006) Postnatal remodeling of dendritic structure and spine density in gonadotropin-releasing hormone neurons. Endocrinology 147:3652–3661

DeFazio RA, Heger S, Ojeda SR, Moenter SM (2002) Activation of A-type gamma-aminobutyric acid receptors excites gonadotropin-releasing hormone neurons. Mol Endocrinol 16:2872–2891

Di Castro MA, Chuquet J, Liaudet N, Bhaukaurally K, Santello M, Bouvier D, Tiret P, Volterra A (2011) Local Ca(2+) detection and modulation of synaptic release by astrocytes. Nat Neurosci 14:1276–1284

Donato J Jr, Cravo RM, Frazao R, Elias CF (2011) Hypothalamic sites of leptin action linking metabolism and reproduction. Neuroendocrinology 93:9–18

Donoso AO, Lopez FJ, Negro-Vilar A (1990) Glutamate receptors of the non-N-methyl-D-aspartic acid type mediate the increase in luteinizing hormone-releasing hormone release by excitatory amino acids in vitro. Endocrinology 126:414–420

Dziedzic B, Prevot V, Lomniczi A, Jung H, Cornea A, Ojeda SR (2003) Neuron-to-glia signaling mediated by excitatory amino acid receptors regulates ErbB receptor function in astroglial cells of the neuroendocrine brain. J Neurosci 23:915–926

Eroglu C, Barres BA (2010) Regulation of synaptic connectivity by glia. Nature 468:223–231

Eskay RL, Warberg J, Mical RS, Porter JC (1975) Prostaglandin E2-induced release of LHRH into hypophysial portal blood(1). Endocrinology 97:816–824

Fonnum F, Johnsen A, Hassel B (1997) Use of fluorocitrate and fluoroacetate in the study of brain metabolism. Glia 21:106–113

Gallardo E, Ramirez VD (1977) A method for the superfusion of rat hypothalami: secretion of luteinizing hormone-releasing hormone. Proc Soc Exp Biol Med 155:79–84

Garcia-Segura LM, McCarthy MM (2004) Minireview: role of glia in neuroendocrine function. Endocrinology 145:1082–1086

Gearing M, Terasawa E (1991) Prostaglandin E2 mediates the stimulatory effect of methoxamine on in vivo luteinizing hormone-releasing hormone (LH-RH) release in the ovariectomized female rhesus monkey. Brain Res 560:276–281

Glanowska KM, Moenter SM (2011) Endocannabinoids and prostaglandins both contribute to gonadotropin-releasing hormone (GnRH) neuron-GABAergic afferent local feedback circuits. J Neurophysiol 106:3073–3081

Gordon GR, Iremonger KJ, Kantevari S, Ellis-Davies GC, MacVicar BA, Bains JS (2009) Astrocyte-mediated distributed plasticity at hypothalamic glutamate synapses. Neuron 64:391–403

Halassa MM, Haydon PG (2010) Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. Annu Rev Physiol 72:335–355

Han SK, Abraham IM, Herbison AE (2002) Effect of GABA on GnRH neurons switches from depolarization to hyperpolarization at puberty in the female mouse. Endocrinology 143:1459–1466

Harms PG, Ojeda SR, McCann SM (1973) Prostaglandin involvement in hypothalamic control of gonadotropin and prolactin release. Science 181:760–761

Hatton GI, Wang YF (2008) Neural mechanisms underlying the milk ejection burst and reflex. Prog Brain Res 170:155–166

Haydon PG, Carmignoto G (2006) Astrocyte control of synaptic transmission and neurovascular coupling. Physiol Rev 86:1009–1031

Henneberger C, Papouin T, Oliet SH, Rusakov DA (2010) Long-term potentiation depends on release of D-serine from astrocytes. Nature 463:232–236

Herbison AE, Moenter SM (2011) Depolarising and hyperpolarising actions of GABA(A) receptor activation on gonadotrophin-releasing hormone neurones: towards an emerging consensus. J Neuroendocrinol 23:557–569

Herbison AE, Neill JD (2006) Physiology of the gonadotropin-releasing hormone neuronal network. In: Knobil E, Neill JD (eds) Knobil and Neill's physiology of reproduction. Elsevier, New York, pp 1415–1482

Hirst WD, Young KA, Newton R, Allport VC, Marriott DR, Wilkin GP (1999) Expression of COX-2 by normal and reactive astrocytes in the adult rat central nervous system. Mol Cell Neurosci 13:57–68

Hynes NE, Lane HA (2005) ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 5:341–354

Iadecola C, Nedergaard M (2007) Glial regulation of the cerebral microvasculature. Nat Neurosci 10:1369–1376

Jasoni CL, Todman MG, Han SK, Herbison AE (2005) Expression of mRNAs encoding receptors that mediate stress signals in gonadotropin-releasing hormone neurons of the mouse. Neuroendocrinology 82:320–328

Junier MP, Ma YJ, Costa ME, Hoffman G, Hill DF, Ojeda SR (1991) Transforming growth factor alpha contributes to the mechanism by which hypothalamic injury induces precocious puberty. Proc Natl Acad Sci U S A 88:9743–9747

Labhsetwar AP, Zolovick A (1973) Hypothalamic interaction between prostaglandins and catecholamines in promoting gonadotrophin secretion for ovulation. Nat New Biol 246:55–56

Ma YJ, Junier MP, Costa ME, Ojeda SR (1992) Transforming growth factor-alpha gene expression in the hypothalamus is developmentally regulated and linked to sexual maturation. Neuron 9:657–670

Ma YJ, Costa ME, Ojeda SR (1994a) Developmental expression of the genes encoding transforming growth factor alpha and its receptor in the hypothalamus of female rhesus macaques. Neuroendocrinology 60:346–359

Ma YJ, Hill DF, Junier MP, Costa ME, Felder SE, Ojeda SR (1994b) Expression of epidermal growth factor receptor changes in the hypothalamus during the onset of female puberty. Mol Cell Neurosci 5:246–262

Ma YJ, Berg-von der Emde K, Rage F, Wetsel WC, Ojeda SR (1997) Hypothalamic astrocytes respond to transforming growth factor-alpha with the secretion of neuroactive substances that stimulate the release of luteinizing hormone-releasing hormone. Endocrinology 138:19–25

Ma YJ, Hill DF, Creswick KE, Costa ME, Cornea A, Lioubin MN, Plowman GD, Ojeda SR (1999) Neuregulins signaling via a glial erbB-2-erbB-4 receptor complex contribute to the neuroendocrine control of mammalian sexual development. J Neurosci 19:9913–9927

Malpaux B (2006) Seasonal regulation in mammals. In: Knobil E, Neill JD (eds) Knobil and Neill's physiology of reproduction. Raven Press, New York

Martineau M, Baux G, Mothet JP (2006) D-serine signalling in the brain: friend and foe. Trends Neurosci 29:481–491

Mayer C, Acosta-Martinez M, Dubois SL, Wolfe A, Radovick S, Boehm U, Levine JE (2010) Timing and completion of puberty in female mice depend on estrogen receptor alpha-signaling in kisspeptin neurons. Proc Natl Acad Sci U S A 107:22693–22698

McCarthy MM, Schwarz JM, Wright CL, Dean SL (2008) Mechanisms mediating oestradiol modulation of the developing brain. J Neuroendocrinol 20:777–783

Milatovic D, Montine TJ, Aschner M (2011) Prostanoid signaling: dual role for prostaglandin E2 in neurotoxicity. Neurotoxicology 32:312–319

Mong JA, Blutstein T (2006) Estradiol modulation of astrocytic form and function: implications for hormonal control of synaptic communication. Neuroscience 138:967–975

Murai KK, Nguyen LN, Irie F, Yamaguchi Y, Pasquale EB (2003) Control of hippocampal dendritic spine morphology through ephrin-A3/EphA4 signaling. Nat Neurosci 6:153–160

Nishida H, Okabe S (2007) Direct astrocytic contacts regulate local maturation of dendritic spines. J Neurosci 27:331–340

Ojeda SR, Campbell WB (1982) An increase in hypothalamic capacity to synthesize prostaglandin E2 precedes the first preovulatory surge of gonadotropins. Endocrinology 111:1031–1037

Ojeda SR, Skinner MK (2006) Puberty in the rat. In: Neill JD (ed) Knobil and Neill's physiology of reproduction. Academic, San Diego, pp 2026–2126

Ojeda SR, Harms PG, McCann SM (1975a) Effect of inhibitors of prostaglandin synthesis on gonadotropin release in the rat. Endocrinology 97:843–854

Ojeda SR, Wheaton JE, McCann SM (1975b) Prostaglandin E2-induced release of luteinizing hormone-releasing factor (LRF). Neuroendocrinology 17:283–287

Ojeda SR, Jameson HE, McCann SM (1977) Hypothalamic areas involved in prostaglandin (PG)-induced gonadotropin release I: effects of PGE2 and PGF2alpha implants on luteinizing hormone release. Endocrinology 100:1585–1594

Ojeda SR, Negro-Vilar A, McCann SM (1979) Release of prostaglandin Es by hypothalamic tissue: evidence for their involvement in catecholamine-induced luteinizing hormone-releasing hormone release. Endocrinology 104:617–624

Ojeda SR, Negro-Vilar A, McCann SM (1982) Evidence for involvement of alpha-adrenergic receptors in norepinephrine-induced prostaglandin E2 and luteinizing hormone-releasing hormone release from the median eminence. Endocrinology 110:409–412

Ojeda SR, Urbanski HF, Katz KH, Costa ME (1985) Stimulation of cyclic adenosine 3′,5-′-monophosphate production enhances hypothalamic luteinizing hormone-releasing hormone release without increasing prostaglandin E2 synthesis: studies in prepubertal female rats. Endocrinology 117:1175–1178

Ojeda SR, Urbanski HF, Katz KH, Costa ME (1986a) Activation of estradiol-positive feedback at puberty: estradiol sensitizes the LHRH-releasing system at two different biochemical steps. Neuroendocrinology 43:259–265

Ojeda SR, Urbanski HF, Katz KH, Costa ME, Conn PM (1986b) Activation of two different but complementary biochemical pathways stimulates release of hypothalamic luteinizing hormone-releasing hormone. Proc Natl Acad Sci U S A 83:4932–4936

Ojeda SR, Urbanski HF, Costa ME, Hill DF, Moholt-Siebert M (1990) Involvement of transforming growth factor alpha in the release of luteinizing hormone-releasing hormone from the developing female hypothalamus. Proc Natl Acad Sci U S A 87:9698–9702

Oliet SH, Bonfardin VD (2010) Morphological plasticity of the rat supraoptic nucleus–cellular consequences. Eur J Neurosci 32:1989–1994

Panatier A (2009) Glial cells: indispensable partners of hypothalamic magnocellular neurones. J Neuroendocrinol 21:665–672

Panatier A, Vallee J, Haber M, Murai KK, Lacaille JC, Robitaille R (2011) Astrocytes are endogenous regulators of basal transmission at central synapses. Cell 146:785–798

Parpura V, Zorec R (2010) Gliotransmission: exocytotic release from astrocytes. Brain Res Rev 63:83–92

Pfrieger FW (2010) Role of glial cells in the formation and maintenance of synapses. Brain Res Rev 63:39–46

Plant TM (2006) Puberty in non human primates and humans. In: Neill JD (ed) Knobil and Neill's physiology of reproduction. Academic, San Diego, pp 2177–2230

Plant TM, Gay VL, Marshall GR, Arslan M (1989) Puberty in monkeys is triggered by chemical stimulation of the hypothalamus. Proc Natl Acad Sci U S A 86:2506–2510

Prevot V, Rio C, Cho GJ, Lomniczi A, Heger S, Neville CM, Rosenthal NA, Ojeda SR, Corfas G (2003) Normal female sexual development requires neuregulin-erbB receptor signaling in hypothalamic astrocytes. J Neurosci 23:230–239

Prevot V, Lomniczi A, Corfas G, Ojeda SR (2005) erbB-1 and erbB-4 receptors act in concert to facilitate female sexual development and mature reproductive function. Endocrinology 146:1465–1472

Prevot V, Bellefontaine N, Baroncini M, Sharif A, Hanchate NK, Parkash J, Campagne C, de Seranno S (2010a) GnRH nerve terminals, tanycytes and neurohaemal junction remodeling in the adult median eminence: functional consequences for reproduction and dynamic role of vascular endothelial cells. J Neuroendocrinol 22:639–649

Prevot V, Hanchate NK, Bellefontaine N, Sharif A, Parkash J, Estrella C, Allet C, de Seranno S, Campagne C, de Tassigny X, Baroncini M (2010b) Function-related structural plasticity of the GnRH system: a role for neuronal-glial-endothelial interactions. Front Neuroendocrinol 31:241–258

Rage F, Lee BJ, Ma YJ, Ojeda SR (1997) Estradiol enhances prostaglandin E2 receptor gene expression in luteinizing hormone-releasing hormone (LHRH) neurons and facilitates the LHRH response to PGE2 by activating a glia-to-neuron signaling pathway. J Neurosci 17:9145–9156

Raskin K, de Gendt K, Duittoz A, Liere P, Verhoeven G, Tronche F, Mhaouty-Kodja S (2009) Conditional inactivation of androgen receptor gene in the nervous system effects on male behavioral and neuroendocrine responses. J Neurosci 29:4461–4470

Rettori V, Gimeno M, Lyson K, McCann SM (1992) Nitric oxide mediates norepinephrine-induced prostaglandin E2 release from the hypothalamus. Proc Natl Acad Sci U S A 89:11543–11546

Roland AV, Moenter SM (2011) Glucosensing by GnRH neurons: inhibition by androgens and involvement of AMP-activated protein kinase. Mol Endocrinol 25:847–858

Ronnekleiv OK, Kelly MJ (2005) Diversity of ovarian steroid signaling in the hypothalamus. Front Neuroendocrinol 26:65–84

Sandau US, Mungenast AE, Alderman Z, Sardi SP, Fogel AI, Taylor B, Parent AS, Biederer T, Corfas G, Ojeda SR (2011a) SynCAM1, a synaptic adhesion molecule, is expressed in astrocytes and contributes to erbB4 receptor-mediated control of female sexual development. Endocrinology 152:2364–2376

Sandau US, Mungenast AE, McCarthy J, Biederer T, Corfas G, Ojeda SR (2011b) The synaptic cell adhesion molecule, SynCAM1, mediates astrocyte-to-astrocyte and astrocyte-to-GnRH neuron adhesiveness in the mouse hypothalamus. Endocrinology 152:2353–2363

Sang N, Zhang J, Marcheselli V, Bazan NG, Chen C (2005) Postsynaptically synthesized prostaglandin E2 (PGE2) modulates hippocampal synaptic transmission via a presynaptic PGE2 EP2 receptor. J Neurosci 25:9858–9870

Sharif A, Duhem-Tonnelle V, Allet C, Baroncini M, Loyens A, Kerr-Conte J, Collier F, Blond S, Ojeda SR, Junier MP, Prevot V (2009) Differential erbB signaling in astrocytes from the cerebral cortex and the hypothalamus of the human brain. Glia 57:362–379

Sharif A, Baroncini M, Prevot V (2013) Role of glia in the regulation of gonadotropin-releasing hormone neuronal activity and secretion. Neuroendocrinology 98:1–15

Terasawa E, Fernandez DL (2001) Neurobiological mechanisms of the onset of puberty in primates. Endocr Rev 22:111–151

Theodosis DT, Poulain DA, Oliet SH (2008) Activity-dependent structural and functional plasticity of astrocyte-neuron interactions. Physiol Rev 88:983–1008

Urbanski HF, Ojeda SR (1987) Activation of luteinizing hormone-releasing hormone release advances the onset of female puberty. Neuroendocrinology 46:273–276

Urbanski HF, Ojeda SR (1990) A role for N-methyl-D-aspartate (NMDA) receptors in the control of LH secretion and initiation of female puberty. Endocrinology 126:1774–1776

van den Pol AN, Trombley PQ (1993) Glutamate neurons in hypothalamus regulate excitatory transmission. J Neurosci 13:2829–2836

Vidensky S, Zhang Y, Hand T, Goellner J, Shaffer A, Isakson P, Andreasson K (2003) Neuronal overexpression of COX-2 results in dominant production of PGE2 and altered fever response. Neuromolecular Med 3:15–28

Voigt P, Ma YJ, Gonzalez D, Fahrenbach WH, Wetsel WC, der Berg-von EK, Hill DF, Taylor KG, Costa ME, Seidah NG, Ojeda SR (1996) Neural and glial-mediated effects of growth factors acting via tyrosine kinase receptors on luteinizing hormone-releasing hormone neurons. Endocrinology 137:2593–2605

Wintermantel TM, Campbell RE, Porteous R, Bock D, Grone HJ, Todman MG, Korach KS, Greiner E, Pérez CA, Schütz G, Herbison AE (2006) Definition of estrogen receptor pathway critical for estrogen positive feedback to gonadotropin-releasing hormone neurons and fertility. Neuron 52:271–280

Witkin JW, O'Sullivan H, Ferin M (1995) Glial ensheathment of GnRH neurons in pubertal female rhesus macaques. J Neuroendocrinol 7:665–671

Woolley CS, McEwen BS (1992) Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. J Neurosci 12:2549–2554

Woolley CS, McEwen BS (1994) Estradiol regulates hippocampal dendritic spine density via an N-methyl-D-aspartate receptor-dependent mechanism. J Neurosci 14:7680–7687

Wright CL, McCarthy MM (2009) Prostaglandin E2-induced masculinization of brain and behavior requires protein kinase A, AMPA/kainate, and metabotropic glutamate receptor signaling. J Neurosci 29:13274–13282

Zhang C, Roepke TA, Kelly MJ, Rønnekleiv OK (2008) Kisspeptin depolarizes gonadotropin-releasing hormone neurons through activation of TRPC-like cationic channels. J Neurosci 28:4423–4434

Zonta M, Angulo MC, Gobbo S, Rosengarten B, Hossmann KA, Pozzan T, Carmignoto G (2003a) Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. Nat Neurosci 6:43–50

Zonta M, Sebelin A, Gobbo S, Fellin T, Pozzan T, Carmignoto G (2003b) Glutamate-mediated cytosolic calcium oscillations regulate a pulsatile prostaglandin release from cultured rat astrocytes. J Physiol 553:407–414

Gonadotropic Axis Deficiency: A Neurodevelopmental Disorder

Lukas Huijbregts, Brooke Tata, and Nicolas de Roux

Abstract The onset of puberty remains a mysterious mechanism despite several recent major breakthroughs in the field of neuroendocrinology. Novel neuropeptides, along with new regulation networks of gene expression, have been described; however, the intimate molecular mechanisms defining the age of puberty onset have yet to be characterized. This article proposes to broaden the reflection surrounding the mechanisms that determine the onset of puberty as a developmental process, similar to those described for other neuronal networks. This new and original approach will bring further insight into the intricate interactions between the brain and the gonads that drive and maintain normal reproductive function.

Puberty: A Neurodevelopmental Process That Starts Earlier Than Usually Thought

The thelarche is defined by the appearance of breasts in girls. It results from an increase in estrogen secretion by the ovaries and corresponds to the stage of the Tanner classification defined by the somatic changes occurring up to the end of puberty. Tanner stage 5 corresponds to a fully developed, mature individual with a functional reproductive activity. Although it is usually considered to be the first marker of puberty, Tanner stage 2 actually reflects a re-activation of the gonadotropic axis that had already started several months earlier. Indeed, biological puberty starts before clinical puberty through nocturnal elevation of plasma luteinizing hormone (LH), without changes during the day. Moreover, pituitary response to GnRH injection appears concomitantly to thelarche. In other words, the pituitary activation started several months before, probably by the nocturnal activation of the gonadotropic axis. The molecular mechanism of this circadian activation of the gonadotropic axis at the end of childhood is unknown. Further, from

L. Huijbregts • B. Tata • N. de Roux (✉)
Inserm U1141, Univ Paris Diderot. Hôpital Robert Debré, 48 Boulevard Sérurier, 75019 Paris, France
e-mail: nicolas.deroux@inserm.fr

J.-P. Bourguignon et al. (eds.), *Brain Crosstalk in Puberty and Adolescence*,
Research and Perspectives in Endocrine Interactions 13,
DOI 10.1007/978-3-319-09168-6_11

stages 2 to 5, plasma LH undergoes important variations as diurnal LH reaches the nocturnal levels and the circadian cycle completely disappears. In the meantime, the negative feedback by steroid hormones in both sexes as well as the positive feedback by estrogen in females only become functional on the hypothalamo-pituitary unit. From a biological point of view, puberty therefore corresponds to the development of a brain-gonads homeostasis that leads to normal reproductive function. This is unique to the gonadotropic axis.

The recent discovery of kisspeptins and their ability to tightly regulate GnRH neurons has led to a better understanding of the interplay between gonads and the brain, especially in regard to neurons and other hypothalamic cells that are involved in the regulation of GnRH secretion and associated disorders (de Roux et al. 2003; Pinilla et al. 2012). Known as the GnRH network, it is composed of neurosecretory neurons, glial cells, such as astrocytes, and also glia-like cells called tanycytes (Prevot et al. 2010). The main function of this network is to regulate GnRH secretion. For methodological and conceptual reasons, it is difficult to study and conceive of a constantly evolving neuronal network; hence the vast majority of original knowledge on the GnRH network has come from adult animals. The roles of kisspeptin, dynorphin, and/or neurokinin B have been described in detail, from their regulation by sexual hormones to the involvement of glial cells in this network (Lomniczi et al. 2013b; Tena-Sempere 2012). These results were then transposed to pre-pubertal animals and helped define the link between the increase of *KiSS1* expression in the hypothalamus and the onset of puberty (Terasawa et al. 2013).

These novel findings show that pubertal onset is the consequence of a long neurodevelopmental process that starts during fetal life, just like most neuronal networks do. The neurodevelopment of the gonadotropic axis is, however, unique for several reasons. GnRH neurons emerge from the olfactory placode and migrate to the hypothalamus during early pre-natal development. Such migration toward the brain is very uncommon in animal biology and has largely been studied in humans affected with Kallman syndrome and in rodents (Mitchell et al. 2011; Wierman et al. 2011). In primates, gonadotrope cells are functional and can response to GnRH stimulation at midgestation. The hypothalamo-pituitary control of the gonadotropic axis must be functional during fetal life to have complete development of gonads in both sexes and sexual organs in males. However, this control is a complex phenomenon. Indeed, at mid-gestation, blood levels of LH and follicle stimulating hormone (FSH) are elevated and close to those observed in adults, whereas at the end of gestation, LH and FSH blood levels are close to null (Guimiot et al. 2012). Such negative regulation partly depends on kisspeptins expressed in the infundibulum (Guimiot et al. 2012). It is important to note that this timing is specific to the gonadotropic axis since other endocrine axes tend to be activated at the end of gestation. The second part of gestation is therefore crucial to producing normal reproductive function in adults.

Post-natal development of the gonadotropic axis is comprised of several stages that are clinically well defined. Mini-puberty starts a few weeks after birth and is followed by a quiescent phase that goes on during childhood until puberty at the average age of 10. The mechanisms underlying this sequential activation-inactivation surrounding mini-puberty are poorly understood, the majority of

studies having essentially focused on the reactivation at puberty. In rodents, mini-puberty is not well characterized. It may correspond to the FSH surge observed in mice at post-natal day (PND12), knowing that puberty usually starts around PND30. This rather late surge, when compared to humans, may be explained by the fact that the first week of rodent life corresponds to the third semester of human in utero development. It was recently shown that the quiescent phase partly depends on a reduced kisspeptin tonus in non-human primates (Ramaswamy et al. 2013). The timing of this central activation of the gonadotropic axis in the first months of life is comparable to other neurodevelopmental processes.

In recent years, studies on puberty onset have brought to light that puberty is associated with a vast genes and transcription factors network whose hypothalamic expression either increases or decreases before pubertal onset (Lomniczi et al. 2013b). Indeed, this complex network notably depends on epigenetic regulations that modulate kisspeptin expression (Lomniczi et al. 2013a). These studies have been of tremendous importance to understanding puberty onset proceedings but have failed to bring answers to the question of pubertal timing. In other words, the molecular events leading to variations of hypothalamic genes expression through time remain unknown.

Neurodevelopment is not limited to the embryonic development of neurons. It also corresponds to the development of synaptic connections or synaptogenesis, which starts during fetal life and goes on throughout adulthood. After birth, neurons are myelinated, further modifying neuronal network activity. Synaptogenesis is composed of well-timed stages, and adolescence is a very important period of synaptic remodeling during which synaptic contacts between neurons get simplified to maximize efficacy. It is interesting to note that puberty happens concomitantly with such complex and intense regulation of synaptic activity in the brain (Sisk and Foster 2004). The link between these two events has essentially been studied through the effect of sexual hormones on brain organization (Ahmed et al. 2008). Nonetheless, it appears that the plasticity of cortical neurons during puberty only partially depends on sexual hormones. These results suggest that the GnRH network is organized and timely determined by similar processes to those involved in the normal plasticity of cortical neurons. This maturation is initially independent of sexual hormones, then progressively shifts under the control of estradiol and testosterone during puberty. Inhibitory input from GABA and excitatory input from glutamate on GnRH and Kisspeptin neurons during the early phase of puberty have hence been demonstrated in rodents. These inputs radically change during the childhood-to-puberty transition. We assume that this change is the consequence of the neurodevelopmental process that probably began during the second half of gestation and just after puberty.

The characterization of the neurodevelopmental mechanisms shared by both hypothalamic and cortical neurons will have a double impact. Firstly, it should provide further understanding of pubertal onset and its associated diseases. Secondly, it should provide new hypotheses to explain neurodevelopmental disorders in children.

Congenital Gonadotropic Deficiency: A Neurodevelopmental Anomaly of the Gonadotropic Axis

We initially studied isolated gonadotropic deficiency, which led us to characterize mutations in *GnRHR* and *KiSS1R* (de Roux et al. 1999, 2003). Mutations in *KISS1* (Topaloglu et al. 2012), *GnRH1* (Bouligand et al. 2009), and also *TAC3* encoding neurokinin B, and *TACR3,* the receptor of neurokinin B (Topaloglu et al. 2009) have also been described in isolated hypogonadotropic hypogonadism. The phenotype observed in these patients has confirmed that the central control of the gonadotropic axis during fetal life is required for normal development of reproductive function. Investigation of the neuroendocrine functions of Kisspeptin and Neurokinin B has revealed their role in the secretion of GnRH. In addition, several genetic defects have been described in Kallmann syndrome (for review see Pitteloud et al. 2010). All of these genes encode proteins required for the development of the olfactory bulbs (Pitteloud et al. 2010). The gonadotropic deficiency observed in these patients is mainly the consequence of the agenesis of the olfactory tracts, although a direct action of these proteins on the migration and maturation of GnRH neurons has also been proposed. We now have evidence that isolated gonadotropic deficiency is not only an endocrine disorder but is also a neurodevelopmental disorder due to a defect in the migration of GnRH neurons or the neuroendocrine control of GnRH neurons.

We have thus suspected that studying complex diseases comprising both neurological disorders and a delayed puberty due to a congenital gonadotropic deficiency should be very informative to delineating the mechanism of the initiation of puberty and related disorders. Indeed, we believe both conditions originate from the same neurodevelopmental anomaly. The literature shows that gonadotropic deficiency has been described in many neurological syndromes, some of which are extremely rare (Alazami et al. 2008; Bem et al. 2011; Bernard et al. 2011; Margolin et al. 2013; Nousbeck et al. 2008; Synofzik et al. 2014; Tetreault et al. 2011). Apart from Prader-Willi and CHARGE syndromes, which are well known by pediatric endocrinologists, most of these syndromes are only followed in pediatric neurology departments. Indeed, hypogonadotropic hypogonadism is rarely diagnosed at birth in these children.

The neurological disease can be neurodegenerative, hence progressive. Signs of dementia have been observed in some adults (Margolin et al. 2013), and 4H syndrome, which associated a leucodystrophia with hypodontia and hypogonadotropic hypogonadism, has been described (Bernard et al. 2011; Tetreault et al. 2011). Mental retardation is frequently observed, as well as cerebellar or proprioceptive ataxia. Some signs are specific to one of these syndromes, such as a microphtalmia associated with a cataract in Warburg microsyndrome (Handley et al. 2013). The endocrine phenotype has been more or less described, with in utero or post-natal growth retardation. In most cases, the endocrine deficit is limited to the gonadotropic axis and is of hypothalamic origin.

Several genes have been described via genetic linkage or exome analysis. These genes encode proteins with various and sometime unexpected functions. For instance, 4H syndrome is due to mutations in two type III RNA polymerase sub-units encoding genes, *POLR3A* and *POLR3B* (Bernard et al. 2011; Tetreault et al. 2011). This is a surprising result, since type III RNA polymerase is ubiquitous. Its main function is to transcribe transfer RNA genes, which will further participate in protein translation. Interestingly, other leucodystrophias involving a defective protein synthesis have been described, but no gonadotropic deficiency was observed. This indicates that the gonadotropic deficiency in 4H syndrome does not depend on white matter disease but on POLR3A or POLR3B dysfunction. Mutations in other genes, such as *RNF216* and *OTUD4*, were shown to cause a complex phenotype associating a gonadotropic deficiency with dementia. Again, it seems that synaptic activity may be impaired, though RNF216 and ITUD4 have been shown to be involved in protein ubiquitination, a major mechanism of protein degradation (Margolin et al. 2013). Very recently, mutations in *PNPLA6* were identified in Boucher-Neuhauser and Gordon Holmes syndromes (Synofzik et al. 2014). PNPLA6 belongs to the family of proteins with a phospholipid esterase domain. It is interesting to note that *PNPLA6* mutations were characterized in different neurodegenerative diseases with ataxia, suggesting that Boucher-Neuhauser syndrome is a phenotypic variant of an important group of neurodegenerative diseases.

Warburg microsyndrome is caused by mutations in *RAB3GAP1*, *RAB3GAP2* and *RAB18* (Handley et al. 2013). RAB3GAP1 and RAB3GAP2 are proteins with a GTPase activity on RAB3 and RAB27. RAB18 is a small monomeric G protein. These results suggest that the gonadotropic deficiency may result from a defective neuronal function such as a neuropeptide or neurosecretion defect.

Syndromic gonadotropic deficiency has thus been described in many neurological pathologies, some of which are extremely rare. Human genetics have led to the identification of several genes but the mechanisms leading to the gonadotropic deficiency remain poorly understood. Nonetheless, hypotheses arising from these results must be validated in animal models by neuroendocrinology laboratory teams to delineate the exact function of these proteins in the central control of the gonadotropic axis. To go further in this direction, our group has decided to characterize the molecular mechanism involved in a phenotype from three siblings. This particular phenotype regroups an intra-uterine and post-natal growth retardation, an impaired glucose metabolism leading to hypoglycemic episodes during childhood and insulino-dependent diabetes during adolescence, a proprioceptive ataxia, mental retardation, central hypothyroidism and a gonadotropic deficiency without anosmia. Our results showed that the haploinsuffisiency of DMXl2 was the cause of this familial phenotype (Tata et al. 2014).

Conclusions and Perspectives

The development of the molecular genetics of puberty disorders over the last 15 years has been critical to delineate puberty onset mechanisms. The constant technical evolution of molecular biology and close collaboration with pediatric endocrinology and pediatric neurology hospital departments will provide opportunities to identify novel genes involved in hypogonadotropic hypogonadism. Initially focused on isolated gonadotropic deficiency, recent promising results have thus been obtained from the study of syndromic forms. This new gene inventory will be of utmost interest for screening familial and sporadic cases of IHH, thus providing better information for genetic counseling.

Initially classified as an endocrine defect, isolated gonadotropic deficiency must now be considered as a neurodevelopmental disorder due to a defect in neuron migration, a defect in the neuroendocrine control of the GnRH network and also a defect in synaptic communication between neurons. This new view of the etiopathogenicity of pubertal disorders also offers new perspectives on the concept of neurodegenerative diseases specific to the GnRH network. Moreover, beyond the fundamental contribution to the mechanisms of puberty onset, they represent an opportunity to study extremely rare phenotypes. Finally, these works will help to understand the development of the homeostasis of the brain-gonad axis during childhood and adolescence that will lead to normal fertility throughout adult life.

References

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

Alazami AM, Al-Saif A, Al-Semari A, Bohlega S, Zlitni S, Alzahrani F, Bavi P, Kaya N, Colak D, Khalak H, Baltus A, Peterlin B, Danda S, Bhatia KP, Schneider SA, Sakati N, Walsh CA, Al-Mohanna F, Meyer B, Alkuraya FS (2008) Mutations in C2orf37, encoding a nucleolar protein, cause hypogonadism, alopecia, diabetes mellitus, mental retardation, and extrapyramidal syndrome. Am J Hum Genet 83:684–691

Bem D, Yoshimura S, Nunes-Bastos R, Bond FC, Kurian MA, Rahman F, Handley MT, Hadzhiev Y, Masood I, Straatman-Iwanowska AA, Cullinane AR, McNeill A, Pasha SS, Kirby GA, Foster K, Ahmed Z, Morton JE, Williams D, Graham JM, Dobyns WB, Burglen L, Ainsworth JR, Gissen P, Müller F, Maher ER, Barr FA, Aligianis IA (2011) Loss-of-function mutations in RAB18 cause Warburg micro syndrome. Am J Hum Genet 88:499–507

Bernard G, Chouery E, Putorti ML, Tetreault M, Takanohashi A, Carosso G, Clement I, Boespflug-Tanguy O, Rodriguez D, Delague V, Abou Ghoch J, Jalkh N, Dorboz I, Fribourg S, Teichmann M, Megarbane A, Schiffmann R, Vanderver A, Brais B (2011) Mutations of POLR3A encoding a catalytic subunit of RNA polymerase Pol III cause a recessive hypomyelinating leukodystrophy. Am J Hum Genet 89:415–423

Bouligand J, Ghervan C, Tello JA, Brailly-Tabard S, Salenave S, Chanson P, Lombes M, Millar RP, Guiochon-Mantel A, Young J (2009) Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. N Engl J Med 360:2742–2748

de Roux N, Young J, Misrahi M, Schaison G, Milgrom E (1999) Loss of function mutations of the GnRH receptor: a new cause of hypogonadotropic hypogonadism. J Pediatr Endocrinol Metab 12(Suppl 1):267–275

de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E (2003) Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci U S A 100:10972–10976

Guimiot F, Chevrier L, Dreux S, Chevenne D, Caraty A, Delezoide AL, de Roux N (2012) Negative fetal FSH/LH regulation in late pregnancy is associated with declined kisspeptin/KISS1R expression in the tuberal hypothalamus. J Clin Endocrinol Metab 97:E2221–E2229

Handley MT, Morris-Rosendahl DJ, Brown S, Macdonald F, Hardy C, Bem D, Carpanini SM, Borck G, Martorell L, Izzi C, Faravelli F, Accorsi P, Pinelli L, Basel-Vanagaite L, Peretz G, Abdel-Salam GM, Zaki MS, Jansen A, Mowat D, Glass I, Stewart H, Mancini G, Lederer D, Roscioli T, Giuliano F, Plomp AS, Rolfs A, Graham JM, Seemanova E, Poo P, García-Cazorla A, Edery P, Jackson IJ, Maher ER (2013) Mutation spectrum in RAB3GAP1, RAB3GAP2, and RAB18 and genotype-phenotype correlations in Warburg micro syndrome and Martsolf syndrome. Hum Mutat 34:686–696

Lomniczi A, Loche A, Castellano JM, Ronnekleiv OK, Bosch M, Kaidar G, Knoll JG, Wright H, Pfeifer GP, Ojeda SR (2013a) Epigenetic control of female puberty. Nat Neurosci 16:281–289

Lomniczi A, Wright H, Castellano JM, Sonmez K, Ojeda SR (2013b) A system biology approach to identify regulatory pathways underlying the neuroendocrine control of female puberty in rats and nonhuman primates. Horm Behav 64:175–186

Margolin DH, Kousi M, Chan YM, Lim ET, Schmahmann JD, Hadjivassiliou M, Hall JE, Adam I, Dwyer A, Plummer L, Aldrin SV, O'Rourke J, Kirby A, Lage K, Milunsky A, Milunsky JM, Chan J, Hedley-Whyte ET, Daly MJ, Katsanis N, Seminara SB (2013) Ataxia, dementia, and hypogonadotropism caused by disordered ubiquitination. N Engl J Med 368:1992–2003

Mitchell AL, Dwyer A, Pitteloud N, Quinton R (2011) Genetic basis and variable phenotypic expression of Kallmann syndrome: towards a unifying theory. Trends Endocrinol Metab 22:249–258

Nousbeck J, Spiegel R, Ishida-Yamamoto A, Indelman M, Shani-Adir A, Adir N, Lipkin E, Bercovici S, Geiger D, van Steensel MA, Steijlen PM, Bergman R, Bindereif A, Choder M, Shalev S, Sprecher E (2008) Alopecia, neurological defects, and endocrinopathy syndrome caused by decreased expression of RBM28, a nucleolar protein associated with ribosome biogenesis. Am J Hum Genet 82:1114–1121

Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M (2012) Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol Rev 92:1235–1316

Pitteloud N, Durrani S, Raivio T, Sykiotis GP (2010) Complex genetics in idiopathic hypogonadotropic hypogonadism. Front Horm Res 39:142–153

Prevot V, Bellefontaine N, Baroncini M, Sharif A, Hanchate NK, Parkash J, Campagne C, de Seranno S (2010) Gonadotrophin-releasing hormone nerve terminals, tanycytes and neurohaemal junction remodelling in the adult median eminence: functional consequences for reproduction and dynamic role of vascular endothelial cells. J Neuroendocrinol 22:639–649

Ramaswamy S, Dwarki K, Ali B, Gibbs RB, Plant TM (2013) The decline in pulsatile GnRH release, as reflected by circulating LH concentrations, during the infant-juvenile transition in the agonadal male rhesus monkey (Macaca mulatta) is associated with a reduction in kisspeptin content of KNDy neurons of the arcuate nucleus in the hypothalamus. Endocrinology 154:1845–1853

Sisk CL, Foster DL (2004) The neural basis of puberty and adolescence. Nat Neurosci 7:1040–1047

Synofzik M, Gonzalez MA, Lourenco CM, Coutelier M, Haack TB, Rebelo A, Hannequin D, Strom TM, Prokisch H, Kernstock C et al (2014) PNPLA6 mutations cause Boucher-Neuhauser and Gordon Holmes syndromes as part of a broad neurodegenerative spectrum. Brain: J Neurol 137:69–77

Tâta B, Huijbregts L, Jacquier S, Csaba Z, Genin E, Meyer V, Leka S, Dupont J, Charles P, Chevenne D, Carel JC, Leger J, de Roux N (2014) Haploinsufficiency of Dmxl2, encoding a synaptic protein, causes infertility associated with a loss of GnRH neurons in mouse. PloS Biol 12(9):e1001952

Tena-Sempere M (2012) Deciphering puberty: novel partners, novel mechanisms. Eur J Endocrinol 167:733–747

Terasawa E, Guerriero KA, Plant TM (2013) Kisspeptin and puberty in mammals. Adv Exp Med Biol 784:253–273

Tetreault M, Choquet K, Orcesi S, Tonduti D, Balottin U, Teichmann M, Fribourg S, Schiffmann R, Brais B, Vanderver A, Bernard G (2011) Recessive mutations in POLR3B, encoding the second largest subunit of Pol III, cause a rare hypomyelinating leukodystrophy. Am J Hum Genet 89:652–655

Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Ozbek MN, Imamoglu S, Akalin NS, Yuksel B, O'Rahilly S, Semple RK (2009) TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. Nat Genet 41:354–358

Topaloglu AK, Tello JA, Kota LD, Ozbek MN, Yilmaz MB, Erdogan S, Gurbuz F, Temiz F, Millar RP, Yuksel B (2012) Inactivating KISS1 mutation and hypogonadotropic hypogonadism. N Engl J Med 366:629–635

Wierman ME, Kiseljak-Vassiliades K, Tobet S (2011) Gonadotropin-releasing hormone (GnRH) neuron migration: initiation, maintenance and cessation as critical steps to ensure normal reproductive function. Front Neuroendocrinol 32:43–52

Changes in Pubertal Timing: Past Views, Recast Issues

Jean-Pierre Bourguignon, Françoise Domine, Fabienne Glowacz, Marie-Christine Lebrethon, and Anne-Simone Parent

Abstract The aim of this article is to review some common opinions on changes in pubertal timing and shed new light both on the indicators used in assessing pubertal timing and the underlying mechanisms. While emphasis is usually placed on the advancement in timing of female puberty, it appears that timing also changes in males, both towards earliness for the initial pubertal stages and towards lateness for the final stages. Such findings suggest that the environmental influences on pubertal timing are more complex than initially thought. Moreover, self-evaluated pubertal timing versus peers provides information that is not always consistent with observations at physical examination, suggesting that both approaches should be considered, especially when studying the correlation between pubertal timing and psychosocial aspects.

The mechanisms of changes in pubertal timing may involve both central neuroendocrine control and peripheral effects in tissues targeted by gonadal steroids. Though energy availability is certainly a clue to the mechanism of pubertal development, changes in the control of both energy balance and reproduction may vary under the influence of common determinants such as endocrine-disrupting chemicals. These effects can take place right before puberty as well as much earlier, during fetal and neonatal life. Finally, environmental factors can interact with genetic factors in determining changes in pubertal timing. Therefore, the variance in pubertal timing is no longer to be considered under the absolutely separate control of environmental and genetic determinants.

J.-P. Bourguignon (✉) • M.-C. Lebrethon • A.-S. Parent
Department of Pediatrics, CHU de Liège, Site ND Bruyères, 600, rue de Gaillarmont, B-4030 Liège, Belgium

Developmental Neuroendocrinology unit, GIGA Neurosciences, University of Liège, Liège, Belgium
e-mail: jpbourguignon@ulg.ac.be

F. Domine
Department of Pediatrics, CHU de Liège, Site CHR de la Citadelle, B-4000 Liège, Belgium

F. Glowacz
Clinic of Delinquency, Social Maladjustment and Integration Processes, University of Liège, Liège, Belgium

J.-P. Bourguignon et al. (eds.), *Brain Crosstalk in Puberty and Adolescence*,
Research and Perspectives in Endocrine Interactions 13,
DOI 10.1007/978-3-319-09168-6_12

Introduction

We review here how studies of the physiological variations in pubertal timing have changed recently with emphasis on the indicators and the mechanisms. The findings suggest that the respective roles of genetic and environmental factors in the control of pubertal timing are complex because they can take place during late prepubertal life as well as fetal/neonatal life. Moreover, both types of factors likely involve intricate mechanisms.

Changes in Indicators of Pubertal Timing

Since the mid-nineteenth century, a so-called secular trend towards earlier menarcheal age has been observed in many countries in Europe and North America. An overall advancement in female pubertal timing that averaged 4 years in a century was derived from those observations (Parent et al. 2003). During the past 50 years, menarcheal age has stabilized in some countries, such as Belgium (Jeurissen 1969; Vercauteren and Susanne 1985; Roelants et al. 2009), where it seemed that the secular advancement in pubertal timing was leveling off as opposed to countries where socio-economical status was still markedly progressing (Parent et al. 2003). It is notable, however, that recent publications have highlighted a persistent but moderate decrease in average menarcheal age in countries like Denmark and the Netherlands (Aksglaede et al. 2009; Talma et al. 2013). Around 2000, a new pattern of changes was observed in several countries such as USA, Denmark and Belgium (Roelants et al. 2009; Aksglaede et al. 2009; Herman-Giddens et al. 1997), with a heterogeneity among pubertal events, i.e., advancement in age at onset of breast development and less or no change in menarcheal age. This finding has led us to revise some issues about indicators and mechanisms of changes in pubertal timing.

Studies of female puberty have been prioritized (Table 1) due to the availability of menarcheal age as a precise timer, and focus on the female has been reinforced

Table 1 Changes in pubertal timing: INDICATORS

Common opinion	Revision
More relevance in females (studies using menarcheal age as timer, female predominance of precocity, weight as a critical factor)	Reality in males (few studies based on testicular volume increase, voice break and growth spurt; association with BMI)
Mean or median age: the clue	Changes in pattern of timing distribution indicating involvement of subgroups
Single pubertal signs prioritized (the “onset” of puberty)	Changes possibly divergent among pubertal signs
Self-evaluation of pubertal development (practical and ethical reasons)	Self-perceived timing providing information different from physical exam

by the critical role of weight and the predominance of sexual precocity in girls (Parent et al. 2003). Based on findings about testicular volume increase, voice break and growth spurt, it has appeared recently, however, that male pubertal timing is advancing as well (Roelants et al. 2009; Aksglaede et al. 2008; Sorensen et al. 2010; Juul et al. 2007; Herman-Giddens et al. 2012). As already pointed out in the female, the initial pubertal signs and signs of completion of puberty could show secular changes that are different, suggesting heterogeneity in the response of pubertal events to modulating factors. For example, the first 3–10 % of boys with evidence of initial pubertal increase in testicular volume ($\geq$4 ml) are younger than in the past (Roelants et al. 2009; Herman-Giddens et al. 2012; Mul et al. 2001) whereas the final 3–10 % (centile 90 and 97) appear to attain adult testicular volume ($\geq$15 ml) later (Roelants et al. 2009; Mul et al. 2001). Thus, besides the changes in mean or median ages at a given pubertal sign, subtle changes in the pattern of distribution of timing of pubertal signs may occur, extending the age range beyond the physiological 4–5-year period for both the initial and the late stages of puberty. The latter finding is also consistent with the secular increase in latency between menarche and the occurrence of regular (ovulatory) cycling that was reported in France together with the classical secular trend towards earlier menarche (Clavel-Chapelon et al. 2002).

In the evaluation of pubertal development, two distinctive issues can be considered: pubertal status, which refers to the degree of physical changes, and pubertal timing, which refers to the age at attainment of a given degree of physical change. The adolescents are understandably reluctant to allow assessment of pubertal status if not justified by the chief complaint at consultation. Therefore, methods of self-assessment of pubertal status (Table 2) were designed, in comparison with Tanner stages, provided either as pictures or drawings (Duke et al. 1980; Morris and Udry (1980), or as written statements (Petersen et al. 1988). Controversial data have been published regarding the validity of these methods that cannot substitute for a physician's examination (Hergenroeder et al. 1999; Brooks-Gunn et al. 1987; Bond et al. 2006; Dick et al. 2001; Dorn and Biro 2011). Moreover, the significance of self-assessed pubertal status in determining pubertal timing has been limited to the interpretation of current status with respect to age. In some instances, retrospective assessment of pubertal timing could be interesting. Berg-Kelly and Erdes (1997) proposed an age-independent method of self-assessment of pubertal timing through a global multiple choice question rating maturation versus peers. The answer to this question was found to result in 94 % agreement with physician examination and normal distribution of timing in the studied population (Berg-Kelly and Erdes 1997). We have used a similar question: "Between 8 and 18 years, boys and girls change physically but not all at the same time. If you consider your physical development, how do you evaluate it in comparison with friends of the same age: Very early? A bit early? Same as most friends? A bit late? Very late?" Such a question enables self-evaluation of pubertal timing based on current status, as done in the Swedish study (Berg-Kelly and Erdes 1997), or retrospectively, as done in our study. As shown in Fig. 1, the distribution of responses in two separate studies was found to be asymmetrical, with more adolescents estimating they had

Table 2 Comparison of some features of different methods of self-assessment of pubertal status and timing in healthy adolescents

	Pubertal status			Pubertal timing
	Sexual maturation scale (SMS)		Pubertal developmental score (PDS)	Global question
	Tanner's pictures	Tanner's drawings		
References	Duke et al. 1980	Morris and Udry 1980; Petersen et al. 1988; Hergenroeder et al. 1999; Dorn and Biro 2011	Petersen et al. 1988; Brooks-Gunn et al. 1987; Bond et al. 2006	Berg-Kelly and Erdes 1997
N items in girls (F) & boys (M)	F: 2 M: 2	F: 2 M: 2	F: 6 M: 5	F & M: 1
Items assessed	Sex characteristic staging (five Tanner's stages)	Sex characteristic staging (five Tanner's stages)	Sex characteristic staging (five items)	Global timing (five qualifications)
Method of self-assessment	Current development versus photographs and descriptive text	Current development versus drawings	Current development versus descriptive text + menarcheal age	Current and retrospective development versus peers
Acceptability/ response rate	Fairly good	Fairly good/64 %	Good/66 %, 70 %	Good/82 %
Usable for different races	No	Yes	Yes	Yes
Agreement with clinical assessment	$\kappa > 0.81$–0.91 (Duke et al. 1980)	r 0.59–0.81 (Morris and Udry 1980) or κ 0.34–0.37 (Hergenroeder et al. 1999) or 0.06–0.43/27–59 % (Schlossberger et al. 1992); r = 0.77–0.91 and κ 0.33–0.50 (Dorn and Biro 2011)	α 0.65–0.82 (Petersen et al. 1988) F: 31–62 % (Brooks-Gunn et al. 1987)	94 % (Berg-Kelly and Erdes 1997)
		F : κ :0.57 M : κ: 0.42 (Bond et al. 2006)		
Biases		Adiposity		

F females, *M* males, *κ* Kappa coefficient (Cohen's rule of thumb), *r* Pearson's correlation coefficient, *α* Cronbach's alpha coefficient

matured early than late. Interestingly enough, in a study comparing adolescent self-perception of pubertal timing using both a global question (peer normative) and the pubertal developmental scale (PDS; stage normative), a similar proportion of late timing (12–13 %) was found using both tools whereas the global question resulted in a greater proportion of early timing (28 %) as opposed to the PDS (13 %; Cance et al. 2012). This finding suggests that the comparison with peers involves a factor

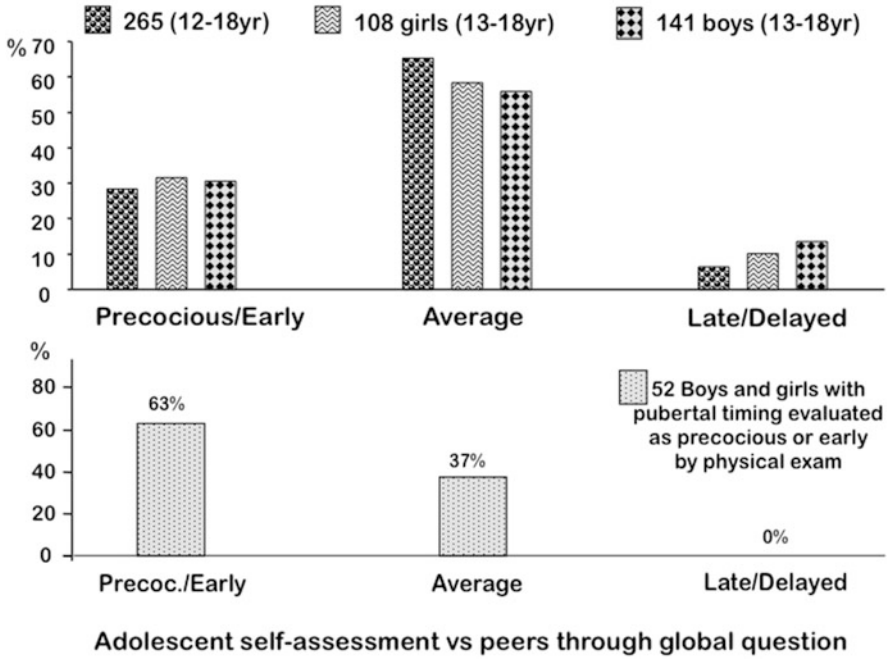

Fig. 1 *Upper panel*: self evaluation of pubertal timing versus peers of the same age in two separate groups of 265 adolescents irrespective of gender and 249 adolescents (108 girls and 141 boys). *Lower panel*: self evaluation of pubertal timing versus peers of the same age in 52 adolescents with early or precocious pubertal timing, based on physical exam

that accounts for increased self-perception of early timing of maturation. Desirability could play some role: evaluating oneself as early versus peers could be felt as a plus whereas being late would be a minus. Some discrepancies in self-evaluation of pubertal timing could be related to the conditions of evaluation. Agreement with physician evaluation was greater when the sexual maturation scale was administered in a clinical setting rather than at school (Schlossberger et al. 1992). These authors also found a greater proportion of early-maturing subjects based on adolescent global self-rating than based on physician evaluation.

Our data also raise the question as to whether an increased proportion of self-evaluated earliness is actually consistent with early or precocious puberty. The response is negative since, among a group of adolescents with early or precocious puberty confirmed by physical examination, about 2/3 had a concordant self-evaluated timing whereas 1/3 were felt to be average maturers. Thus, self-perceived pubertal development does not reliably match data from physical exam and provides different information that can have its own significance for adolescent behavior. Such a conclusion has been drawn by Deppen et al. (2012), who reported that adolescent girls with average timing of menarche but early self-perceived pubertal timing were at increased risk of adolescent exploratory behaviors.

Changes in Mechanisms of Pubertal Timing

In searching for possible causes and mechanisms of changes in pubertal timing, the default hypothesis is hypothalamic-pituitary maturation, possibly because central precocity is much more frequent than peripheral precocity (Table 3). It appears, however, that peripheral mechanisms can coexist with central mechanisms or secondarily facilitate them. Such a concept is supported by the dissociation between advancement in age at onset of breast development in Denmark, the Netherlands and Belgium without a parallel change in menarcheal age, suggesting that both events are influenced by different factors, including possible breast development due to estrogenic effects independent of hypothalamic-pituitary maturation (Roelants et al. 2009; Aksglaede et al. 2009; Mul et al. 2001). That dissociation of breast development and menarche could account for the secular reduction in correlation coefficient between the ages at occurrence of the two pubertal events (Biro et al. 2006). The interpretation of the mechanistic role of sex steroids or related environmental factors is complex due to the multiple sites where they can interact between the hypothalamus and the peripheral target tissues. In particular, sex steroids exert potent inhibitory feedback on the pituitary gonadotrophins. As an illustration (Fig. 2), a girl with androgen excess caused by an adrenal adenoma has developed no breasts at 13, possibly due to the androgen/estrogen ratio as well as the negative feedback caused at the pituitary level. However, hypothalamic maturation should have progressed silently since, 6 months after withdrawal from the androgen excess, breast development is nearly adult and menarche occurs. This condition is somehow comparable with the mechanism that we have delineated in a female rodent model (Rasier et al. 2007, 2008) to explain sexual precocity in girls who were previously exposed to the estrogenic insecticide DDT and then withdrawn from that environment after migration to Belgium (Krstevska-Konstantinova et al. 2001).

Table 3 Changes in pubertal timing: MECHANISMS

Common opinion	Revision
Central puberty as default hypothesis	Peripheral mechanisms likely coexisting with or preceding secondary central puberty
Direct causal role of nutrition in onset of puberty established	Factors such as EDCs affect both energy balance and pubertal timing
Focus on environmental conditions right before onset of puberty	Fetal and neonatal origin of changes in pubertal timing
Variance of pubertal timing explained by genetic factors for 70–80 % and environmental factors for 20–30 %	Environmental and genetic factors interaction through polymorphisms and epigenetics
Increase in age at pubertal onset is associated with reduction in pubertal growth and little or no change in final height	In the male, promotion of bone growth by androgens and bone maturation by estrogens can be dissociated, e.g., using aromatase inhibitors

EDCs endocrine-disrupting chemicals

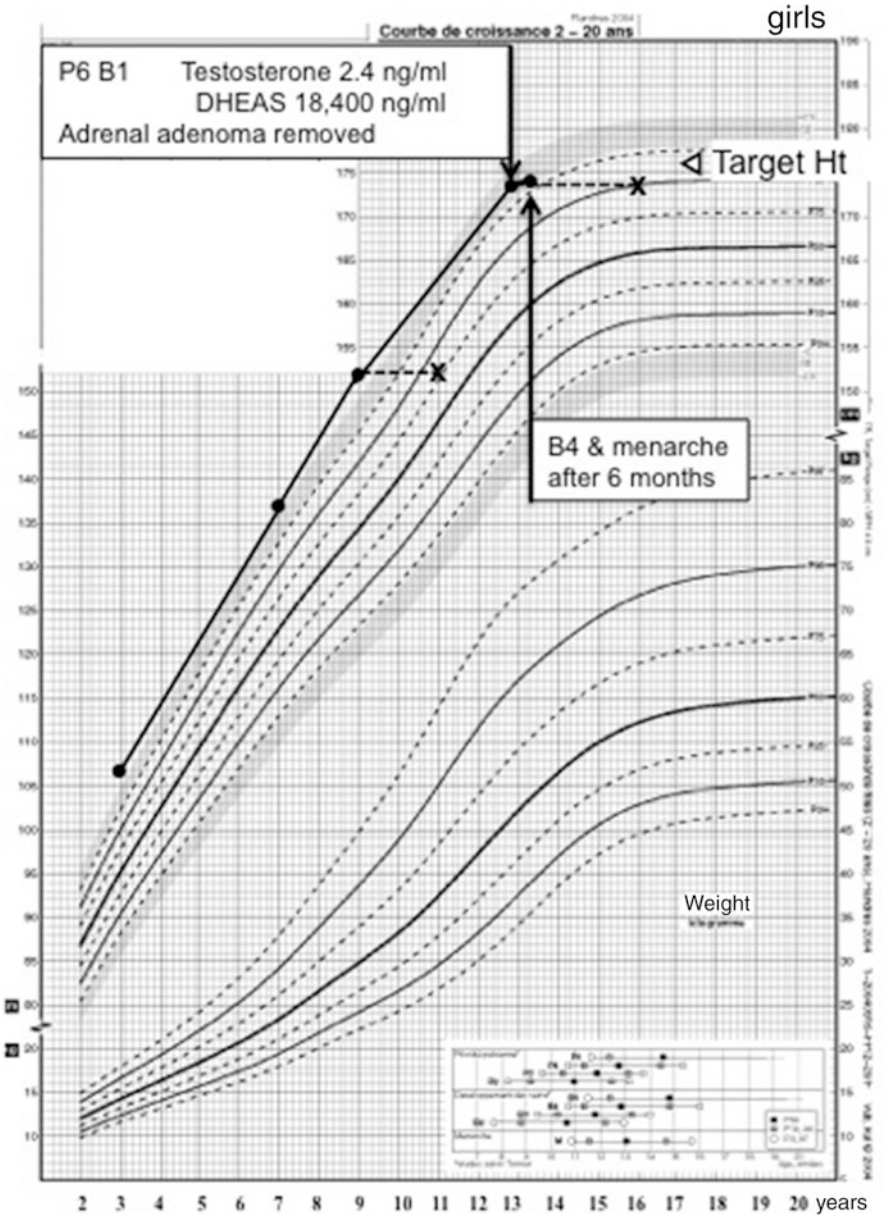

Fig. 2 Growth curve of a female patient presenting at 13 years with no breast development (B1 Tanner's stage) and adult pubic hair (P6 Tanner's stage) due to an androgen-secreting adrenal adenoma. Within 6 months after surgical removal of the adenoma, breasts attained B4 stage and menarche occurred. Target height and bone age (*X*) are indicated

Because nutrition has long been known to be a determinant of puberty, especially in females, a direct causal relationship between obesity and earlier onset of puberty has been hypothesized (Parent et al. 2003). Evidence has indeed accumulated that a sufficient amount of fat mass signaling to the neuroendocrine system through leptin is a prerequisite to the onset of puberty. Leptin can stimulate pulsatile GnRH secretion and is indeed necessary but not sufficient to account for the onset of puberty (Lebrethon et al. 2007). It appears also that both energy balance and pubertal timing share common regulatory factors that could be jointly influenced during prenatal or neonatal organization of the neuroendocrine control of homeostasis (Bourguignon et al. 2010, 2013) Along the same lines, most studies on the role of environmental factors in triggering the onset of puberty were centered on the immediately preceding period. Now, the impact of events during fetal and neonatal periods appears to be equally important. For instance, Ibanez et al. (2011) have provided evidence that, in girls with premature pubarche, menarche is advanced as a function of reduction in birth weight. Studies of the effects of neonatal exposure to endocrine disrupters such as diethylstilbestrol (DES) in the female rat showed opposing effects on GnRH secretion and pubertal timing, depending on the dose (Franssen et al. 2014). Moreover, both prenatal food restriction and neonatal DES exposure resulted in a reduced response of pulsatile GnRH secretion to leptin, with both effects being additive (Franssen et al. 2014). Though the mechanism of variations in pubertal timing caused in fetal or neonatal life is not fully elucidated, it could involve epigenetics that, together with polymorphisms, teach us that the contribution of genetic and environmental factors is intricate in explaining the variance in pubertal timing.

While the pubertal growth spurt is decreasing with age at onset of puberty, the impact of differences in pubertal timing on final height is limited (Bourguignon 1988). Such a finding, however, may be revised based on the dissociated gain in height and progression of bone maturation in two prismatic male cases with estrogen receptor or aromatase deficiency (Smith et al. 1994; Carani et al. 1997). Those observations have highlighted the specific role of estrogens in bone maturation and led some authors to use aromatase inhibitors in short adolescents and demonstrated a significant gain in adult stature (Hero et al. 2006). Such treatment, however, may involve undesirable effects such as vertebral deformities and should be restricted to research protocols that also have to delineate the possible effects of such treatment on brain and behavior.

Acknowledgments Supported by the “Belgian Fonds de la Recherche Scientifique Médicale” , the University of Liège and the Belgian Study Group for Pediatric Endocrinology.

References

Aksglaede L, Olsen LW, Sorensen TI, Juul A (2008) Forty years trends in timing of pubertal growth spurt in 157,000 Danish school children. PLoS One 3:e2728

Aksglaede L, Sorensen K, Petersen JH, Skakkebaek NE, Juul A (2009) Recent decline in age at breast development: the Copenhagen Puberty Study. Pediatrics 123:e932–e939

Berg-Kelly K, Erdes L (1997) Self-assessment of sexual maturity by mid-adolescents based on a global question. Acta Paediatr 86:10–17

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

Bond L, Clements J, Bertalli N, Evans-Whipp T, McMorris BJ, Patton GC, Toumbourou JW, Catalano RF (2006) A comparison of self-reported puberty using the Pubertal Development Scale and the Sexual Maturation Scale in a school-based epidemiologic survey. J Adolesc 29:709–720

Bourguignon JP (1988) Linear growth as a function of age at onset of puberty and sex steroid dosage: therapeutic implications. Endocr Rev 9:467–488

Bourguignon JP, Rasier G, Lebrethon MC, Gerard A, Naveau E, Parent AS (2010) Neuroendocrine disruption of pubertal timing and interactions between homeostasis of reproduction and energy balance. Mol Cell Endocrinol 324:110–120

Bourguignon JP, Franssen D, Gerard A, Janssen S, Pinson A, Naveau E, Parent AS (2013) Early neuroendocrine disruption in hypothalamus and hippocampus: developmental effects including female sexual maturation and implications for endocrine disrupting chemical screening. J Neuroendocrinol 25:1079–1087

Brooks-Gunn J, Warren MP, Rosso J, Gargiulo J (1987) Validity of self-report measures of girls' pubertal status. Child Dev 58:829–841

Cance JD, Ennett ST, Morgan-Lopez AA, Foshee VA (2012) The stability of perceived pubertal timing across adolescence. J Youth Adolesc 41:764–775

Carani C, Qin K, Simoni M, Faustini-Fustini M, Serpente S, Boyd J, Korach KS, Simpson ER (1997) Effect of testosterone and estradiol in a man with aromatase deficiency. N Engl J Med 337:91–95

Clavel-Chapelon F, E3N-Epic group, European Prospective Investigation into Cancer (2002) Evolution of age at menarche and at onset of regular cycling in a large cohort of French women. Hum Reprod 17:228–232

Deppen A, Jeannin A, Michaud PA, Alsaker F, Suris JC (2012) Subjective pubertal timing and health-compromising behaviours among Swiss adolescent girls reporting an on-time objective pubertal timing. Acta Paediatr 101:868–872

Dick DM, Rose RJ, Pulkkinen L, Kaprio J (2001) Measuring and understanding its impact: a longitudinal study of adolescent twins. J Youth Adolesc 30:385–399

Dorn LD, Biro FM (2011) Puberty and its measurement: a decade in review. J Res Adolesc 21:180–195

Duke PM, Litt IF, Gross RT (1980) Adolescents' self-assessment of sexual maturation. Pediatrics 66:918–920

Franssen D, Ioannou YS, Alvarez-Real A, Gerard A, Mueller JK, Heger S, Bourguignon JP, Parent AS (2014) Pubertal timing after neonatal diethylstilbestrol exposure in female rats: neuroendocrine vs peripheral effects and additive role of prenatal food restriction. Reprod Toxicol 44:63–72

Hergenroeder AC, Hill RB, Wong WW, Sangi-Haghpeykar H, Taylor W (1999) Validity of self-assessment of pubertal maturation in African American and European American adolescents. J Adolesc Health 24:201–205

Herman-Giddens ME, Slora EJ, Wasserman RC, Bourdony CJ, Bhapkar MV, Koch GG, Hasemeier CM (1997) Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. Pediatrics 99:505–512

Herman-Giddens ME, Steffes J, Harris D, Slora E, Hussey M, Dowshen SA, Wasserman R, Serwint JR, Smitherman L, Reiter EO (2012) Secondary sexual characteristics in boys: data from the Pediatric Research in Office Settings network. Pediatrics 130:e1058–e1068

Hero M, Wickman S, Dunkel L (2006) Treatment with the aromatase inhibitor letrozole during adolescence increases near-final height in boys with constitutional delay of puberty. Clin Endocrinol 64:510–513

Ibanez L, Lopez-Bermejo A, Diaz M, Marcos MV (2011) Endocrinology and gynecology of girls and women with low birth weight. Fetal Diagn Ther 30:243–249

Jeurissen A (1969) Age of the establishment of the menarche and its evolution in Belgium during the last 40 years. Acta Paediatr Belg 23:319–330

Juul A, Magnusdottir S, Scheike T, Prytz S, Skakkebaek NE (2007) Age at voice break in Danish boys: effects of pre-pubertal body mass index and secular trend. Int J Androl 30:537–542

Krstevska-Konstantinova M, Charlier C, Craen M, Du Caju M, Heinrichs C, de Beaufort C, Plomteux G, Bourguignon JP (2001) Sexual precocity after immigration from developing countries to Belgium: evidence of previous exposure to organochlorine pesticides. Hum Reprod 16:1020–1026

Lebrethon MC, Aganina A, Fournier M, Gerard A, Parent AS, Bourguignon JP (2007) Effects of in vivo and in vitro administration of ghrelin, leptin and neuropeptide mediators on pulsatile gonadotrophin-releasing hormone secretion from male rat hypothalamus before and after puberty. J Neuroendocrinol 19:181–188

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

Mul D, Fredriks AM, van Buuren S, Oostdijk W, Verloove-Vanhorick SP, Wit JM (2001) Pubertal development in The Netherlands 1965–1997. Pediatr Res 50:479–486

Parent AS, Teilmann G, Juul A, Skakkebaek NE, Toppari J, Bourguignon JP (2003) The timing of normal puberty and the age limits of sexual precocity: variations around the world, secular trends, and changes after migration. Endocr Rev 24:668–693

Petersen AC, Crockett L, Richards M, Boxer A (1988) A self-report measure of pubertal status: reliability, validity, and initial norms. J Youth Adolesc 17:117–133

Rasier G, Parent AS, Gerard A, Lebrethon MC, Bourguignon JP (2007) Early maturation of gonadotropin-releasing hormone secretion and sexual precocity after exposure of infant female rats to estradiol or dichlorodiphenyltrichloroethane. Biol Reprod 77:734–742

Rasier G, Parent AS, Gerard A, Denooz R, Lebrethon MC, Charlier C, Bourguignon JP (2008) Mechanisms of interaction of endocrine-disrupting chemicals with glutamate-evoked secretion of gonadotropin-releasing hormone. Toxicol Sci 102:33–41

Roelants M, Hauspie R, Hoppenbrouwers K (2009) References for growth and pubertal development from birth to 21 years in Flanders, Belgium. Ann Hum Biol 36:680–694

Schlossberger NM, Turner RA, Irwin CE Jr (1992) Validity of self-report of pubertal maturation in early adolescents. J Adolesc Health 13:109–113

Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS (1994) Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med 331:1056–1061

Sorensen K, Aksglaede L, Petersen JH, Juul A (2010) Recent changes in pubertal timing in healthy Danish boys: associations with body mass index. J Clin Endocrinol Metab 95:263–270

Talma H, Schonbeck Y, van Dommelen P, Bakker B, van Buuren S, Hirasing RA (2013) Trends in menarcheal age between 1955 and 2009 in the Netherlands. PLoS One 8:e60056

Vercauteren M, Susanne C (1985) The secular trend of height and menarche in Belgium: are there any signs of a future stop? Eur J Pediatr 144:306–309

Index

J.-P. Bourguignon et al. (eds.), *Brain Crosstalk in Puberty and Adolescence*,
Research and Perspectives in Endocrine Interactions 13,
DOI 10.1007/978-3-319-09168-6