Brain Magnetic Resonance evaluation and pubertal development variations among female adolescents

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ABSTRACT

Aim: There is evidence that adolescents with distinct rhythms of pubertal maturation have different psychosocial behaviours. However, a relation between pubertal maturation and the development of the prefrontal cortex and limbic system was not yet established. The aim of this study was to apply brain magnetic resonance imaging to investigate brain development of teenagers with similar age but distinct timing of pubertal maturation.

Methods: We compared the hormonal profiles and brain structure and volume using magnetic resonance imaging of two groups of 14 year-old girls, four with an early pubertal development (group 1) and three with an average pubertal development (group 2).

Results: The age at onset of puberty was the only biological variable that distinguished the two groups (p = 0,05). There were no differences regarding global brain volume. However, early-maturers showed a tendency for an increased volume of all subcortical structures except for nucleus accumbens.

Conclusion: To our knowledge, this is the first work to address the relation of pubertal maturation timing and central nervous system development using brain magnetic resonance imaging. The observed tendency for an increased volume of the subcortical structures may be related to a possible delayed development of the nucleus accumbens in early-maturers, and may explain the increased vulnerability of this group to risk behaviours.

Keywords: Adolescence; brain development; magnetic resonance imaging; neuroimaging; puberty

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TRADUÇÃO NEUROIMAGIOLÓGICA DOS RITMOS DE MATURAÇÃO PUBERTÁRIA EM ADOLESCENTES DO SEXO FEMININO

RESUMO

Introdução: Há evidência que adolescentes com a mesma idade cronológica mas com estádios pubertários distintos têm comportamentos psicossociais diferentes. Desconhece-se, no entanto, se existe alguma relação entre o ritmo de maturação pubertária e o desenvolvimento do córtex pré-frontal e sistema límbico.

Objetivo: Pretendeu-se com este trabalho avaliar através de ressonância magnética, o desenvolvimento cerebral de adolescentes do sexo feminino com a mesma idade cronológica mas com distintos ritmos de maturação pubertária.

Material e Métodos: Avaliaram-se parâmetros hormonais e imagiológicos (idade óssea, ultrassonografia pélvica e ressonância magnética cerebral estrutural) a adolescentes de sexo feminino de 14 anos de idade.

Resultados: Foram incluídas no estudo quatro adolescentes maturadoras precoces (grupo 1) e três maturadoras médias (grupo 2), A idade de início da puberdade foi a única variável biológica que distinguiu os 2 grupos (p=0,05).

Apesar de não se encontrar significado estatístico, verificou--se que as maturadoras precoces, apresentavam volumes de todas as estruturas subcorticais estudadas superiores às maturadoras médias, com exceção do núcleo accumbens.

Conclusões: Com base na pesquisa bibliográfica efetuada, este estudo foi o primeiro a tentar relacionar os ritmos de maturação pubertária e o desenvolvimento cerebral através da ressonância magnética. A tendência observada para o aumento do volume das estruturas subcorticais poderá estar relacionado com um possível atraso na maturação do núcleo accumbens nas maturadoras precoces e poderá explicar o aumento da vulnerabilidade deste grupo para comportamentos de risco.

Consideramos que poderá constituir o ponto de partida para investigações futuras, de forma a compreender melhor o desenvolvimento adolescente e assim permitir encontrar formas mais eficazes de intervenção.

Palavras-chave: Adolescência; desenvolvimento cerebral; neuroimagem, puberdade; ressonância magnética

INTRODUCTION

Adolescence is defined by marked physical and psychosocial changes.[1-6] It is a period of great vulnerability, particularly associated to experimentation and risk behaviours.[7] The transition between childhood and adulthood is not straightforward and it is well known that adolescents with distinct rhythms of pubertal maturation have different psychosocial behaviours.[2,8-10] Three distinct theories try to explain such differences: the "stressful change hypothesis", the "off-time hypothesis" and the "early-timing hypothesis".[9] The first theory predicts that regardless of timing, girls experiencing the pubertal transition will manifest higher levels of emotional disturbance than pre or post-pubertal girls. The "off-time hypothesis" explains that both early and late maturing girls will manifest more psychological problems than their on-time age-mates. Finally, the "early-time hypothesis" advocates that girls maturing earlier than their peers will be less emotionally stable and will have more behaviour problems. Indeed, several studies have shown that early-maturer girls have a lower self-esteem, a higher risk of drug use and misuse and tend to carry a psychopathological profile, being more vulnerable to peer pressure.[3,5,6]

Although influenced by numerous factors such as genetics, nutrition and ethnicity, which may influence physical development, puberty is essentially a hormonal and neuroendocrinological process.[1,4,7,11,12] Among girls, puberty starts between eight and 13 years of age (mean age: 10 years) with the onset of the thelarche.[2,5,7,13,14]

Notably, adolescence is recognized as the largest and most dynamic period of neuronal development. Longitudinal studies have revealed that grey matter maturation is represented by an inverted U-shape curve during childhood and adolescence, occurring from posterior to anterior regions of the brain. Accordingly, the prefrontal cortex (PFC), which participates in risk-benefit assessment and decision-making, is described as the last brain region to mature.[6,13,15-19] As a result, the immature motivational circuit – limbic system/PFC – favours activity in brain emotional areas, including the nucleus accumbens and the amygdala.[4,13,15] These data suggest that there may be a brain developmental explanation for the impulsivity and risk behaviours of adolescence.[8]

Magnetic resonance imaging (MRI) has been invaluable in the study of the central nervous system in humans *in vivo*, since it is an innocuous, reproducible and repeatable technique that offers high tissue contrast and spatial resolution.[18-20]

The purpose of this study was to investigate with MRI the brain development of female adolescents with the same chronological age but distinct rhythms of pubertal maturation.

To avoid potential confounding variables (such sex or endocrinology pathology) only healthy female adolescents were selected.

METHODS

Sample

The sample was identified and recruited from the Outpatient Clinic of the Pediatric Endocrinology Unit of Centro Hospitalar São João - EPE, Porto, Portugal. All the volunteers were previously followed in this unit by suspected (not confirmed) pathology.

The inclusion criteria were the following: adolescent females of 14 years of age who had the thelarche between eight and nine years of age (early-maturers) or between 10 and 11 years of age (average-maturers). Exclusion criteria included having an associated pathology and / or being under pharmacological therapy. The study was approved by the Ethics Committee of the Centro Hospitalar São João - EPE. All volunteers and their legal representatives signed an informed consent according to the Helsinki Declaration.[21]

Clinical data

The following data was collected from the individual clinical files: weight, height, body mass index (BMI), the age at thelarche and menarche, sexual maturity rating (Tanner stage) (TS), hormonal profile previously performed within three months (including FSH – follicle-stimulating hormone; LH – luteinizing hormone; estradiol; 17OHP – 17 – hydroxyprogesterone; testosterone; DHEAS – dehydroepiandrosterone sulphate; androstenedione; IGF1 – insulin-like growth factor 1 (somatomedin C); FT4 – free thyroxine; TSH – thyroid stimulating hormone), bone age and pelvic ultrasound (including ovary volume, uterine volume and presence of endocavitary line).

Magnetic resonance imaging and brain volumetry

MRI brain examinations were conducted in the Department of Radiology of the Centro Hospitalar São João, EPE, using a 3.0T system (MAGNETOM® Tim Trio, Siemens Medical Solutions, Erlangen, Germany), equipped with a 12-channel head phased-array coil. All the datasets were acquired in a single session, with the presence of a neuroradiologyst. A 3D-T1-w pulse sequence (magnetisation prepared rapid acquisition gradient echo – MPRAGE) was acquired with the following parameters: 160 slices, 265 mm field-of-view (FOV); spatial resolution $1x1x1mm^3$, TR/TE/f.a. = 2030/2.96ms/9º.

Image processing was performed using the FMRIB Software Library (FSL) analysis tools.[22,24] Brain volumetry (total brain, white matter and grey matter volumes) were calculated with the Sienax package from FSL, while the volume of subcortical structures (hippocampus, amygdala, thalamus, caudate nucleus, putamen, globus pallidus and nucleus accumbens) were measured with the FIRST toolbox, also part of FSL.[22,26,27]

Statistical analysis

Frequency distributions were generated for every categorical variable for the descriptive statistics. For non-homogenous variances, the *Mann-Whitney* test was adopted. Relations between hormone levels and subcortical structures were assessed using Spearman correlation coefficients. Statistical analyses were performed using SPSS 21 (IBM SPSS software). Results of the brain volumetry and of the volume of subcortical structures are presented as median and standard deviation (SD). Statistical significance was considered for $p \leq 0.05$.

RESULTS

Thirteen adolescents were identified. Four were excluded due to no parental authorization (n=3) and participation refusal (n=1). From the nine volunteers that were included in the study, five were early-maturers (group 1) and four were average-maturers (group 2). The demographic characteristics of the sample are detailed in Table1.

The mean age of onset of puberty was 8,75 years for the early-maturers (range 8-11y) and 10,3 years for the average-maturers (range 10-11y), respectively. Menarche was at mean age of 11y for group 1 (minimum 11 years, maximum 12 years) and 12y for group 2 (minimum 11 years, maximum 13 years). All adolescents were TS 5, except for volunteer 5 who was stage 4. There were no differences between groups regarding age at menarche (p = 0,229), weight (p = 0,857) and BMI (p = 0,857).

Pelvic ultrasound evaluation was compatible with the respective pubertal development in all cases. Bone age was higher than chronological age in all adolescents (mean 15.4 ± 0.73 y). The hormonal profile was obtained for eight of the total of nine adolescents (Table 2). Hormonal results of all adolescents were within the reference values for age and gender, except for volunteer 1, 4 and 5, highlighted in Table 1, which were slightly above the reference values but without pathological relevance. Group 1 had higher levels than group 2 (not statistically significant) for FSH (p = 0,400), FT4 (p = 0,400), androstenedione (p = 1) and estradiol (p = 1); and lower levels of LH (p = 0,400), testosterone (p = 0,400), DHEAS (p = 0,400), IgF1 (p = 1), 17OHP (p = 0,700) and TSH (p = 0,629).

Regarding brain MRI, one adolescent did not complete the brain scan (V9). A diencephalic lesion was incidentally detected in another volunteer (V3), which was diagnosed later as a pilocytic astrocytoma. Threrefore both volunteer (V3 and V9) were excluded.

Global brain and subcortical structures volumetry results are presented in Table 2. There were no statistical differences between the two groups for total brain volume, as well as for grey matter and white matter volumes (Figure 1). Concerning the volume of the subcortical structures, group 1 evidenced higher volumes for all subcortical structures (left and right-sided) than group 2, except for the nucleus accumbens (Figures 2 and 3).

Correlation of the subcortical brain structures volume and hormone levels was performed (Table 3). We found a positive correlation between FSH levels and the volume of the putamina (r = 0,886), and as well as between DHEAS levels and the volume of the left nucleus accumbens (r = 0,829). Negative correlations were found between LH levels and the left (r = -0,943) and right hippocampi volume (r = -1); between testosterone levels and the left caudate nucleus (r = -0,829), left and right putamina (r = -0,829) and globus pallidus (left r = -0,943; right r = -0,886); between estradiol and the left nucleus accumbens (r = -0,886); and between androstenedione levels and the right nucleus accumbens volume (r = -0,943).

DISCUSSION

Our research aimed at assessing brain neurodevelopment by using brain MRI on a clinical sample of adolescents with distinct maturing rhythms. To our knowledge, this study is among the first to address the relation of pubertal maturation timing and the central nervous system development using MRI.

Regarding total brain volume, there were no differences between the early and average-maturers, despite early-maturers had had higher volumes for all subcortical structures except for the nucleus accumbens. It is well known that this nucleus

Table 1 – Characteristics of the study population.

ID	Group	T	M	W	BMI	P	TS	FSH (1.4-40)	LH (1.1-45)	Estrad (19-528)	Test (11-72)	DHEAS (144-285)	Andros (0.8-3,3)	17-OHP (0.2-2,3)	IGF1 (261-540)	FT4 (0,6-1,6)	TSH (0.2-5,4)
V1	2	10	11	51,5	22,1	NP	5	3,44	5,61	284	**90**	191	**5,37**	1,3	277	0,93	1,91
V2	1	8	11	47,0	17,9	NP	5	7,88	5,53	62	24	98	1.2	0,87	327	0,69	2,02
V3	1	8	12	54,5	22,3	NP	5	0,29	0,21	12	43	188	2,02	0,34	138	0,74	1,72
V4	1	9	11	53,0	22,6	NP	5	7,09	4,72	116	45	83	**4,75**	1,07	476	0,99	1,18
V5	2	10	12	51,5	21,2	NP	4	6,09	3,59	48	37	**317**	2,78	1,34	518	0,82	2,74
V6	2	11	13	58,0	22,1	NP	5	5,51	6,64	54	49	154	2,36	1,1	550	0,69	1,86
V7	1	9	11	55,0	21,7	NP	5	5,15	3,33	42	46	219	3,11	1,86	524	0,9	2,41
V8	1	9	11	66,0	29,7	O	5	-	-	-	-	-	-	-	502	0,99	0,92
V9	2	11	12	95,4	35,5	O	5	5,22	8,02	53,4	25	132,2	-	2,2	-	1,15	2,24

Legend: [ID: identification, T: thelarche (years), M: menarche (years), W: weight (kgs), BMI: body mass index, P: percentile, NP: normoponderal, O: obesity, TS: Tanner stage, FSH: follicle stimulating hormone (mU/ml), LH:- luteinizing hormone (mU/ml), Estrad: estradiol (pg/ml), Test: testosterone (ng/dl), DHEAS: dehydroepiandrosterone sulphate (ug/dl), Andros: androstenedione (ng/ml), 17OHP: 17 hydroxyprogesterone (ng/ml), IGF1: insulin-like growth factor 1 (somatomedin C) (ng/ml), FT4: free thyroxine (ng/dl), TSH: thyroid stimulating hormone (uU/ml). Normal values between brackets. The highlighted values in bold are slightly above the reference values.]

**Table 2 –** Total brain volume and subcortical structures volume in mm³.

	V1	V2	V4	V5	V6	V7	V8
Group	2	1	1	2	2	1	1
TBV	1638251,24	1673169,48	1613117,76	1646785,79	1562173,12	1554448,93	1627019.53
GM	898179,44	950950,46	924326,55	943836,23	896585,23	868779,82	911730.66
WM	740071,80	722219,02	688791,21	702949,56	665587,89	685669,11	715288.87
hipoL	3513,61	3668,98	3935,80	4145,82	2949,98	3963,66	3539,32
hipoR	3726,85	3889,72	3993,66	4179,04	3569,33	4449,07	3716,13
talL	7178,30	8218,78	7470,83	8104,12	7076,51	8202,70	7622,99
talR	7305,82	8108,41	7634,78	7959,46	7034,72	8035,54	7661,57
cauL	3137,49	4647,31	3368,95	3526,47	3232,86	3702,20	4135,11
cauR	3552,88	4466,21	3514,68	3902,58	3412,88	3680,77	4202,61
putL	4384,77	5432,75	5688,85	5378,10	4729,81	4770,53	5519,55
putR	4220,83	5703,85	5920,31	5254,87	4593,72	4712,67	5916,02
palL	1638,40	1885,93	1673,76	1820,56	1643,76	1703,76	1719,84
palR	1615,90	1793,77	1755,20	1818,42	1611,61	1700,55	1643,76
amyL	1172,27	1401,59	1449,81	1055,48	893,67	1120,84	1339,44
amyR	1144,41	1266,57	1624,47	1735,91	1173,35	1295,50	1165,84
accuL	415,76	486,48	403,97	648,29	527,20	565,78	491,84
accuR	377,54	523,99	354,68	484,34	498,27	474,70	495,06

Legend: ID: identification of volunteers; TBV: total brain volume; GM: Grey matter; WM: white matter; hipo: hippocampus; tal: thalamus, cau: caudate; put: putamen; pal: pallidus; amy: amygdala; accu: accumbens, L-left, R-right.

**Table 3 –** Hormonal effects on the subcortical structures volume.

Structure	Hormonal effect	r_s	r_s^2	P
Putamen left	FSH +	+0,886	0,784	0,019
	Test -	-0,829	0,687	0,042
Putamen right	FSH +	+0,886	0,784	0,019
	Test -	-0,829	0,687	0,042
Accubens left	DHEAS+	+0,829	0,687	0,042
	Estradiol -	-0,886	0,784	0,019
Accubens right	Androst -	-0,943	0,889	0,005
Hippocampus left	LH -	-0,943	0,889	0,005
Hippocampus right	LH -	-1	1	<0,001
Caudate left	Test -	-0,829	0,687	0,042
Pallidus left	Test -	-0,943	0,889	0,005
Pallidus right	Test -	-0,886	0,784	0,019

Legend: rs: Spearman correlation coefficient, rs2: coefficient of determination.
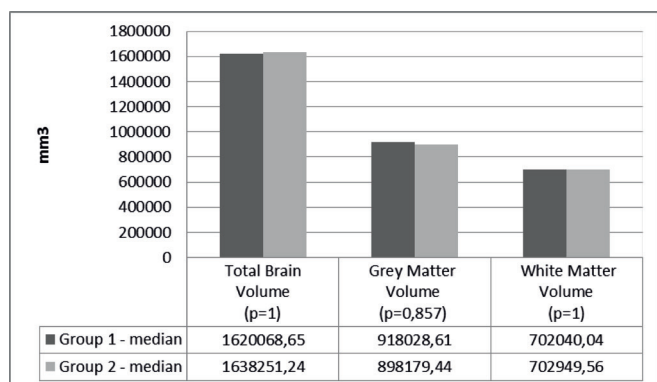
Figure 1 – Volumetry of global brain (total brain, grey matter and white matter).
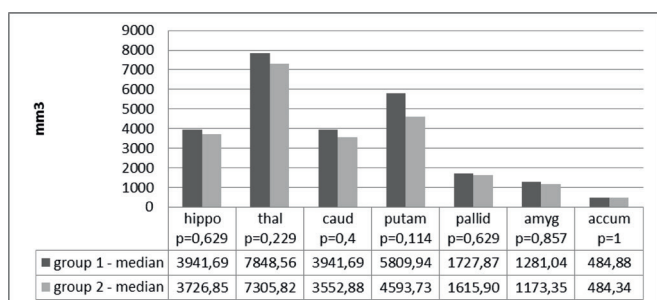Comparison between group 1 (early-maturers) and group 2 (average-maturers).
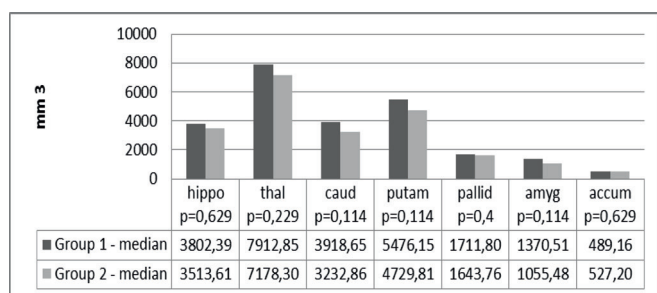
Figure 2 – Volumetry of left subcortical structures.
Comparison between group 1 (early-maturers) and group 2 (average-maturers).
[hippo: hippocampus; thal: thalamus; caud: caudate; putam: putamen; pallid: pallidus; amyg: amygdala; accum: accumbens].

Figure 3 – Volumetry of right subcortical structures.
Comparison between group 1 (early-maturers) and group 2 (average-maturers).
[hippo: hippocampus; thal: thalamus; caud: caudate; putam: putamen; pallid: pallidus; amyg: amygdala; accum: accumbens].

is involved in the connection between the limbic system and the PFC, and is associated with pleasure and reward. (28) The observed tendency for the higher volumes of all other subcortical structures among early-maturers may be explained by a possible delayed development of the nucleus accubens in this group and this could potentially translate into

the increased vulnerability and engagement in risk behaviours typical of early-maturers.

Regarding the hormonal profile, we found that group 1 had higher levels of androstenedione and lower levels of testosterone and DHEAS than group 2, despite not reaching statistical significance. Although no causal linearity has been established between the hormonal profiles and behaviour so far, it has been shown that boys and girls with maladaptive behaviours have higher levels of androstenedione and lower levels of testosterone and DHEAS during their early and middle adolescence, what is in line with the tendency of our results. Furthermore, it is well known the relevant role of the gonadal hormones on the central nervous system maturation, with for example both progesterone and estradiol facilitating myelination in adolescent females, while testosterone has a similar role in males.[4,7] It is known as well that the hippocampus and the amygdala have a higher number of estrogen and androgen receptors, respectively, what may explain the largest volume of the amygdala among males during adolescence and hippocampal volume only among females.[17,18]

While the association between the levels of circulating gonadal hormones and sex-specific differences is well established, the effects of sexual hormones and pubertal stage on brain development, is not yet well understood. Studies are not consensual, with some suggesting a pubertal-related maturation of the hippocampus, amygdala and cortical grey-matter, and others not having been able to find a significant association between sexual hormones and the subcortical structures.[12,29,30]

The strong correlations between hormones with direct influence on the sexual development (FSH, LH, estradiol, testosterone, DHEAS, androstenedione) and the brain subcortical structures found in our study should be further explored.

The most important limitation of our study was the small sample size, which was related to difficulties in recruiting voluntary adolescents. The accidental finding of a diencephalic tumour in an asymptomatic volunteer, allowed timely surgery, with complete resection.

In spite of the small sample, our results support the hypothesis that brain maturation of early-maturers is not proportionate to biological maturation, in particular to the nucleus accumbens. This study should be seen as a starting point for future research for better understanding adolescent's behavior and allowing the design of more efficacious interventions.

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Info box: abbreviations

17OHP	17-Hydroxyprogesterone
BMI	Body mass index
DHEAS	Dehydroepiandrosterone sulphate
IGF1	Insulin-like growth factor 1 (somatomedin C)
FIRST	FMRIB´s integrated registration and segmentation tool
FSH	Follicle-stimulating hormone
FSL	FMRIB´s software library
FT4	Free thyroxine
LH	Luteinizing hormone
MPRAGE	Magnetization-prepared rapid acquisition gradient echo
MRI	Magnetic resonance imaging
PFC	Prefrontal cortex
TS	Tanner stage
TSH	Thyroid stimulating hormone

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