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Transitions in the transcriptome of the serotonergic and dopaminergic systems in the human brain during adolescence

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Abstract
Adolescence is a period of profound neurophysiological, behavioral, cognitive and psychological changes, but not much is known about the underlying molecular neural mechanisms. The aim of this study was to systematically analyze expression levels of the genes forming serotonergic and dopaminergic synapses during adolescence. We analyzed the mRNA expression profiles of genes that code for all components of serotonergic and dopaminergic synapses, in 16 brain areas from human and non-human primates from public domain databases, to detect genes whose expression changes during adolescence. Two serotonin receptors, *HTR1E* and *HTR1B* had expression levels that exhibit a sharp transition in the prefrontal cortex in adolescence, but we found no similar transition in the dopaminergic system. A similar but smoother rise in expression levels is observed in *HTR4* and *HTR5A*, and in *HTR1E* and *HTR1B* in three other expression datasets published. An earlier rise is observed in *HTR1A*, and a smooth and significant rise with age is observed in the expression of *HTR1E* in microarray measurements in macaque monkeys. The expression of *HTR1E* and *HTR1B* is correlated across subjects within each age group, suggesting that they are controlled by common mechanisms. These results point to *HTR1E* and *HTR1B* as major candidate genes involved in adolescence maturation processes, and to their operation through common control mechanisms. The maturation profiles may also involve several other 5-HT receptors, including the genes *HTR5A*, *HTR4* and *HTR1A*.

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1. Introduction

Adolescence is a transitional period of profound changes in many behavioral domains, including mood lability, cognition, decision-making and thereby impulsive and high risk-taking behavior (Arain et al., 2013; Steinberg and Morris, 2001; Steinberg, 2004). With onset of puberty, aggressive impulsive behaviors become more prominent and then later in adolescence, a decline in aggressive impulsive behaviors begins that continues throughout adulthood. Studies in animal models and human subjects indicate that two major families of neurotransmitters are involved in regulating these behaviors: serotonin and dopamine (Duke et al., 2013; Nemoda et al., 2011). Lower brain serotonin increases impulsive behavior (Depue and Collins, 1999; Duke et al., 2013; Fletcher et al., 2007; Katz, 1999; Mann et al., 2001; Winstanley et al., 2005). Dopamine plays a role in impulsivity and aggressive behaviors (Davey et al., 2008; Nemoda et al., 2011; Pattij and Vanderschuren, 2008). The dopaminergic system also interacts with serotonergic system: serotonin inhibits dopamine activity, particularly in relation to dopaminergic role in aggressive and impulsive behaviors (Goveas et al., 2004; van der Vegt et al., 2003; Winstanley et al., 2006).

Despite the large body of evidence suggesting that serotonin and dopamine are related to changes in adolescence behavior (Daws and Gould, 2011; Dillon et al., 1991; Dinopoulos et al., 1997; Nemoda et al., 2011), the specific genes, proteins and mechanisms underlying such changes in impulsive behavior are not well understood. It has been suggested that these behavioral changes are caused by having different brain structures mature at different rates, and specifically, that the prefrontal cortex matures later than the limbic system and myelination of tracts targeting the ventral prefrontal cortex is not complete until late adolescence (Casey et al., 2010). However, details of pathway maturation in terms of neurotransmitter systems and timing of changes in adolescents in the brain in normal development is mostly unknown. Our current study focuses on genes from two systems, serotonin and dopamine, since these systems are tightly linked to behavioral changes. Other pathways and systems, including myelination, plasticity, synaptic formation and pruning will be studied elsewhere.

Receptors for serotonin and dopamine are expressed in numerous brain regions, and it is largely unknown which brain areas are involved in adolescence maturation processes. The candidate brain areas thought to be involved in regulation of impulsivity include the orbital and medial prefrontal cortex, anterior cingulate cortex, amygdala, the nucleus accumbens, ventral tegmentum and the dorsal raphe nucleus (Miyazaki et al., 2012). The dorsal lateral PFC plays a critical role in higher cognitive capacities, including reasoning, planning, social behavior and abstraction (Baxter, 2011; Fuster, 2002). These functions maturate throughout adolescence until adulthood, leading to gradual increase in cognitive control and behavioral inhibition (Casey et al., 2005). Characterizing age-related changes in the transcriptome in various brain structures can provide new insights into the normal process of neurotransmitter development that may underlie these behavioral changes and the psychopathology vulnerability that characterize adolescence.

The current study puts forward the hypothesis that the marked behavioral changes observed in adolescence reflect underlying changes in the gene expression of components of the serotonergic and dopaminergic neurotransmitter pathways. To identify candidate genes underling changes in impulsive behavior, we systematically analyze expression levels of the genes forming serotonergic synapses (Figure 1A) and dopaminergic synapses (Figure 1B), including both pre- and post-synaptic components. We determined the components of these two neurotransmitter systems that undergo a major transition in gene expression from childhood to adolescence by examining expression levels changes over time using public domain databases.

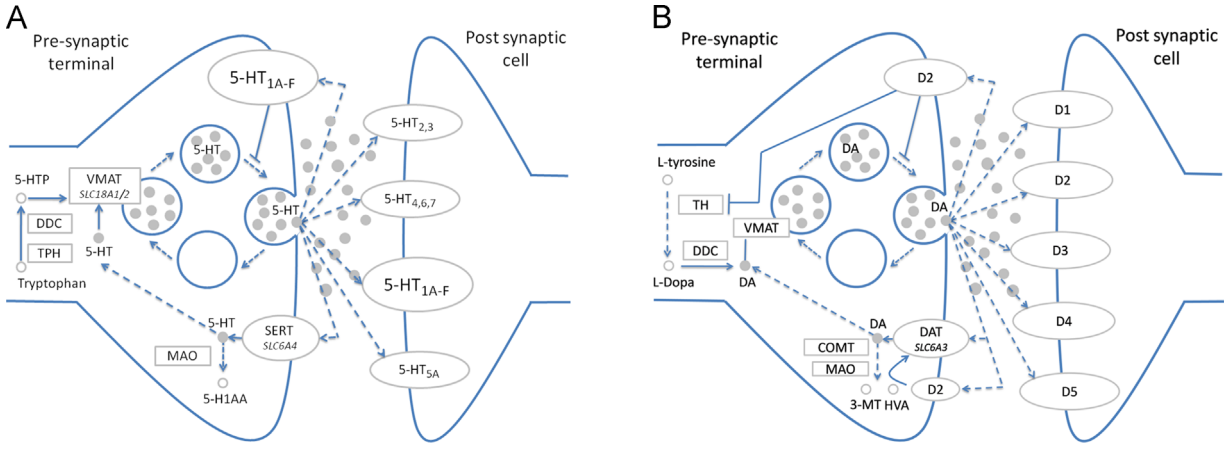

Figure 1 Schematic charts of serotonergic and dopaminergic synapses and genes in this study. (A) Serotonergic synapses include seven families of serotonin receptors: 5-HT_1 operates through G_i proteins; 5-HT_2 is coupled with G_q/G_{11} proteins, and is an excitatory serotonin receptor subtype; 5-HT_3 is a ligand-gated ion channel; 5-HT_4 and 5-HT_7 are also G-protein coupled receptors, but operate through stimulating production of cAMP; 5-HT_5 reduces cAMP through G_i and G_o; and 5-HT_6 is coupled to G_s and mediates excitatory neurotransmission. (B) Dopaminergic synapses consist of two families of dopamine receptors. D1 and D5 are coupled with G_s proteins which activate c-AMP dependent pathway. D2, D3, D4 are coupled with G_i proteins which deactivate adenylyl cyclase and inhibit the production of cAMP.

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2. Experimental procedures

2.1. Gene expression data

We analyzed transcriptome profiles collected using microarrays from the brains of 57 human donors (Kang et al., 2011). The dataset contains exon-level transcriptome of 17,565 mainly protein-coding genes collected from 11 cortical and 5 sub-cortical brain regions, collected using Affymetrix Human Exon 1.0 ST arrays. The RNA quality has been assessed via a battery of quality control experiments, and the results of selected genes were validated through real-time PCR analysis (see detailed description in the Supplementary information of Kang et al. 2011). We included all subjects older than one year and younger than 41, leaving a total of 22 subjects, 6 children, 8 adolescents and 8 adults. The brains of all subjects were inspected for genomic, facial and body abnormalities and were found to be normal. Detailed demographic and clinical data per subject, and the status of the inspected abnormalities is provided in supplemental Table 1 in the Supplementary information section of Kang et al. 2011. The data were quantile-normalized and $\log_2$-transformed.

We further analyzed the transcriptome profiles measured using RNA sequencing made available by Brainspan, an online Atlas of the Developing Human Brain (Sunkin et al., 2013). The set of donors includes a subset of 41 healthy donors out of the 57 donors in the above data, with ages ranging between 8 post-conception weeks and 40 years. After limiting cases to the same age range we were left with 18: 6 children (ages 1–10 years), 7 adolescents (11–24), 5 adults (25–41 years old).

Finally, we also analyzed microarray measures published by two other studies. First, Somel et al. (2010) measured the mRNA expression of 12,396 genes in 23 human postmortem brain samples, taken from the superior frontal gyrus of the prefrontal cortex. The samples were collected from two certified tissue banks (the NICHD Brain and Tissue Bank, and the Chinese Brain Bank Center). The mRNA levels were measured using Affymetrix Human Gene 1.0 ST arrays. The RNA quality was assessed using Agilent® 2100 Bioanalyzer. Considering all donors in the same age range (1 year to 41 years) yielding a total of 9 subjects. For detailed description see supplemental Table 1 in the Supplementary information of Somel et al. 2010. Second, Harris et al. (2009) measured mRNA expression profiles using Affymetrix HG-U133 Plus 2.0 Gene Chip in the prefrontal cortex of 48 normal subjects. RNA quality was evaluated using Agilent high-resolution electrophoresis system, and quantitative real-time PCR was performed using Applied Biosystems 7900 HT Sequence Detection System. Considered all subjects in the same age range (1 year to 41 years) yielded a total of 26 donors. For full demographic details and RNA quality assessment, see supplemental Table 1 in the Supplementary information section of Harris et al., 2009. All reported datasets were assessed for quality, quantile-normalized and $\log_2$transformed. Together, the three human datasets included a total of 93 cases. Tissue was collected after obtaining parental or next of kin consent and with approval by the institutional review boards (Harris et al., 2009; Kang et al., 2011; Somel et al., 2010).

In all three groups of donors we measured the correlation between the expression levels and clinical data including Post Mortem Intervals (PMI). We also measured the correlation between expression levels and tissue pH levels in two datasets that measured them (Kang et al., 2011; Harris et al., 2009). We calculated the correlation per gene in each region, and corrected for multiple comparison using Storey false discovery rate (FDR) (Storey, 2002). We found no significant (q-value <0.05) correlation for either PMI or pH in any of the datasets.

2.2. Non-human primate

For non-human primates, we analyzed microarray measurements collected in rhesus macaque brain and reported by Somel et al. (2011). The data set included mRNA expression levels of 12,000 genes as measured in the prefrontal cortex of 34 monkeys aged 0 to 28 years. The samples were collected from the Suzhou Experimental Animal Center. RNA quality was assessed using Agilent® 2100 Bioanalyzer. We considered macaque aged 3–5 years as adolescents based on Johnson and Kapsalis (1995). Animal tissue acquisition was approved by a review committee (Somel et al., 2011).

2.3. Calibration of gene expression values

To correct for possible variations in global expression levels due to age or inter-subject variability, we computed the mean expression level of a standard group of 575 housekeeping genes (HKG) (Eisenberg and Levanon, 2003). The mean expression level of this baseline group exhibited very little variation with age. The results of analyses were preserved when the expression levels of all genes were normalized by the mean HKG expression in the corresponding brain region and corresponding subjects.

2.4. Gene selection

We considered genes that participate in the serotonin pathway and the dopamine pathway based on KEGG (Aoki-Kinoshita and Kanehisa, 2007). We focused on genes that code for proteins that are specific to the serotonin and dopamine pathways and did not analyze proteins that participate in multiple pathways in more generic functions like signal transduction or calcium channels (a total of 33 genes). For serotonergic synapse (Figure 1A) we analyzed a total of 25 genes (Supplemental Table S1). These included 17 genes that code for seven receptors of 5-hydroxytryptamine and their subtypes: *HTR1A*, *HTR1B*, *HTR1D*, *HTR1E*, *HTR1F*, *HTR2A*, *HTR2B*, *HTR2C*, *HTR3A*, *HTR3B*, *HTR3C*, *HTR3D*, *HTR3E*, *HTR4*, *HTR5A*, *HTR6*, *HTR7*; eight genes that code for pre-synaptic components of the serotonergic synapse including proteins involved in tryptophan metabolism (*DDC*,*TPH1*, *TPH2*) and vesicle recycling (*SLC18A1*, *SLC18A2*), amine oxidases (*MAO-A*, *MAO-B*) and serotonin transporter (*SLC6A4*). The mapping between protein names and gene symbol is given in Supplemental Table 1 and 2.

In the dopaminergic synapse we analyzed a total of 13 genes. These genes included five dopamine receptors: *DRD1*, *DRD2*, *DRD3*, *DRD4*, *DRD5*, five genes that code for pre-synaptic proteins involved in tyrosine, L-dopa, homovanillic acidand3-methoxytyramine metabolism (*TH*, *DDC*, *COMT*, *MAO-A*, *MAO-B*), two genes which products facilitate vesicle recycling (*SLC18A1*, *SLC18A2*) and dopamine transporter (*SLC6A3*).

2.4.1. Statistical analysis

2.4.1.1. Transition magnitude. We used one-way analysis of variance (ANOVA) to quantify the differences in the mean gene expression among three age groups: children (age 1–10 years), adolescence (11–23 years), adults (24–41 years). We conducted a separate ANOVA test for every gene and brain region (a total of $33 \times 16 = 528$ tests). The resulting p-values were corrected for multiple comparisons using Storey false discovery rate (FDR) (Storey, 2002). The FDR outputs, known as q-values, measures the strength of an observed statistic, like the p-values, only this time with respect to the positive false discovery rate.

We also quantified the strength of expression changes in the transition from childhood to adolescence using a Wilcoxon test that tests for a difference in the medians of the two groups. The results were usually similar and are reported in Supplemental Table S2.

2.4.1.2. Correlation and age-corrected correlations. We assessed the relation between gene pairs using two types of correlation measures. First, the Spearman correlation between life-long expression profiles $corr(gene_1, gene_2)$. Second, to capture correlation that does not stem from the overall changes in expression along

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life, we computed the age-conditioned correlation within each age group $corr(gene_1, gene_2 \mid age)$. Note that high overall correlation values do not necessarily yield high conditional-correlation values, and vice versa.

3. Results

To search for genes, whose expression exhibits a distinct transition from childhood to adulthood, we considered genes that participate in the serotonin pathway (25 genes) and dopamine pathway (13 genes) as defined by KEGG (Aoki-Kinoshita and Kanehisa, 2007) (see Experimental procedures for inclusion criteria). Figure 1 shows a schematic plot of the serotonin synaptic (Figure 1A) and the dopamine synaptic (Figure 1B) molecular indices. To detect transition in mRNA expression levels in adolescence, we first analyzed genome-wide measurements of the transcriptome reported by Kang and colleagues (2011). These include mRNA levels measured across 16 brain regions (11 cortical and 5 subcortical regions) in 13 time points (see Experimental procedures).

For every gene and region, we grouped subjects by age into three groups: children (1–10 years old, 6 subjects), adolescents (11–23 years old, 8 subjects) and adults (24–41 years old, 8 subjects). We then quantified the magnitude of the transition in expression between these groups using ANOVA (see Experimental procedures), and used the $-\log_{10}$ of the p-values as a measure of the transition magnitude (similar patterns of results were obtained using a Wilcoxon test for different medians; see Experimental procedures and Supplemental Table S2). Figure 2 shows the transition magnitude for all brain regions and genes tested. The genes with the highest transition index are listed in Table 1.

In the serotonin pathway (Figure 2A) two genes, *HTR1B* and *HTR1E*, exhibit robust sharp increase in expression during adolescence in multiple cortical regions, the ventral, dorsal and occipital prefrontal cortex and the inferior parietal cortex. This transition is particularly strong in the prefrontal cortices (Figure 2A), and much weaker in other cortical and subcortical areas. When considering all samples from prefrontal areas, the adolescent increase becomes even more statistically significant (*HTR1B:* p-value$=4.45\times10^{-15}$, $n=81$, ANOVA, FDR corrected q-value$=1.87\times10^{-14}$, *HTR1E:* p-value$=4.95\times10^{-17}$, $n=81$, ANOVA, FDR corrected q-value$=4.14\times10^{-16}$).

Figure 3A traces the gene expression levels of *HTR1B* and *HTR1E*, in four prefrontal regions, where the changes in expression levels are most significant (Figure 2). These regions are the ventrolateral prefrontal cortex (circles), the dorsolateral prefrontal cortex (triangles), the orbital prefrontal cortex (squares) and the medial prefrontal cortex (downward triangles). In all four regions, the expression levels of *HTR1B* and *HTR1E* notably elevate during adolescence and remain high throughout adulthood.

A significant rise is also observed in *HTR1A*, (Figure 3B, transition in the joint prefrontal areas: p-value$=1.58\times10^{-4}$, $n=81$, ANOVA, FDR corrected q-value$=1.89\times10^{-4}$), but the onset of expression increase appears to be earlier (the q-value for transition at age$=8$ is 2.4×10^{-6}).

A similar, yet more moderate expression increase in prefrontal regions is also observed for the *HTR4* and *HTR5A* genes (Figure 3B, *HTR4*, p-value$=4.8\times10^{-10}$ ANOVA, $n=81$, FDR q-value$=1.2\times10^{-8}$, *HTR5A*, p-value$=9\times10^{-9}$ ANOVA, $n=81$, FDR q-value$=2.3\times10^{-7}$). The expression of these two genes increases by a similar magnitude in the frontal cortical regions: the primary motor cortex (Figure 3C, circles), the primary somatosensory cortex (Figure 3C, triangles) and the posterior inferior parietal cortex (Figure 3C, squares).

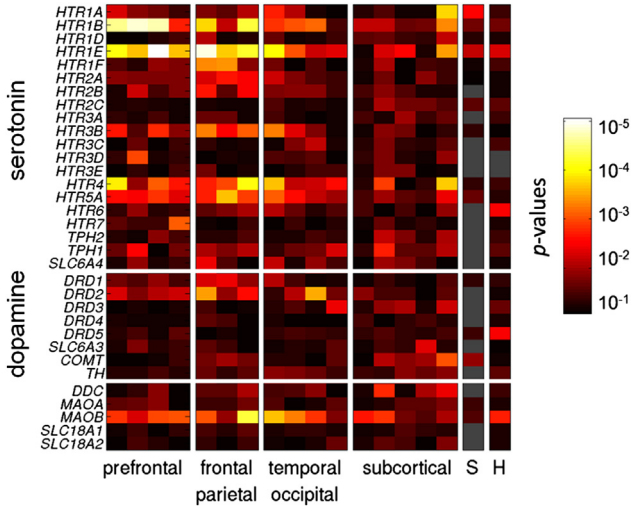

Figure 2 Transition magnitude across serotonergic and dopaminergic genes as measured using $\log_{10}(p\text{-values})$. Each column corresponds to one of 16 brain regions Kang et al. (2011) and two additional datasets (Somel et al., 2010 and Harris et al., 2009 denoted S and H respectively). Brain regions are grouped and sorted as follows: prefrontal: DFC, OFC, VFC, MFC; frontal-parietal: M1C, S1C, IPC; temporal-occipital: ITC, STC, A1C, V1C; subcortical: AMY, CBC, HIP, MD, STR. The genes coding for serotonin receptors, *HTR1B* and *HTR1E* exhibit significant expression transition in prefrontal and frontal areas. The genes coding for serotonin receptor *HTR4* and *HTR5A* exhibits smaller significant transition in multiple cortical regions. Changes in gene expression levels are far less significant in the dopaminergic synapse genes, particularly in the prefrontal and frontal cortex. The color scale denotes the $\log_{10}(p\text{-value})$. Gray denotes expression levels that were not measured.

To correct for possible variations in global expression levels due to age or inter-subject variability, we normalized the expression levels of all genes by the mean expression level of a standard group of 575 housekeeping genes (HKG) (Eisenberg and Levanon, 2003). The results of analyses, including the shape of the expression profile and their significance was highly preserved.

To test the consistency of these results, we analyzed the expression profiles of *HTR1E* and *HTR1B* in two more datasets. First, we analyzed RNA sequencing measurements of the same tissues provided by Brainspan (Sunkin et al., 2013). Figure 4A shows the expression profiles of *HTR1E* and *HTR1B* in the four prefrontal regions, ventrolateral prefrontal cortex (circles), dorsolateral prefrontal cortex (triangles), orbital prefrontal cortex (squares) and medial prefrontal cortex (invert triangle). We further tested the time profile of *HTR1E* in the microarray data from Somel et al. (2010) (Figure 4B) and Harris et al. (2009) (Figure 4C). Both data sets show smooth rise in the expression levels of

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Table 1 Serotonergic synapse genes with highest transition magnitude during adolescence in all brain regions (top ten scores) and in the prefrontal regions aggregated (top seven scores).

	Brain region	*p*-Value	*FDR q-value*
Gene symbol			
HTR1E	Ventrolateral prefrontal cortex(VFC)	4.0×10^{-6}	8.5×10^{-4}
HTR1E	Primary motor cortex (M1)	1.5×10^{-5}	0.0013
HTR1B	Orbital prefrontal cortex (OFC)	1.9×10^{-5}	0.0013
HTR1B	Ventrolateral prefrontal cortex(VFC)	2.7×10^{-5}	0.0014
HTR1B	Dorsolateral prefrontal cortex (DFC)	4.0×10^{-5}	0.0017
MAOB	Posterior inferior parietal cortex(IPC)	9.0×10^{-5}	0.0029
HTR1B	Posterior inferior parietal cortex(IPC)	1.0×10^{-4}	0.0029
HTR1E	Posterior inferior parietal cortex(IPC)	1.1×10^{-4}	0.0029
HTR1E	Dorsolateral prefrontal cortex (DFC)	1.5×10^{-4}	0.0036
HTR4	Posterior inferior parietal cortex(IPC)	1.7×10^{-4}	0.0036
Aggregated prefrontal regions			
HTR1E	Prefrontal cortex	4.95×10^{-17}	4.1×10^{-16}
HTR1B	Prefrontal cortex	4.5×10^{-15}	1.9×10^{-14}
HTR4	Prefrontal cortex	4.8×10^{-10}	1.3×10^{-9}
MAOB	Prefrontal cortex	6.5×10^{-10}	1.4×10^{-9}
HTR5A	Prefrontal cortex	9.3×10^{-9}	1.6×10^{-8}
HTR3B	Prefrontal cortex	5.3×10^{-5}	7.4×10^{-5}
HTR1A	Prefrontal cortex	1.6×10^{-4}	1.9×10^{-4}

Significance was corrected for multiple hypotheses using Storey FDR (2002).

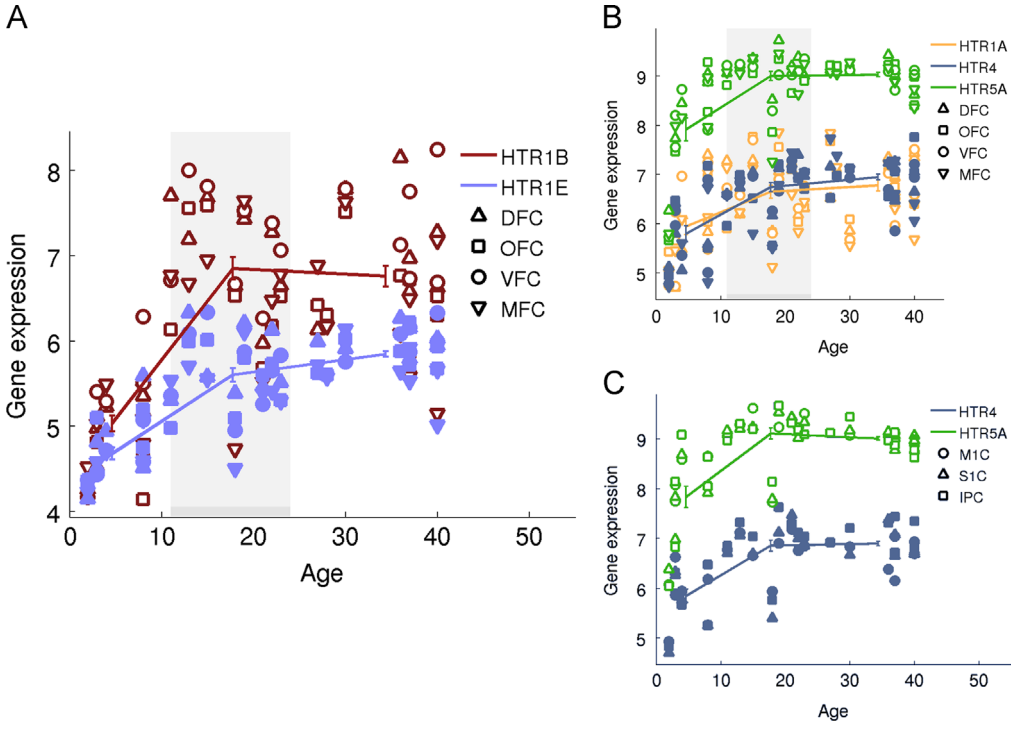

Figure 3 Expression profiles of genes encoding serotonin receptors in several cortical regions. Adolescence period (ages 11–24) is marked in gray. (A) Expression levels of *HTR1B* and *HTR1E* in four prefrontal regions: circles—ventrolateral prefrontal cortex (VFC), triangles—dorsolateral prefrontal cortex (DFC), squares—orbital prefrontal cortex (OFC), downward triangles—medial prefrontal cortex (MFC). (B) Expression levels of *HTR1A*, *HTR4* and *HTR5A* in prefrontal regions. Markers are the same as in (A). (C) Expression levels of *HTR4* and *HTR5A* in frontal and parietal regions: circles—primary motor cortex (M1C), triangles—primary somatosensory cortex (S1C), squares—posterior inferior parietal cortex (IPC).

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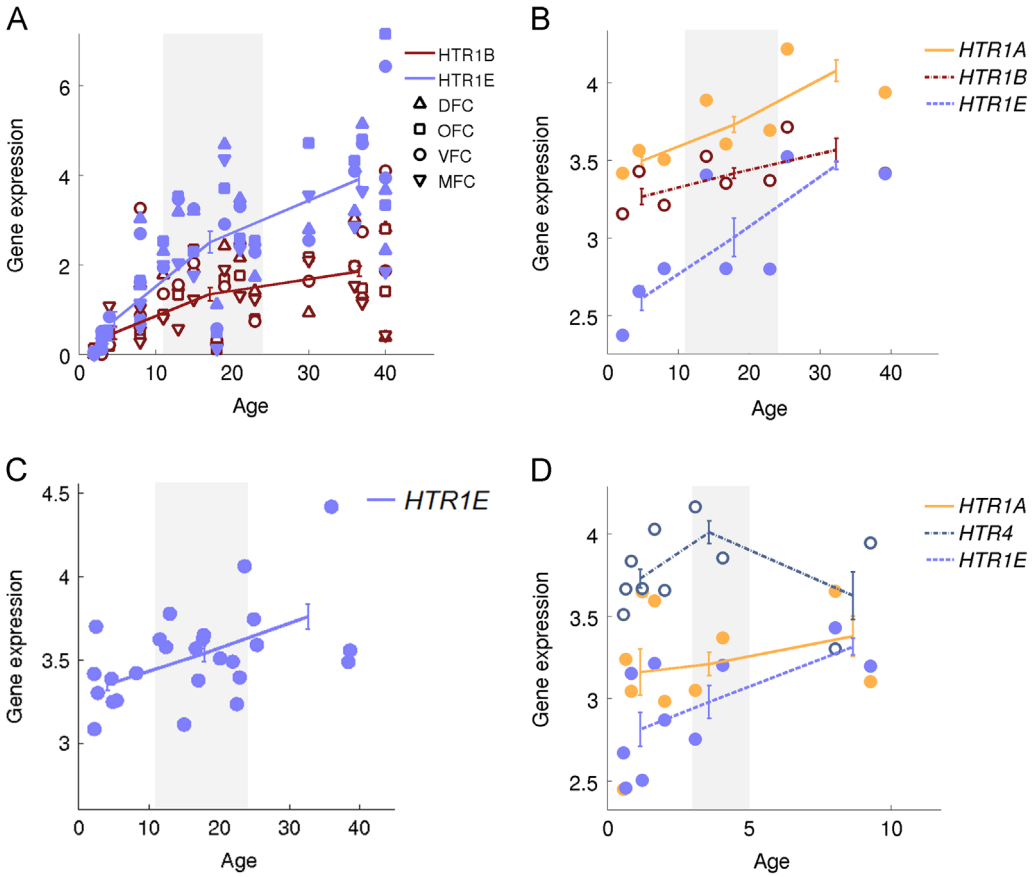

Figure 4 Expression profiles of genes encoding 5-HT receptors as measured in different experiments and organisms. (A) Gene expression levels of *HTR1B* and *HTR1E* in four human prefrontal cortex regions, as measured using RNA sequencing (Sunkin et al., 2013), (B) Gene expression levels of *HTR1A, HTR1B* and *HTR1E* in human superior frontal gyrus of the prefrontal cortex (Somel et al., 2010). (C) Expression of *HTR1E* in human prefrontal cortex (Harris et al., 2009). (D) Expression of levels of *HTR1B* and *HTR1E* in Rhesus macaque prefrontal cortex (Somel et al., 2011).

HTR1E (Somel: p-value<0.041,Harris: p-value <0.033, ANOVA).

To test if these change are specific to human adolescence, we further analyzed expression profile of the homologs of *HTR1A, HTR1B, HTR1E, HTR4* and *HTR5A* measured along the development of macaque monkeys (Somel et al., 2011) (see Experimental procedures). Matching age groups across species is complex, since various maturation processes occur at different rates. We therefore used the cross-species mapping from (Johnson and Kapsalis, 1995), as a guideline, where the adolescence period was taken to be based on puberty. In macaque, we find that *HTR1A, HTR1E* and *HTR4* expression also rises during development from childhood to adulthood (Figure 4D). *HTR1E* exhibits high correlation between gene expression levels and age (Spearman $\rho=0.86$, p-value$=1.2\times10^{-8}$, $n=27$, ages 0-28 Y). Unlike the sharp transition in expression profile observed in humans, expression in macaque (p-value$=0.2$, ANOVA, $n=10$) rises smoothly with age. *HTR1B* and *HTR5A* expression profile is very different from the one expressed in human (supplemental Figure S2).

The above findings show that both *HTR1E* and *HTR1B* exhibit a sharp increase in expression levels from childhood to adolescence. The question remains however, if these two age-related gene expression transitions are part of a common orchestrated process, or can be viewed as two transitions occurring at about the same time, but not directly inter-dependent. To study this question, we tested the correlation between the expression levels of *HTR1E* and *HTR1B*. Clearly, since the expression levels of both follow a similar trend with age, rising from childhood to adolescence, their overall expression profiles across lifespan are strongly correlated ($\rho=0.79$, p-value$<10^{-20}$, Spearman). To evaluate how the expression of the two genes is correlated beyond the effect of age, we tested the subject-to-subject correlation between the two genes *within each age group* (see Experimental procedures). The age-conditioned correlation between *HTR1B* and *HTR1E* was significant during adolescence ($\rho=0.63$,p-value$=1.4\times10^{-4}$) and adulthood ($\rho=0.61$, p-value$<6.1\times10^{-4}$), but much less so in childhood ($\rho=0.42$, p-value$=0.07$). Supplemental Figure S3A depicts the joint expression levels of the two genes illustrating the pronounced correlation in each of three age groups.

We further computed correlations between all pairs of genes studied within the three age-groups. No other genes exhibit consistent high correlation with *HTR1E* and *HTR1B* across all three age groups (supplemental Figure S1), but

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some of the pairs are correlated within one or two age group. *HTR1A* expression is correlated with both *HTR1B* and *HTR1E* in childhood and adolescence but not in adulthood (*HTR1A-HTR1B,* Childhood: $\rho=0.59$, p-value$=7.1\times10^{-3}$, $n=20$; adolescence, $\rho=0.52$, p-value$=2.4\times10^{-3}$, $n=32$; adulthood $\rho=-0.05$, p-value$=0.78$, $n=29$, Spearman. *HTR1A-HTR1E,* Childhood: $\rho=0.62$, p-value$=4.6\times10^{-3}$; adolescence, $\rho=0.41$, p-value $=0.02$; adulthood $\rho=-0.04$, p-value$=0.84$, Spearman, Supplemental Figure S3B-C). *HTR4* and *HTR5A* exhibit a similar correlation profile, they are significantly correlated during childhood and adolescence (Childhood: $\rho=0.65$, p-value$=2.3\times10^{-3}$, $n=20$; adolescence, $\rho=0.47$, p-value $=7.6\times10^{-3}$, $n=32$, Spearman) but not in adulthood ($\rho=0.27$, p-value$=0.16$, $n=29$, Spearman). Therefore, the correlation structure between all genes in the pathway seems to change with age and awaits further study, which is outside the scope of the current paper.

Another interesting question concerns the nature of the change in the serotonin receptor expression. Are the changes in expression levels rapid and limited to a narrow time window, or do they rise smoothly and gradually over time? To investigate this issue we compared the ANOVA scores which quantify sharp changes between mean expression of the childhood and adolescence groups, with the Pearson (linear) correlation between expression levels and age. Supplemental Table S5 lists the q-values from these two measures for the seven genes that exhibit the most significant linear correlation. The corrected q-values of the transition magnitude ANOVA test are considerably more significant than q-values obtained for linear correlation, suggesting that the expression levels of serotonin receptor encoding genes undergo a sharp transition rather than a smooth rise.

4. Discussion

In this study we examined how expression levels of the genes involved in the serotonergic and dopaminergic synaptic pathways change from childhood to adulthood. We analyzed gene expression measured in four different transcriptome public domain data-sets, three using microarrays and one using RNA sequencing. We found that the expression levels of two genes, *HTR1B* and *HTR1E,* that code for serotonin receptors, *5-HT$_{1B}$,* and *5-HT$_{1E}$* rise sharply and significantly from childhood to adolescence. This transition occurs in multiple cortical regions, including the ventral, dorsal and occipital prefrontal cortex and the inferior parietal cortex, and is particularly strong in the prefrontal and frontal areas as compared to other cortical and subcortical areas. A similar but weaker transition is observed in *HTR4* and *HTR5A*. In contrast, none of the genes that we analyzed in the dopaminergic pathway was found to have a similar significant transition in adolescence.

Interestingly, the sharpest rise in *HTR1E* and *HTR1B* expression is observed in prefrontal cortical areas, that is last to go through pruning during adolescence and the last to have white matter tracts myelinated (Giedd et al., 1999). The *HTR1B* receptor is a presynaptic autoreceptor and the *HTR1E* receptor is an index of serotonin synapses and both may reflect the process of synaptic pruning. Serotonin receptors as a whole, are mostly highly expressed in the human PFC and exert control over its excitability (Lambe et al., 2011). However, the existing data regarding developmental regulation of their expression levels in these areas are limited. Lambe et al. (2011) studied the expression levels of six genes coding for serotonin receptor using RT-PCR (*HTR1A*, *HTR2A*, *HTR2C*, *HTR4*, *HTR5A* and *HTR6*) in the dorsolateral PFC across human life-span. That analysis, not focusing on the period of adolescence, revealed a fairly constant expression level of the *HTR1A* and *HTR2A* genes, whereas the *HTR4* and *HTR6* genes showed significant changes during adolescence. Our findings are consistent with these results, as here we find a robust expression increase for *HTR4* in the posterior inferior parietal cortex. However, we also find a weak transition increase for *HTR1A*. *HTR1B* and *HTR1E* were not examined by Lambe et al. (2011).

Serotonin receptors from the family of 5-HT$_1$ receptors are known to play a particularly complex role in regulating response to serotonin. Their action was linked to many behaviors that are typically presented during adolescence, such as impulsivity and risk taking, and also to several related psychiatric pathologies. Importantly, 5-HT$_1$ receptors are expressed both post-synaptically where they initiate a post synaptic signaling cascade; and pre-synaptically where they operate as auto-receptors hyperpolarizing the presynaptic membrane to inhibit firing and the release of serotonin. For this reason, it is possible that abrupt changes in expression of *HTR1* genes may be involved in functional changes in behaviors observed in adolescence. PET studies have linked the binding levels of the 5-HT$_{1A}$ receptor to aggressive behavioral trait severity (Parsey et al., 2002).

The first receptor whose expression level we found rise sharply, 5-HT$_{1B}$, is expressed both pre- and post- synaptically in multiple brain areas. In the prefrontal cortex, as a heteroreceptor located on dopamine terminals, it regulates dopamine release. As a presynaptic autoreceptor it regulates serotonin release from serotonin terminals. Animal studies demonstrated that ablating 5-HT$_{1B}$ receptor leads to impulsive-aggression and thus suggest that it has anti-impulsive and aggressive role (Crabbe et al., 1996). Consequently, it may be a regulator of normal behavior and contribute to psychiatric disorders (Drago et al., 2010). Recently, it was shown in humans that its regulation may play a role in aggression-related phenotypes (Conner et al., 2010) and PET imaging studies showed lower binding potential of 5-HT$_{1B}$ in depressed patients and suicide victims (Murrough et al., 2011). Our finding that the 5-HT$_{1B}$ receptor gene shows a sharp expression increase in the PFC during adolescence seems consistent with a model whereby a failure of this developmental change may contribute to pathologically higher aggressive and impulsive behaviors in adolescence and young adults.

The second receptor with expression rising sharply, 5-HT$_{1E}$, was the most recent to be identified of the 5-HT$_1$ auto-receptor family (Leonhardt et al., 1989). Its encoding gene, *HTR1E*, was found to be expressed in the cortex and hippocampus (Barnes and Sharp, 1999). Since only a few agonists and antagonists have been designed for this receptor (Janssen et al., 2004), its specific function is largely unknown. Our findings that *HTR1E* expression levels show a similar profile to *HTR1B*, and that *HTR1B* and *HTR1E* are correlated independently of age, suggests that the 5-HT$_{1E}$

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and 5-HT_{1B} receptors or genes share common regulatory mechanisms.

The above findings are important in light of the dearth of similar previous studies. In a search for candidate genes for schizophrenia, whose onset peaks during late adolescence (ages 15–25), Harris et al. (2009) conducted microarrays of prefrontal cortex tissue and identified a large number of genes with various functions, but they detected no specific serotonergic or dopaminergic gene expression changes. Lambe et al. (2011) looked at development patterns of only six serotonin receptors using RT-PCR, and detected significant expression changes in some serotonin receptors from infancy to childhood, but much weaker changes in adolescence.

To-date, only a single positron emission tomography (PET) imaging study evaluated age-related changes in 5-HT_{1B} receptor density in healthy humans (Matuskey et al., 2012). It demonstrated an age-related decrease of 5-HT_{1B} receptor in the cortices of 48 adult subjects. However, the age range in that study (18–61 years) limited this preliminary report in terms of detecting changes in 5-HT_{1B} receptor density during adolescence that might have reflected on our findings.

Our findings are consistent with the hypothesis that sharp rises in expression of serotonin receptor subtypes B and E of the 5-HT_1 autoreceptor family during adolescence may be linked to emergence of more pronounced aggressive impulsive behaviors during adolescence. Specifically, multiple studies indicated that lower serotonin signaling during adolescence could lead to promoted impulsive behavior (Duke et al., 2013; Mann et al., 2001). Some authors, including Katz (1999), suggested that the primary function of the serotonergic system is to reduce impulsive over-responding to proximal affective stimuli (Depue and Collins, 1999). In animal models, forebrain serotonin depletion (Bizot et al., 1999; Denk et al., 2005) and treatment with a 5-HT_{1A} receptor agonist (Winstanley et al., 2005) were showed to increase impulsivity. The latter observation is consistent with our finding using PET showing a positive correlation with aggressive traits and level of 5-HT_{1A} receptor binding (Parsey et al., 2002).

Our finding of the rise in *HTR1A* expression is partially supported by a previous report that the function of the 5-HT_{1A} receptor in early age is different than in adulthood in mice (Gross et al., 2002). The earlier rise of *HTR1A* expression, as compared to the transitions in *HTR1B* and *HTR1E*, suggests that the role of the 5-HT_{1A} receptor in brain maturation may precede that of the 5-HT_{1B} and 5-HT_{1E} receptors in the yet unknown sequence of neurobiological events underlying adolescence. Serotonin is most often implicated also in the pathogenesis of depression, which typically appears post-pubertally (American Psychiatric Association, 2000). The most studied serotonin receptor, 5-HT_{1A}, has been shown to be involved in depression and anxiety (Caliendo et al., 2005), with greater autoreceptor binding observed during and between episodes of major depression, consistent with a biological endophenotype. This gene and its expression are also affected by stress. The relation between genetics, stress and mood disorders in this context is thought to be stronger during adolescence because onset of mood disorders appears to emerge during this developmental period (Rice et al., 2002; Scourfield et al., 2003). It remains an important question how the transitions in expression levels found in this paper may be related to serotonin-related pathogenesis of mood disorders.

The present study has some limitations. The datasets we used were designed to study brain development in general and not adolescence in particular. Biological markers were not available to determine the onset of adolescence (e.g. menarche, coitus) and therefore the childhood and adolescence groups were separated based on their chronological age. The data does not include specific behavioral traits of the subjects such as impulsivity and aggression. Despite these limitations, the rise in expression levels that we detected are robust statistically, and replicated in four different transcriptome datasets using different methods and samples.

The analysis presented in this paper was conducted using data collected from primates. It would be interesting to test if these effects are specific to primates, or can be found in other mammals including rodents. Such tests could shed light on the evolution of the serotonin system and its role in development, but are beyond the scope of this paper, and will await further study.

This study focuses on the serotonergic and dopaminergic neurotransmitter systems, since they play a pivotal role in control of mood and behavior during adolescence, in the evolution of adolescent psychopathology, and have relatively high potential to be pharmacologically manipulated. Studying changes in expression of genes involved in other systems including myelination and plasticity, could contribute further to our understanding of the CNS maturation process, and awaits further study.

In summary, this is the first study to comprehensively evaluate transitions in the transcriptome of both the serotonergic and dopaminergic neurotransmitter systems across the brain during adolescence. Our findings suggest that *HTR1B* and *HTR1E* genes play a central role in adolescence maturation processes. Since understanding healthy brain maturation is essential for evaluation of deviations from normal development, it is suggested that *HTR1B* and *HTR1E* genes may be candidate molecular mechanisms to be examined further in future studies of adolescent-onset psychopathology.

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Contributors

GS, OBS and GC designed the study. GC, GZ and JJM managed the literature search. OBS and GC managed the analyses. GS, OBS and GC wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare no conflict of interest.

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None.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.euroneuro.2014.02.009 and at http://chechiklab.biu.ac.il/.

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