Catechol-β-Methyltransferase Enzyme Activity and Protein Expression in Human Prefrontal Cortex across the Postnatal Lifespan

The prefrontal cortex (PFC) dopamine system, which is critical for modulating PFC function, undergoes remodeling until at least young adulthood in primates. Catechol-β-methyltransferase (COMT) alters extracellular dopamine levels in PFC, and its gene contains a functional polymorphism (Val158Met) that has been associated with variation in PFC function. We examined COMT enzyme activity and protein immunoreactivity in the PFC during human postnatal development. Protein was extracted from PFC of normal individuals from 6 age groups: neonates (1–4 months), infants (5–11 months), teens (14–18 years), young adults (20–24 years), adults (31–43 years), and aged individuals (68–86 years; n = 5–8 per group). There was a significant 2-fold increase in COMT enzyme activity from neonate to adulthood, paralleled by increases in COMT protein immunoreactivity. Furthermore, COMT protein immunoreactivity was related to Val158Met genotype, as has been previously demonstrated. The significant increase in COMT activity from neonate to adulthood complements previous findings of protracted postnatal changes in the PFC dopamine system and may reflect an increasing importance of COMT for PFC dopamine regulation during maturation.

Keywords: COMT, development, dopamine, schizophrenia

Introduction

The prefrontal cortex (PFC) shows a protracted postnatal developmental course, with refinement of synaptic innervation and connectivity occurring well into adulthood (Lewis 1997; Levitt 2003). This extended time course means that PFC function may, relative to other brain regions, be particularly sensitive to neurodevelopmental lesions (Levitt 2003) and, moreover, their functional consequences might not be manifest until adulthood (Weinberger 1987). Thus, the postadolescent age of onset of certain neurodevelopmental disorders that relate to dysfunction of the PFC, such as schizophrenia, may only become clinically apparent once this brain region has fully matured (Weinberger 1987; Weinberger and Marenco 2003). Therefore, elucidating the maturation of the PFC from birth until adulthood is critical both to our understanding of its normal function and also its dysfunction in neurodevelopmental disorders such as schizophrenia.

Dopamine plays a critical role in modulating normal PFC function (Goldman-Rakic and others 2000), and the PFC dopamine system undergoes extensive refinement during postnatal life (Lambe and others 2000). The primate PFC does not receive its full complement of synaptic dopamine innervation until around adolescence. Thus, the density of catecholamine-positive fibers and varicosities in the rhesus monkey PFC peaks in adolescence, before receding to adult levels (Rosenberg and Lewis 1995). Additionally, the density of catecholamine appositions onto pyramidal cells, but not onto interneurons, doubles from birth to adolescence in rhesus monkey PFC, suggesting that the increase in dopamine input might differentially affect excitatory versus inhibitory transmission in cortical circuits (Lambe and others 2000). Furthermore, the abundances of dopamine receptors and tyrosine hydroxylase, the rate-limiting enzyme for dopamine biosynthesis, are also dynamically regulated during primate postnatal development (Lidow and Rakic 1992; C.S. Weickert, M.J. Webster, P. Gondipalli, D. Rothmond, R.J. Fatula, M.M. Herman, J.E. Kleinman, and M. Akil, unpublished data). Mirroring these anatomical changes, working memory performance, which is known to be critically dependent on PFC dopamine function (Goldman-Rakic 2000), does not peak until after adolescence (reviewed in Lewis 1997; Luna and others 2004), consistent with the increase in dopaminergic signaling during this period. Interestingly, information on the expression of molecules responsible for dopamine inactivation during this period is sparse, despite the critical role that such molecules likely play in modulating PFC dopaminergic function.

The activity of the catechol-β-methyltransferase (COMT) enzyme modulates dopamine levels in the PFC (Karoum and others 1994; Gogos and others 1998; Tunbridge and others 2004). Elimination of dopamine via catabolism by COMT is thought to be particularly important in the PFC, relative to the striatum, because dopamine transporters in the former region are extrasynaptic, permitting greater neurotransmitter diffusion (Lewis and others 2001). The COMT gene contains a polymorphism (Val158Met) that affects the activity of the enzyme: Met158 homozygotes have approximately one-third less COMT enzyme activity in PFC than Val158 homozygotes (Chen and others 2004), and the reduced enzyme activity determined by the Met158 allele presumably results in increased PFC dopamine relative to Val158 carriers because COMT inhibition results in elevated dopamine efflux (Tunbridge and others 2004). Consistent with its role in modulating PFC dopamine levels, the Val158Met polymorphism is associated with performance on tests of working memory and executive function, which depend on PFC function. Thus, the high-activity Val158 allele is linked with relatively poorer performance on such tasks, relative to the Met158 allele (e.g., Egan and others 2001; Bilder and others 2002; Joobber and others 2002; Malhotra and others 2002), presumably as a result of increased PFC dopamine catabolism. Paralleling these findings, administration of tolcapone, a COMT inhibitor, improves performance of extradimensional set shifting in rats (Tunbridge and others 2004), a task dependent on medial PFC in rats (Birrell and Brown 2000) and the dorsolateral PFC in primates (Dias and others 1996). Tolcapone also improves cognitive function in humans (Gasparini and others 1997; Mattay and others 2004). COMT may also be genetically associated with schizophrenia (Egan and others 2001; 2002).
Shifman and others 2002; discussed in Tunbridge, Harrison, and Weinberger 2006). Thus, knowledge of the developmental profile of COMT activity is relevant to understanding the development of the normal PFC dopamine system, as well as its dysfunction in schizophrenia and other neurodevelopmental disorders.

Studies of the postnatal expression profile of COMT are few (Agathopolous and others 1971; Stanton and others 1975; Brust and others 2004; see Discussion), and none have been conducted in human brain. Thus, we sought to determine COMT activity and expression during postnatal maturation in the PFC of normal humans. We demonstrate a dramatic increase in PFC COMT activity across the human postnatal lifespan, which correlates with increases in COMT protein immunoreactivity determined by immunoblotting.

Materials and Methods

Tissue Collection and Protein Extraction
Specimens were obtained through the Office of the Medical Examiners of the District of Colombia and were fresh frozen and processed as previously described (Kleinman and others 1995) in the Clinical Brain Disorders Branch of the National Institute of Mental Health. All subjects were free of psychiatric and neurologic disorders and of significant neuropathological findings. The cohort used in this study (see Table 1) consisted of neonates (1-4 months, n = 8), infants (5-11 months, n = 5), teens (14-18 years, n = 8), young adults (20-24 years, n = 6), adults (31-43 years, n = 7), and aged (68-86 years, n = 6). All individuals were of African American origin, except for one individual of Asian descent, included in the aged group. Pulverized dorsolateral PFC tissue (Brodmann’s area 46) was thawed and homogenized over wet ice in extraction buffer (0.6% Tris and 50% glycerol containing protease inhibitors [0.024% AEBSF, 0.005% aprotinin, 0.001% pepstatin A, and 0.001% leupeptin]). Protein concentrations were determined using the Bradford method, and crude homogenates were stored in single-use aliquots (20 μL of a 1 μg/μL dilution) at -80° C.

COMT Activity Assay
COMT enzyme activity was assayed by measuring the incorporation of a radioactive methyl group into a catechol substrate in crude tissue homogenates, as previously described (Chen and others 2004). Crude tissue homogenates (2.5 mg tissue; Chen and others 2004), we used 20 μg of crude protein, which we found to yield a linear relationship between measured COMT activity and reaction time (r = 0.995; data not shown). Background levels of radioactivity were determined by adding 10 mM tolcapone to randomly selected samples (4 per assay), and this background was subtracted from measured levels of COMT activity.

COMT enzyme activity was assayed in all samples at one time. The assay was repeated 4 times, and data presented are the mean values for these 4 experiments, with each sample normalized to mean adult COMT activity levels, considered to be 100%. The raw data are presented in the supplementary information.

Western Blotting
Crude tissue homogenates (2.5 μg total protein) were taken from the same protein samples used for the enzyme activity assay (determined during pilot studies to be within the linear range of protein quantification for both COMT and β-actin) and diluted to a volume of 10 μL in sample buffer (LDS; Invitrogen, Carlsbad, CA) and sterile water and boiled for 7 min. They were then run on 15% Tris glycine polyacrylamide gels (120 V for ~2 h) and transferred to polyvinylidene fluoride membrane (16 h at 30 V). Membranes were labeled with anti-COMT antibody (Chemicon, Temecula, CA; Catalog number AB8873; 1:5000 dilution) as previously described (Tunbridge, Weinberger, and Harrison 2006) and were apposed to Kodak Biomax MR film. Membranes were then rinsed and incubated with anti-actin antibody (Chemicon; Catalog number MAB1501; 1:5000 dilution) to monitor variations in sample handling and western blotting. Films were digitized, and individual bands were quantified using NIH Image (v1.32; profile plots function). COMT immunoreactivity was normalized to the corresponding β-actin measurement, although analysis of raw data produced similar results. Western blotting was performed 3 times, and data presented are the mean normalized values for these 3 experiments.

Genotyping
Genomic DNA was extracted from pulverized cerebellar hemisphere using Puregene reagents (Genta, Minneapolis, MN). Samples were genotyped for the COMT Val158Met polymorphism using a Taqman 5’ exonuclease assay as previously described (Chen and others 2004). The distribution of COMT Val158Met genotypes in the different age groups is shown in Table 1. Allele frequencies are as expected for a cohort with this racial profile (Palmatier and others 2004).

Data Analysis
All experiments and image analyses were performed blind to the identities of the samples. One-way analysis of variance (ANOVA) was used to investigate whether age groups were matched for demographic variables (Table 1), and the effect of brain pH and postmortem interval on COMT enzyme activity and protein expression was investigated using Pearson’s correlations. The effect of gender was examined by t-test in the infant and neonate groups combined (because older age groups consisted largely, or entirely, of males). The effect of age group on COMT enzyme activity was explored using one-way ANOVA, with pH and postmortem interval included as covariates (although inclusion or exclusion of these covariates had no effect on the findings), and correlation. The relationship between COMT enzyme activity and

Table 1
Demographics of the developmental cohort

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Val158Met genotypes</th>
<th>Postmortem intervala</th>
<th>Brain pH</th>
<th>Cause of death (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonates (8)</td>
<td>0.20 ± 0.03</td>
<td>3M/5F</td>
<td>V/V: n = 2, V/M: n = 6, M/M: n = 0</td>
<td>48.8 ± 9.4</td>
<td>6.34 ± 0.11</td>
<td>SIDS (5); endocardial fibroelastosis (1); bronchopneumonia (1); undetermined (1).</td>
</tr>
<tr>
<td>Infants (5)</td>
<td>0.60 ± 0.10</td>
<td>3M/2F</td>
<td>V/V: n = 3, V/M: n = 2, M/M: n = 0</td>
<td>46.8 ± 5.8</td>
<td>6.53 ± 0.08</td>
<td>SIDS (2); broncholitisis (1); diphenhydramine intoxication (1); cardiomyogly, atrial septal defect (1).</td>
</tr>
<tr>
<td>Adolescents (8)</td>
<td>16.8 ± 0.62</td>
<td>8M</td>
<td>V/V: n = 4, V/M: n = 4, M/M: n = 0</td>
<td>25.5 ± 5.0</td>
<td>6.48 ± 0.06</td>
<td>GSW to chest (3), back (2), abdomen (1); multiple GSW (1); stab wound to chest (1).</td>
</tr>
<tr>
<td>Young adults (6)</td>
<td>22.2 ± 0.65</td>
<td>6M</td>
<td>V/V: n = 3, V/M: n = 2, M/M: n = 1</td>
<td>37.2 ± 4.7</td>
<td>6.34 ± 0.12</td>
<td>GSW to torso (2), chest (1); stab wound to chest (1); fibrinous pericarditis (1); pulmonary embolism (1).</td>
</tr>
<tr>
<td>Adults (7)</td>
<td>37.7 ± 1.71</td>
<td>7M</td>
<td>V/V: n = 4, V/M: n = 3, M/M: n = 0</td>
<td>27.4 ± 6.2</td>
<td>6.43 ± 0.06</td>
<td>ASCVD (1); stab wound to chest (1); acute asthma attack (1); GSW to chest (1); pulmonary embolism (1); multiple GSW (1); GSW to torso (1).</td>
</tr>
<tr>
<td>Aged (6)</td>
<td>76.7 ± 2.85</td>
<td>5M/1F</td>
<td>V/V: n = 5, V/M: n = 1, M/M: n = 0</td>
<td>45.8 ± 6.2</td>
<td>6.33 ± 0.11</td>
<td>Pulmonary embolism (1).</td>
</tr>
</tbody>
</table>

Note: SIDS, sudden infant death syndrome; GSW, gunshot wound; ASCVD, arteriosclerotic cardiovascular disease; M, male; F, female. *Differs between groups at trend significance level.
aspects of COMT protein expression was investigated using Pearson’s correlations. The effect of Val¹⁵⁸Met genotype on COMT enzyme activity and protein immunoreactivity was determined using t-tests or one-way ANOVA in order to investigate the effect of including age as a covariate (the single Met¹⁵⁸ homozygote in the cohort was excluded from these analyses).

Results

COMT Enzyme Activity during the Postnatal Lifespan

COMT enzyme activity was successfully detected in all individuals, and there was no significant effect of brain pH or postmortem interval (F values < 1). There was a significant increase in COMT enzyme activity from the neonate period to adulthood (main effect of age group: F₅,₂₅ = 4.0, P < 0.01; Fig. 1; see Table 2 for post hoc comparisons), which continued into adulthood (see Table 2) and was also reflected as a significant correlation between COMT enzyme activity and increasing age (r = 0.64, P < 0.0001). There was no significant effect of gender on COMT activity in infants and neonates (t = 1.1, P > 0.1).

Correlations between COMT Enzyme Activity and Protein Immunoreactivity

Western blotting for COMT revealed the presence of 4 major bands at approximately 39, 32, 26, and 24 kDa. Whereas the 39-, 32-, and 24-kDa bands were present in all individuals, the 26-kDa band was not visible in some samples (Fig. 2). Based on its size, the 32-kDa band is presumed to be the membrane-bound form of catechol-o-methyltransferase (MB-COMT), and we have recently identified the 39-kDa band as a novel variant of COMT (presumably an isoform of MB-COMT, based on its size and relative abundance; Tunbridge, Weinberger, and Harrison 2006). The finding of a second doublet at around 24 and 26 kDa is novel, and, given that these bands are the approximate size of soluble COMT (S-COMT), these may represent isoforms of S-COMT, although it is also possible that they represent breakdown products of the larger COMT bands. None of the western blotting immunoreactive bands showed significant correlations with either brain pH (0.30 < r < 0.29) or postmortem interval (−0.26 < r < −0.11), except for expression of the 39-kDa band, which was significantly correlated with brain pH (r = 0.42, P < 0.01).

There were significant correlations between COMT enzyme activity and immunoreactivity of the 32-kDa (r = 0.51, P < 0.001; Fig. 3b) and 24-kDa (r = 0.61, P < 0.001; Fig. 3d) bands, normalized to β-actin, and a trend correlation between COMT enzyme activity and immunoreactivity of the 39-kDa band (r = 0.28, P < 0.1; Fig. 3a), normalized to β-actin. However, COMT enzyme activity significantly correlates neither with immunoreactivity of the 26-kDa band (r = 0.21; Fig. 3c) normalized to β-actin nor with β-actin immunoreactivity (r = 0.16; data not shown). The positive correlations between COMT enzyme activity and immunoreactivity were reflected as significant correlations between normalized immunoreactivity and age for the 32-kDa (r = 0.42, P < 0.01) and 24-kDa (r = 0.36, P < 0.05) bands but not for the 39-kDa (r = 0.19, P > 0.1) or 26-kDa (r = 0.11, P > 0.1), or β-actin (r = −0.014, P > 0.1), bands.

We also examined correlations between the immunoreactivity of the 39-kDa band expressed relative to the total MB-COMT (32 + 39 kDa bands) because we have demonstrated this measure to be altered in schizophrenia and bipolar disorder (Tunbridge, Weinberger, and Harrison 2006). There was a significant negative correlation between this measure and COMT enzyme activity (r = −0.536, P > 0.001), which was also reflected as a significant negative correlation with age (r = −0.450, P > 0.005). These data are discussed in more detail in the supplementary information. Protein immunoreactivity of the different bands broken down by group is shown in the supplementary information table.

![Figure 1](https://academic.oup.com/cercor/article-abstract/17/5/1206/346113)  
**Figure 1.** COMT enzyme activity in dorsolateral PFC across human postnatal development. There is a significant increase in COMT enzyme activity (expressed as a percentage of mean adult values) in the PFC during human postnatal development. COMT enzyme activity does not peak until adulthood, at which point it is significantly greater than in all other groups, except aged individuals (see Table 2).

![Figure 2](https://academic.oup.com/cercor/article-abstract/17/5/1206/346113)  
**Figure 2.** Examples of COMT and actin immunoblots in 2 different subjects. Immunoblotting with an anti-COMT antibody produced 4 major bands at 39, 32, 26, and 24 kDa (see left-hand panel). The 26-kDa band was not visible in some individuals (see example in right-hand panel). Immunoblotting with an antiactin antibody produced a single band at 50 kDa, the predicted size of β-actin.

<table>
<thead>
<tr>
<th></th>
<th>Neoneate</th>
<th>Infant</th>
<th>Teen</th>
<th>Young adult</th>
<th>Adult</th>
<th>Aged</th>
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<tr>
<td>Infants</td>
<td>NS</td>
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<tr>
<td>Teens</td>
<td>NS</td>
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<tr>
<td>Young adults</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Adults</td>
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<td>0.006</td>
<td>0.029</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aged</td>
<td>0.011</td>
<td>0.024</td>
<td>0.079</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Note: NS, not significant.
Effect of Val158Met Genotype on COMT Activity and Protein Immunoreactivity

There was no significant difference in COMT enzyme activity between Val158 homozygotes and Val158Met heterozygotes ($t = 0.90, P > 0.1; \text{Table 3}$), and this remained nonsignificant when age was included as a covariate ($F_{1,31} = 0.02, P > 0.1; \text{see Fig. 4 for COMT activity in different genotype groups}$). In contrast, there was a significant difference between genotype groups for immunoreactivity of the 32-kDa band ($t = 2.6, P < 0.05; \text{Table 3}$) and a trend difference for the immunoreactivity of the 39-kDa band ($t = 1.8, P < 0.1; \text{Table 3}$), with Val158 homozygotes showing significantly greater immunoreactivity than heterozygotes. Furthermore, there was a significant effect of genotype on the immunoreactivity of the 39-kDa band, expressed relative to the total MB-COMT (32 + 39 kDa bands; $t = -2.9, P < 0.01; \text{Table 3}$; of interest given that it replicates our recent findings in postmortem PFC tissue taken from psychiatric patients and controls [Tunbridge, Weinberger, and Harrison 2006]; see supplementary online information). However, there were no significant genotype group differences for immunoreactivity of either the 26-kDa ($t = 0.40; \text{Table 3}$) or the 24-kDa ($t = 1.63; \text{Table 3}$) COMT bands, or β-actin ($t = 0.13; \text{Table 3}$).

Discussion

The main finding of this study is that there is a significant increase in COMT enzyme activity in human dorsolateral PFC during postnatal development, which continues into adulthood. Although this study lacks the power to statistically investigate interactions between age group and Val158Met genotype, this increase in COMT enzyme activity with age is unlikely to result from a chance difference in Val158Met genotype frequencies between age groups because the genotypes are fairly evenly distributed among the groups and an increase in COMT enzyme activity...
activity with age is seen in both Val^{158}/Val^{158} and Val^{158}/Met^{158} genotype groups (Fig. 4). Additionally, we demonstrate significant correlations between COMT enzyme activity and immunoreactivity of COMT protein isoforms and replicate our previous finding of an additional 39-kDa COMT immunoreactive band (Tunbridge, Weinberger, and Harrison 2006; see supplementary information).

These data show that, like other PFC dopaminergic markers (e.g., Lidow and Rakic 1992; Rosenberg and Lewis 1995; Lewis and others 1998; Lambe and others 2000; Levitt 2003; Weickert, M.J. Webster, P. Gondipalli, D. Rothmond, R.J. Fatula, M.M. Herman, J.E. Kleinman, and M. Akil, unpublished data), PFC COMT is variable depending on an individual’s age and increases over a protracted postnatal period. However, in contrast to data obtained for other PFC dopaminergic markers (see Introduction and discussed further below), COMT enzyme activity continues to increase through adolescence, peaking only in adulthood, as demonstrated by our finding of a significant difference in this measure between the adult group and teen and young adult groups.

Our data are consistent with the limited available data on COMT activity during postnatal maturation in other tissues and species. In human liver, COMT activity increases postnatally, peaking in adulthood (Agathopolous and others 1971). Similarly, porcine COMT activity has been shown to increase after birth in several tissue types, including brain (Stanton and others 1975). Brust and others (2004) calculated that COMT activity increased in frontal cortex, but not in striatum or mesencephalon, in young adult pigs compared with newborns. However, these findings were extrapolated from positron emission tomography of 18F-fluorodopa utilization, rather than direct measurement of COMT enzyme activity. Interestingly, our findings extend those of Chen and others (2004) who found a weak but positive correlation between age and COMT enzyme activity in dorsolateral PFC in an adult cohort (mean age ± standard deviation: 40.6 ± 14.9 years, r = 0.194).

The increase in COMT activity during postnatal maturation is perhaps related, at least in part, to the general expansion and refinement of the PFC dopamine system that occurs over this period (e.g., Lambe and others 2000). That is, the increased PFC COMT activity from neonates to adulthood might reflect an increased need for dopamine catabolism, accompanying the expansion in dopaminergic innervation that occurs during adolescence. In this respect, increasing COMT expression and activity may be part of the developmental homeostasis that regulates dopamine function in the PFC during this critical postnatal developmental period, in which prefrontal function is maturing, and reward and learning mechanisms subserved by dopamine are also being elaborated and refined. However, our data suggest that the maturational elevation of COMT activity is more prolonged than other aspects of the dopaminergic system. This is in clear contrast to the postnatal profiles of other markers of dopamine function within the primate PFC. Thus, dopaminergic innervation of the monkey PFC peaks during adolescence (Rosenberg and Lewis 1995; Lewis 1997; Lambe and others 2000), and adult levels of the dopamine receptors are obtained by the onset of adolescence, following a peak early in postnatal development (Lidow and Rakic 1992). Similarly, there is no change in monoamine oxidase-A, or monoamine oxidase-B, activity in the human frontal cortex during the period between childhood and adulthood (Kornhuber and others 1989). Finally, although postnatal changes of levels in dopamine and its metabolites have not been systematically studied into full adulthood in a single human cohort, the available data from studies of monkey and human brain suggest that adult levels are reached during adolescence (Adolfsson and others 1979; Goldman-Rakic and Brown 1992). Thus, the increase in PFC COMT activity from the young adult period to adulthood demonstrated in this study stands in contrast to data obtained for a range of other dopaminergic markers within the PFC, which reach adult levels during or before adolescence.

Evidence that the functional role of COMT in PFC changes during adolescence comes from recent data obtained in subjects with velocardiofacial syndrome (VCFS, also known as DiGeorge syndrome; Shprintzen and others 1978). VCFS is a developmental disorder resulting from hemizygous deletion of chromosome 22q11, with the COMT locus comprising part of the critically deleted region; thus, individuals with VCFS are hemizygous for the Val^{158}/Met polymorphism. Of interest to the present study is the fact that the relationship between the Val^{158}/Met allele and cognition in VCFS subjects is critically dependent on the age of the individual (Goethel and others 2005). Thus, prior to adolescence (11–13 years), Met^{158} hemizygotes perform significantly better than Val^{158} hemizygotes on tests of cognition (Bearden and others 2004; Goethel and others 2005), similar to findings in normal adults (e.g., Egan and others 2001), whereas in late adolescence (16–18 years) Val^{158} hemizygotes outperform Met^{158} hemizygotes (Baker and others 2005; Goethel and others 2005), due to a significant deterioration in performance in the Met^{158} hemizygote group (Goethel and others 2005). The better PFC performance of Val^{158} hemizygotes compared with Met^{158} hemizygotes in the older VCFS cohorts is consistent with the suggested inverted-U-shaped relationship between PFC dopamine levels and PFC function (Goldman-Rakic and others 2000, Mattay and others 2003) because the presence of the Met^{158} allele on a hemizygous background combined with age-related increases in dopamine signaling likely represents suboptimal COMT function and superoptimal dopamine levels, compared with the more optimal dopamine levels found in Val^{158} hemizygotes (for further discussion, see Tunbridge, Harrison, and Weinberger 2006). It might be envisaged that the changing relationship
between the COMT Val158Met polymorphism and PFC function in VCFS is related to fluctuations in the global dopaminergic state of the PFC at different postnatal developmental time points, determined by COMT enzyme activity and other dopaminergic markers. Such fluctuations might be expected to result from alterations in the balance between dopamine innervation and catabolism, as these opposing actions concurrently increase during PFC maturation.

It is important to note that the increase in COMT activity may, in part, be related to its methylation of substrates other than the catecholamines, such as catecholestrogens (Männistö and Kaakkola 1998). Certain catecholestrogens are toxic, and, therefore, the upregulation of COMT activity during ontogenesis may represent an attempt to metabolize any accumulation of these compounds. Catecholestrogens are bioactive, for example, in the periphery they modulate angiogenesis (Zhu and Conney 1998). However, although they are known to be present in the cortex, their function in brain remains unknown (Zhu and Conney 1998).

Western blotting using a previously described antibody (Chen and others 2004; Tunbridge, Weinberger, and Harrison 2006) identified several COMT immunoreactive bands. The band at approximately 32 kDa is of the anticipated size for MB-COMT, and we recently described the 39-kDa isoform and demonstrated that it is a COMT allozyme (Tunbridge, Weinberger, and Harrison 2006). Thus, based on their size and abundance relative to the lower bands (e.g., Chen and others 2004), we believe the 32- and 39-kDa bands to be isoforms of MB-COMT. This interpretation is consistent with the data above, showing their abundance to be correlated with COMT enzyme activity. In our previous study (Tunbridge, Weinberger, and Harrison 2006), S-COMT was expressed at too low an abundance to be reliably quantified; thus, our current report of a doublet of COMT immunoreactive bands at 24 and 26 kDa, approximately the reported size of S-COMT (25 kDa), represents a novel finding. It is currently not clear whether both of these bands are isoforms of S-COMT, breakdown products of the larger COMT isoforms, or whether one or both represent nonspecific antibody binding. However, the significant correlation between the abundance of the 24-kDa band and COMT enzyme activity supports the interpretation that the 24-kDa band is a COMT allozyme. Thus, correlations between COMT enzyme activity and western blotting results suggest that both MB-COMT and S-COMT allozymes contribute to measured COMT enzyme activity.

Although COMT enzyme activity was higher in the Val/Val homozygote group than the Val/Met heterozygote group, we found no significant effect of the Val158Met polymorphism on this measure in the current samples of human brain tissue. This is likely due primarily to the absence of a Met/Met homozygote group than the Val/Met heterozygote group, we found no significant effect of Val158Met genotype on expression of the 24- or 26-kDa bands, possibly due to the small sample size and their relatively lower abundance. Thus, although Val158Met genotype affected abundance of COMT immunoreactive bands as previously described (Shield and others 2003; Chen and others 2004), this failed to manifest as a significant effect on enzyme activity in this study.

In conclusion, we demonstrate that COMT enzyme activity increases during human postnatal development in the PFC, likely as the result of increased COMT protein. These results complement previous data showing a protracted development of the PFC dopamine system in primates.

**Supplementary Material**

Supplementary material can be found at: [http://www.cercor.oxfordjournals.org/](http://www.cercor.oxfordjournals.org/)

**Notes**

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Address correspondence to Elizabeth M. Tunbridge. Department of Psychiatry, Neurosciences Building, Oxford University, Warneford Hospital, Oxford OX3 7JX, UK. Email: elizabeth.tunbridge@psych.ox.ac.uk.

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Chen J, Lipska BK, Halin M, Ma QD, Matsumoto M, Melhem S, Kolachana BS, Hyde TM, Herman MM, Apud J, Egan MF, Kleinman JE, Weinberger DR. 2004. Functional analysis of genetic variation in COMT immunoreactivity, and the Val158Met polymorphism (see supplementary online material; Tunbridge, Weinberger, and Harrison 2006). However, we found no significant effect of Val158Met genotype on expression of the 24- or 26-kDa bands, possibly due to the small sample size and their relatively lower abundance. Thus, although Val158Met genotype affected abundance of COMT immunoreactive bands as previously described (Shield and others 2003; Chen and others 2004), this failed to manifest as a significant effect on enzyme activity in this study.

In conclusion, we demonstrate that COMT enzyme activity increases during human postnatal development in the PFC, likely as the result of increased COMT protein. These results complement previous data showing a protracted development of the PFC dopamine system in primates.

**Supplementary Material**

Supplementary material can be found at: [http://www.cercor.oxfordjournals.org/](http://www.cercor.oxfordjournals.org/)


