Altered Cortical Expression of GABA-Related Genes in Schizophrenia: Illness Progression vs Developmental Disturbance

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Background: Schizophrenia is a neurodevelopmental disorder with altered expression of GABA-related genes in the prefrontal cortex (PFC). However, whether these gene expression abnormalities reflect disturbances in postnatal developmental processes before clinical onset or arise as a consequence of clinical illness remains unclear. Methods: Expression levels for 7 GABA-related transcripts (vesicular GABA transporter [vGAT], GABA membrane transporter [GAT1], GABAα receptor subunit α1 [GABRA1] [novel in human and monkey cohorts], glutamic acid decarboxylase 67 [GAD67], parvalbumin, calretinin, and somatostatin [previously reported in human cohort, but not in monkey cohort]) were quantified in the PFC from 42 matched pairs of schizophrenia and comparison subjects and from 49 rhesus monkeys ranging in age from 1 week postnatal to adulthood. Results: Levels of vGAT and GABRA1, but not of GAT1, messenger RNAs (mRNAs) were lower in the PFC of the schizophrenia subjects. As previously reported, levels of GAD67, parvalbumin, and somatostatin, but not of calretinin, mRNAs were also lower in these subjects. Neither illness duration nor age accounted for the levels of the transcripts with altered expression in schizophrenia. In monkey PFC, developmental changes in expression levels of many of these transcripts were in the opposite direction of the changes observed in schizophrenia. For example, mRNA levels for vGAT, GABRA1, GAD67, and parvalbumin all increased with age. Conclusions: Together with published reports, these findings support the interpretation that the altered expression of GABA-related transcripts in schizophrenia reflects a blunting of normal postnatal developmental changes, but they cannot exclude a decline during the early stages of clinical illness.

Key words: schizophrenia/prefrontal cortex/ neurodevelopment/parvalbumin/GABA

Introduction

Deficits in certain cognitive processes, such as working memory, are common in individuals with schizophrenia and have been attributed to dysfunction of the prefrontal cortex (PFC).¹ This dysfunction appears to reflect, at least in part, alterations in molecular markers of GABA neurotransmission.²,³ For example, multiple postmortem studies of schizophrenia subjects have documented lower messenger RNA (mRNA) levels of the principal enzyme responsible for cortical GABA synthesis, the 67-kDa isoform of glutamic acid decarboxylase 67 (GAD67).⁴⁻¹⁰ Although less well studied, mRNA levels of the presynaptic GABA membrane transporter (GAT1), which is responsible for the reuptake of extracellular GABA, have also been reported to be lower,¹¹ whereas mRNA levels of the vesicular GABA transporter (vGAT), which loads GABA into presynaptic vesicles, have been reported to be unchanged¹² in the PFC of schizophrenia subjects. On the postsynaptic side, mRNA levels of 2 of the most common ionotropic GABAα receptor subunits, α1 and α2 (GABRA1 and 2), appear to be lower and higher, respectively, in some¹³⁻¹⁵ but not all¹⁷ studies of schizophrenia.

These alterations may be more common in particular subsets of cortical GABA neurons. For example, parvalbumin mRNA, which is expressed in ~25% of PFC GABA neurons, is lower in schizophrenia.¹⁶⁻¹⁹ Parvalbumin-containing neurons also exhibit lower expression of GAD67 mRNA¹⁸ and contain lower levels of GAD67 protein in their axon terminals.²⁰ Importantly, these differences appear to reflect disease-related reductions in gene expression and not a deficit in the number of parvalbumin-containing neurons.¹⁴,¹⁸,²¹ A separate subset (~25%) of cortical GABA neurons that express somatostatin also show lower levels of the
the PFC of individuals with schizophrenia, but expressed in >40% of PFC GABA neurons that contain neither parvalbumin nor somatostatin, are not lower in the PFC of individuals with schizophrenia, but see reference.

Understanding the potential contributions of alterations in GABA-related transcripts (defined here as gene products that regulate GABA neurotransmission or are selectively expressed by subsets of GABA neurons) to working memory impairments in schizophrenia requires knowledge of whether they represent causes, consequences, or confounds of the underlying disease process. The findings cited above do not appear to be confounds due to either a nonspecific downregulation or general degradation of cortical mRNAs, because different GABA-related transcripts are lower, higher, or unchanged in the illness. Other findings suggest that the observed transcript alterations are not likely to be attributable to confounding factors such as medications or substance use.

Given that altered levels of GABA-related transcripts were observed in postmortem studies of schizophrenia subjects with varying illness duration, they could reflect the consequences of illness chronicity. If so, then the magnitude of the GABA-related transcript alterations would be expected to co-vary with illness duration. Alternatively, these alterations could be part of a causal developmental pathway leading to PFC dysfunction and working memory impairments in schizophrenia. For example, working memory performance and the associated activation of the PFC undergo a protracted maturation with adult levels of performance not achieved until late adolescence. Interestingly, a recent longitudinal prospective cohort study reported that in individuals who were later diagnosed with schizophrenia working memory function did not differ from comparison subjects at age 7, but then failed to improve with age at the normal rate. Thus, disturbances in the developmental trajectories of GABA-related transcripts prior to the clinical onset of schizophrenia could be causal factors contributing to working memory impairments in the illness.

In order to shed light on the “chronic illness consequence” vs “developmental cause” explanations of altered GABA-related gene expression in schizophrenia, we conducted 2 studies. First, we quantified mRNA levels of vGAT, GABRA1, GAT1, and the other GABA-related transcripts in the PFC from 42 matched pairs of schizophrenia and comparison subjects, and evaluated these data as a function of illness duration. The other GABA-related transcripts were quantified in previous studies and included GAD67, parvalbumin, calretinin, and somatostatin. Second, because it is not possible to assess the developmental trajectories of GABA-related transcripts in subjects with schizophrenia, we quantified the expression of these same transcripts in the PFC from 49 rhesus monkeys ranging in age from 1 week postnatal to adulthood. We then compared the developmental trajectory of each transcript with its expression status in schizophrenia to examine whether a disturbance during development could be a plausible explanation for the pattern of GABA-related transcript alterations seen in the illness. While this approach has been recently explored by other groups using a developmental human cohort, rhesus monkey studies provide the advantage of less between-subject variance due to factors other than age.

Methods

Human Studies

Brain specimens (n = 84) were obtained during autopsies conducted at the Allegheny County Office of the Medical Examiner (Pittsburgh, PA) after consent was obtained from the next-of-kin. Consensus Diagnostic and Statistical Manual of Mental Disorders-IV-Text Revision diagnoses for each subject were made using structured interviews with family members and review of medical records; the absence of a psychiatric diagnosis was confirmed in healthy comparison subjects using the same approach. To reduce the effects of biological variance and control for experimental variance, subjects with schizophrenia or schizoaffective disorder (n = 42) were matched individually to one healthy comparison subject for gender, and as closely as possible for age, and samples from both subjects in a pair were processed together throughout all stages of the study. The mean age, postmortem interval, brain pH, RNA integrity number (RIN), and tissue storage time did not differ between subject groups (supplementary tables T1 and T2). The University of Pittsburgh's Committee for the Oversight of Research Involving the Dead and Institutional Review Board for Biomedical Research approved all procedures.

Frozen tissue blocks from each subject were confirmed to contain PFC area 9 using Nissl-stained tissue sections cut on a cryostat at 40 μm thickness. Gray matter from adjacent sections was separately collected into a tube containing TRIzol reagent using a method that ensured minimal white matter contamination and excellent RNA preservation.

Levels of vGAT, GAT1, and GABRA1 mRNAs were quantified by real-time quantitative PCR (qPCR) using previously described methods (see supplementary methods for details). The geometric mean of 3 normalizers (β-actin, cyclophilin-A, and glyceraldehyde 3-phosphate dehydrogenase [GAPDH]) were used and these Ct values did not differ between the schizophrenia and comparison subject groups as reported previously. The results of similar studies for GAD67, parvalbumin, calretinin, and somatostatin mRNAs in the same cohort of subjects have been recently reported.
Monkey Studies
Forty-nine rhesus monkeys (Macaca mulatta) ranging in age from postnatal 1 week to 11.5 years were used (supplementary tables T3 and T4). Animals were housed according to age as previously described. All housing and experimental procedures were conducted in accordance with the guidelines of the US Department of Agriculture and the NIH Guide for the Care of Animals, and with approval from the University of Pittsburgh’s Institutional Animal Care and Use Committee.

Twenty-one animals were perfused transcardially with ice-cold artificial cerebrospinal fluid following deep anesthesia induced with ketamine and pentobarbital; in 5 of these animals, a small tissue block from the left principal sulcus had been surgically excised for in vitro electrophysiology studies 2–4 weeks prior to perfusion. The remaining 28 animals were experimentally naive. After deep anesthesia with ketamine and pentobarbital was induced, the brains were removed. For all animals, the right hemisphere of each brain was blocked coronally and each block was frozen and stored at −80°C.

Frontal pole sections (40 µm) composed entirely of gray matter were collected into tubes containing TRIzol reagent in a manner that ensured excellent RNA preservation. Area 10 was used due to the availability of tissue from that cortical region for each animal. qPCR was conducted as described for the human study with the following differences. Primer sets were designed against the macaque sequences of the same 7 target genes used in the human studies, as well as two reference genes, β-actin and cyclophillin-A, which exhibited stable relative expression levels across the postnatal developmental ages studied ($F_{7,41} = 1.594; P = .16$ in the present study, and as previously reported). GAPDH was not used as a reference gene because its mRNA levels appeared to be unstable across early postnatal development.

Statistical Analyses
Two ANCOVA models were used to examine the effect of diagnosis on vGAT, GABRA1, and GAT1 mRNA levels. The unpaired ANCOVA model included mRNA level as the dependent variable, diagnostic group as the main effect, and any relevant covariates that were significantly related to mRNA level. Since subjects were also paired to account for the parallel processing of tissue samples from a pair and to balance diagnostic groups for sex and age, a second paired ANCOVA model including subject pair as a blocking factor was also employed. In building the analytical models, graphics were used to assess model fit and to assure the absence of outliers. The statistical results (both test statistics and $P$ values with degrees of freedom varying depending on the numbers of covariates used) are reported for both models.

Because the monkey data suggested that log(age) might perhaps provide a better fit to the monkey data, the human model was also reanalyzed using log(age) instead of age. Results of the 2 models did not differ, with $P$ values being very similar; thus, subject log(age) results for humans are not presented.

To explore whether the alterations in GABA-related transcript levels in schizophrenia subjects were a consequence of the disease process, the correlations between illness duration (the difference between age at clinical onset and age at death) and the within-pair percent difference (schizophrenia subject minus comparison subject) in expression of each mRNA transcript were computed and Bonferroni-Holm adjusted $P$ values are reported. In addition, the age by diagnosis interactions were assessed in both ANCOVA models.

The relationship of the within-subject pair differences for transcript levels to each of the following potential confounds were examined by chi-square tests: diagnosis of schizoaffective disorder, death by suicide, substance use diagnosis at time of death, or antidepressant, anti-psychotic, or benzodiazepine use at time of death. The substance use and medication data were obtained from toxicology studies at time of death and records of prescriptions at time of death. Subjects with negative toxicology and a reported history of medication nonadherence were considered as not taking medications at time of death, regardless of active prescriptions. In addition, independent living status at the time of death and the Hollingshead Two-Factor Index of Social Position were used as surrogate measures for cognitive functioning (supplementary table T2). Independent samples 2-tailed $t$ tests were used to examine the relationship between transcript expression level and independent living status at the time of death, while Pearson’s correlation coefficient was used to determine the relationship between transcript expression and Hollingshead Two-Factor Index of Social Position.

For the monkey studies, the correlations between age and each mRNA level were determined using Pearson’s correlation coefficient (with Bonferroni-Holm adjusted $P$ values). A second analysis used an ANCOVA model in which animals were placed into 1 of 4 age groups based on existing data regarding probable inflection points in the maturation of primate PFC circuitry. Such inflection points have been best studied for the postnatal developmental trajectory of excitatory synapse density, which exhibits 4 distinct phases in both macaque and human PFC. Therefore, each animal in the present study was assigned to 1 of the following 4 age groups: (1) perinatal, 0.25–1 month of age, within the period of a rapid increase in excitatory synaptic density, (2) prepubertal, 3–9 months, within the period when the density of excitatory synapses is at a plateau, (3) juvenile, 15–37 months, within the period of excitatory synapse pruning and (4) postpubertal, 42–138 months, during the period when the density of excitatory synapses is at stable adult levels. We use the term developmental trajectory to characterize the pattern of change in a transcript level over postnatal
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deviation from 1 week after birth to midlife, which
corresponds to the age range of the monkey cohort. The
ANCOVA model for gene expression used age group as
a factor. Levels of vGAT, GAT1, and calretinin mRNAs
significantly differed (all $F_{1,44} > 7.95$, all $P < .01$) as
a function of tissue storage time, which was included as a
covariate in the ANCOVA analyses, as well as any condi-
tional analysis, for these transcripts. Pairwise compar-
sion tests with $\alpha = 0.05$ were conducted for post hoc compari-
sions between age groups. Bonferroni-Holm adjusted $P$
values were computed to control for multiple comparison
tests among mRNAs.

Results

**GABA-Related Transcript Expression in Schizophrenia**

In PFC area 9, mean levels of vGAT (figure 1A; unpaired: $F_{1,78} = 4.13$, $P = .046$; paired: $F_{1,40} = 4.76$, $P = .04$) and GABRA1 (figure 1B; unpaired: $F_{1,79} = 8.39$, $P < .01$; paired: $F_{1,41} = 8.33$, $P < .01$) mRNAs were modestly but significantly lower in the subjects with schizophre-
nia. In contrast, GAT1 mRNA levels did not differ (fig-
ure 1C; unpaired: $F_{1,76} = 0.39$, $P = .53$; paired: $F_{1,40} = 0.72$, $P = .40$) between groups. Levels of vGAT (unpaired: $F_{1,76} = 6.94$, $P = .01$) and GAT1 (unpaired: $F_{1,76} = 9.07$, $P < .01$) mRNAs were negatively associated with tissue storage time. Therefore, tissue storage time was included in the paired and unpaired statistical models for vGAT and GAT1. Other covariates including pH and RIN were not related to vGAT, GABRA1, or GAT1 mRNA lev-
els. In the subjects with schizophrenia, levels of vGAT, GABRA1 and GAT1 mRNAs also did not differ as a function of diagnosis of schizoaffective disorder, death
by suicide, substance use diagnosis at time of death, or antidepressant, antipsychotic, or benzodiazepine use at
time of death (all $|r| < 1.96$, $P > .05$).

In this same cohort of 42 subject pairs, using the same
qPCR method, we recently reported that mRNA levels
for GAD67, parvalbumin, and somatostatin were lower,
and mRNA levels for calretinin were higher, in the sub-
jects with schizophrenia.19,20 Transcript expression levels
for vGAT, GABRA1, GAT1, GAD67, parvalbumin, cal-
retinin, or somatostatin were not significantly related to
independent living status at time of death (all $|r| < 0.423;
all $P > .69$) or Hollingshead Two-Factor Index of Social
Position (all $|r| < .27$; all $P > .08$).

To determine if levels of the 6 GABA-related tran-
scripts altered in schizophrenia (vGAT, GABRA1,
GAD67, parvalbumin, calretinin, and somatostatin) were
correlated with illness duration, the within-pair percent
difference in transcript expression was plotted against ill-
ness duration for each schizophrenia subject. The percent
differences of the expression levels of all transcripts did
not significantly correlate with illness duration (all $|r| < .27$, Bonferroni-Holm corrected $P > .52$) (figure 2). In
addition, the percent differences of the expression levels

![Fig. 1. Vesicular GABA transporter (vGAT) (A), GABA receptor subunit α1 (GABRA1) (B), and GABA membrane transporter (GAT1) (C) messenger RNA levels in prefrontal cortex from schizophrenia and comparison subjects. Scatter plots show the transcript levels for each matched pair of a comparison and either a schizophrenia (open circles) or schizoaffective disorder subject (open triangles). Values below the dashed unity line reflect pairs in which transcript levels are lower in the schizophrenia (or schizoaffective) subject relative to the comparison subject.](http://schizophreniabulletin.oxfordjournals.org/)

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Fig. 2. Within-pair percent difference (schizophrenia minus comparison subject) in prefrontal cortex transcript levels vs illness duration in schizophrenia subjects. Pearson’s correlation coefficient ($r$) was not statistically significant for any of the GABA-related messenger RNA levels examined that were altered in schizophrenia (A–F). Bonferroni-Holm corrected $P$ values are shown for each transcript. For each panel, each matched pair of schizophrenia and comparison subjects is shown as an open black circle.
for these same transcripts did not significantly correlate with age at illness onset (all $|r| < .25$, Bonferroni-Holm corrected $P > .69$).

We further examined if any of the 6 GABA-related transcripts altered in schizophrenia exhibited an age-related effect that differed between diagnostic groups. There was no significant age by diagnosis interaction on mRNA levels for any of these GABA-related markers (nominal $P$ values: vGAT: $F_{1,77} = 1.26, P = .26$; GAD67: $F_{1,78} = 3.65, P = .06$; GABRA1: $F_{1,78} = 0.02, P = .88$; parvalbumin: $F_{1,76} = 0.38, P = .54$; calretinin: $F_{1,76} = 0.24, P = .63$; somatostatin: $F_{1,78} = 0.29, P = .59$; all Bonferroni-Holm corrected $P > .36$) (figure 3).

Together, these findings suggest that changes in GABA-related transcripts in schizophrenia are not a consequence of illness chronicity.

Postnatal Trajectories of GABA-Related Transcripts in Monkey PFC

To investigate whether the pattern of altered cortical GABA-related mRNA levels in schizophrenia could be consistent with developmental abnormalities occurring before clinical illness onset, we determined the normative postnatal developmental trajectories of these GABA-related transcripts in monkey PFC. Due to the significant effect of tissue storage time found in the human studies, preliminary analysis was done in the monkey studies and showed that tissue storage time was a significant covariate for the vGAT, GAT1, and calretinin mRNAs. Thus, the following analyses for these 3 transcripts were adjusted for tissue storage time.

For the 4 markers of GABA neurotransmission, log(age) was significantly positively correlated with mRNA levels for GABRA1 ($r = .87, P < .0001$, Bonferroni-Holm corrected $P < .001$) and GAD67 ($r = .41, P = .004$, Bonferroni-Holm corrected $P = .01$), but not with vGAT (partial $r = .30, P = .04$, Bonferroni-Holm corrected $P = .07$) or GAT1 (partial $r = .01, P = .94$) mRNAs (figure 4A–D). When analyzed by age group, GAD67 ($F_{3,45} = 3.73, P = .02$, Bonferroni-Holm corrected $P = .05$) and GABRA1 ($F_{3,45} = 63.8, P < .0001$, Bonferroni-Holm corrected $P < .001$) significantly differed across the 4 age groups. The mean mRNA levels were higher in the postpubertal relative to perinatal group for vGAT (+16%, $P = .09$, Bonferroni-Holm corrected $P = .17$), GAD67 (+21%, $P = .006$, Bonferroni-Holm corrected $P = .02$), and GABRA1 (+78%, $P < .0001$, Bonferroni-Holm corrected $P < .001$) (figure 4E–H).

For the 3 markers of GABA neuron subpopulations, mRNA levels were positively correlated with log(age) for parvalbumin ($r = .63, P < .0001$, Bonferroni-Holm corrected $P < .001$), negatively correlated with log(age) for somatostatin ($r = -.55, P < .0001$, Bonferroni-Holm corrected $P < .001$), and marginally negatively correlated with log(age) for calretinin (partial $r = -.25, P = .08$) (figure 5A–C). When analyzed by age group, parvalbumin ($F_{1,45} = 27.02, P < .0001$, Bonferroni-Holm corrected $P < .001$) and somatostatin ($F_{1,45} = 13.47, P < .0001$, Bonferroni-Holm corrected $P < .001$) significantly differed across the 4 age groups. Mean levels in the postpubertal group were significantly higher than in the perinatal group for parvalbumin (+78%, $P < .0001$, Bonferroni-Holm corrected $P < .001$), lower for somatostatin (−33%, $P < .0001$, Bonferroni-Holm corrected $P < .001$), and marginally lower for calretinin (−23%, $P = .06$, Bonferroni-Holm corrected $P = .06$) (figure 5D–F).

Given that a number of environmental exposures from birth through adolescence are associated with increased risk for schizophrenia,44 and may have a particularly strong influence on neural circuits during sensitive periods of cortical development,45 we determined when during postnatal development transitions in the expression levels of GABA-related mRNAs might occur. From the perinatal to prepubertal period, parvalbumin (+578%, $P < .0001$, Bonferroni-Holm corrected $P < .001$) and GABRA1 (+54%, $P < .0001$, Bonferroni-Holm corrected $P < .001$) mRNA levels significantly increased, while vGAT (+10%, $P = .19$) mRNA levels marginally increased (supplementary table T5). GABRA1 mRNA expression also increased significantly from the juvenile to postpubertal period (+12%, $P = .001$, Bonferroni-Holm corrected $P = .003$), albeit more modestly than the earlier rise. Although GAD67 mRNA levels increased progressively from the perinatal to postpubertal period, there were no observable inflection points between adjacent age groups. Somatostatin mRNA levels decreased significantly from the perinatal to prepubertal and prepubertal to juvenile periods (−17%, $P = .007$, Bonferroni-Holm corrected $P = .01$ and −18%, $P = .005$, Bonferroni-Holm corrected $P = .014$, respectively). Calretinin mRNA levels decreased significantly (−23%, $P = .02$, Bonferroni-Holm corrected $P = .03$) and parvalbumin mRNA levels increased significantly (+28%, $P = .04$, Bonferroni-Holm corrected $P = .048$) from the prepubertal to juvenile periods. Taken together, the majority of the prominent GABA-related transcript expression changes occurred before the juvenile age period in monkey PFC.

Discussion

We report modest but significantly lower tissue levels of vGAT and GABRA1 mRNAs in the PFC of this 42 pair cohort of schizophrenia subjects. Given these and other recently reported GABA-related transcript abnormalities from the same subject cohort,19,20 we sought to determine whether altered expression of GABA-related mRNAs in schizophrenia could reflect the consequences of illness chronicity. Our analyses suggest that duration of clinical illness does not account for the observed alterations in GABA-related gene expression. To the extent that alterations in GABA neurotransmission contribute to cognitive
Fig. 3. Interaction of age by diagnosis on GABA-related messenger RNA levels in the prefrontal cortex of schizophrenia subjects. For each panel, comparison subjects are shown in open circles and schizophrenia subjects in filled circles. Gray solid and black dashed lines indicate lines of best fit for comparison and schizophrenia subjects, respectively. F- and Bonferroni-Holm adjusted $P$ values represent the age by diagnosis interaction statistics.
Fig. 4. Postnatal developmental trajectories of transcripts regulating GABA neurotransmission in monkey prefrontal cortex. For panels A–D, the black line indicates least squares line of best fit; and Pearson's correlation coefficient \(r\) and corresponding Bonferroni-Holm adjusted \(P\) value are indicated for each panel. For panels E–H, the black bars indicate group means. Age groups that do not share the same letters are significantly different (Bonferroni-Holm adjusted \(P < .05\)).
impairments in schizophrenia, our findings are consistent with reports that cognitive deficits in schizophrenia are not progressive after illness onset.46,47

To explore an alternative possibility that alterations in cortical GABA-related transcripts in schizophrenia might reflect developmental disturbances, we determined the postnatal developmental trajectories of GABA-related transcripts in the PFC from healthy rhesus monkeys. When comparing the mRNA levels between the first week of life and after puberty, the levels of GAD67, GABRA1, and parvalbumin mRNAs significantly increased, vGAT mRNA levels marginally increased, somatostatin mRNA levels significantly decreased, calretinin mRNA levels marginally decreased, and GAT1 mRNA levels remained unchanged. These developmental patterns generally matched those previously reported in human PFC.7,17,48 For example, microarray49 and PCR8 studies (available online at http://www.libd.org/braincloud) using PFC tissue from a human developmental cohort reported developmental trajectories for vGAT, GAD67, GABRA1, parvalbumin, and somatostatin that generally matched those reported here in macaque PFC.

Interestingly, for most transcripts the change in expression level with development was in the opposite direction of the difference observed between schizophrenia and comparison subjects. For example, levels of vGAT,
mental trajectories, resulting in a failure to achieve normal, mature mRNA levels.

This idea of an incomplete maturation of cortical GABA-related transcripts in schizophrenia is supported by previous findings in the literature. For example, the GABA_A receptor α2 subunit declines across postnatal development in monkey and human PFC, and is higher in the PFC of schizophrenia subjects, and the GABA_A receptor δ subunit in the PFC increases across postnatal development and is lower in schizophrenia. In addition, the µ-opioid receptor, which is expressed by parvalbumin neurons and regulates GABA release, declines with postnatal development and is higher in schizophrenia. Furthermore, expression patterns of alternatively spliced transcripts of the GAD1 gene are also consistent with this hypothesis; GAD67 mRNA increases and GAD25 mRNA decreases normally during early postnatal development of human PFC, but in schizophrenia subjects, the relative levels of GAD67 and GAD25 mRNAs were lower and higher, respectively, than in matched comparison subjects.

However, not all GABA-related transcripts with altered expression in schizophrenia fit the pattern predicted by the incomplete maturation hypothesis. For example, somatostatin mRNA levels are lower in the PFC of subjects with schizophrenia, and in accordance with the incomplete maturation hypothesis would be expected to increase in transcript expression during postnatal development. However, this transcript undergoes a pronounced decline with age that begins during early postnatal development and persists throughout adulthood both in macaques and humans. The fact that cortical somatostatin mRNA levels are also lower in other psychiatric disorders suggests that the altered expression of this transcript in schizophrenia may be driven by factors that are common to these disease processes and that could obscure the influence of early developmental events. Given the heterogeneity in disease course in schizophrenia, along with recent findings that a subset of individuals with schizophrenia show marked alterations in the expression of GABA-related transcripts, not all individuals with schizophrenia would be expected to show the molecular signatures of incomplete maturation.

The timing of the largest developmental changes in expression levels differed across GABA-related transcripts (supplementary table T5). These differences may reflect transcript-specific sensitive periods during development, and reveal which transcripts might be most expected to be affected by adverse environmental events that are associated with an increased risk of schizophrenia. For example, risk factors such as birth complications and urbanicity may be particularly likely to affect parvalbumin, GABRA1, and somatostatin expression since these transcripts change substantially during the perinatal to prepubertal periods. Similarly, risk factors occurring later in development, such as cannabis use during the early juvenile period, may predominantly impact the developmental trajectories of parvalbumin, calretinin, and somatostatin, which exhibit significant changes during the prepubertal to juvenile periods.

Such effects of environmental events on the specific GABA-related transcripts are likely to have a broader impact as GABA neurons are involved in shaping the maturation of cortical circuits during these same developmental periods. For example, the timing of the critical period for the development of binocular vision can be manipulated by the enhancement or reduction of GABA transmission in visual cortex, especially via GABRA1-containing neurons postsynaptic to parvalbumin-containing GABA neurons. Thus, the substantial increases in GABRA1 and parvalbumin mRNA levels in the primate PFC during postnatal development suggest that parvalbumin-containing inputs to GABRA1-containing postsynaptic structures may be especially susceptible to environmental exposures during development.

It is important to note that not all of the findings of the present study are in agreement with some reports in the literature. For example, recent postmortem studies using a different subject cohort failed to find the significantly lower levels of vGAT and GABRA1 mRNAs observed in the present study. This discrepancy could reflect differences in subject composition of the cohorts because we recently reported that altered expression of GABA-related transcripts is particularly marked in a subset of individuals with schizophrenia.

In concert, our findings suggest that altered expression of certain GABA-related transcripts in the PFC of subjects with schizophrenia is not the consequence of cumulative illness effects and may be due to blunted or incomplete developmental trajectories of these transcripts. However, these data cannot rule out a decline in GABA-related transcripts during the early stages of clinical illness after onset of psychosis. These findings may provide clues as to what molecular systems might be targeted for preemptive interventions at-at-risk individuals, and when such interventions might be most effective.
Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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