Biological Psychiatry

Archival Report

Transcriptome Alterations in Prefrontal Pyramidal Cells Distinguish Schizophrenia From Bipolar and Major Depressive Disorders

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ABSTRACT

BACKGROUND: Impairments in certain cognitive processes (e.g., working memory) are typically most pronounced in schizophrenia (SZ), intermediate in bipolar disorder, and least in major depressive disorder. Given that working memory depends, in part, on neural circuitry that includes pyramidal cells in layer 3 (L3) and layer 5 (L5) of the dorsolateral prefrontal cortex (DLPFC), we sought to determine if transcriptome alterations in these neurons were shared or distinctive for each diagnosis.

METHODS: Pools of L3 and L5 pyramidal cells in the DLPFC were individually captured by laser microdissection from 19 matched tetrads of unaffected comparison subjects and subjects with SZ, bipolar disorder, and major depressive disorder, and the messenger RNA was subjected to transcriptome profiling by microarray.

RESULTS: In DLPFC L3 and L5 pyramidal cells, transcriptome alterations were numerous in subjects with SZ, but rare in subjects with bipolar disorder and major depressive disorder. The leading molecular pathways altered in subjects with SZ involved mitochondrial energy production and the regulation of protein translation. In addition, we did not find any significant transcriptome signatures related to psychosis or suicide.

CONCLUSIONS: In concert, these findings suggest that molecular alterations in DLPFC L3 and L5 pyramidal cells might be characteristic of the disease processes operative in individuals diagnosed with SZ and thus might contribute to the circuitry alterations underlying cognitive dysfunction in individuals with SZ.

Keywords: Bipolar disorder, Major depression, Microarray, Prefrontal cortex, Pyramidal neurons, Schizophrenia

http://dx.doi.org/10.1016/j.biopsych.2017.03.018

Dysfunction of the dorsolateral prefrontal cortex (DLPFC) appears to be a core element of the disease process of schizophrenia (SZ) (1). This dysfunction is manifest as certain types of cognitive deficits, such as impairments in working memory (2). However, working memory deficits are also present in individuals with bipolar disorder (BPD) or major depressive disorder (MDD) (3–5). The severity of these impairments typically differs across diagnoses, with impairments most pronounced in SZ, intermediate in BPD, and least in MDD (6,7). These findings, in concert with evidence that certain genetic (8) and environmental risk factors (9) are shared among psychotic and mood disorders, suggest that these diagnoses might represent different points on a spectrum of disease, in contrast to the long-standing view that they are distinct kinds of illnesses (10).

This spectrum of disease hypothesis can be tested, in part, by determining if alterations in the neurobiological substrate for working memory are shared among individuals with SZ, BPD, and MDD. In monkeys, working memory depends on task-specific patterns of activity in pyramidal cells (PCs) located in DLPFC layer 3 (L3) and layer 5 (L5) (11,12). In the DLPFC of individuals with SZ, PCs in these layers exhibit morphological alterations, such as smaller somal size, lower spine density, and truncated dendritic trees (13–18). A lower density of dendritic spines on DLPFC L3 PCs was also detected in subjects with BPD (19) but not in subjects with MDD (16), although the latter did have smaller dendritic trees. At the molecular level, relative to unaffected comparison (UC) subjects, subjects with SZ exhibited transcriptome alterations in DLPFC L3 and/or L5 PCs (20,21) that were not detected in total gray matter samples, suggesting that cell type–specific analyses might reveal diagnosis-related patterns of molecular pathology not detected in transcriptome studies of cortical gray matter (22).

In this study, we assessed gene expression profiles in DLPFC L3 and L5 PCs obtained from matched tetrads of subjects with SZ, subjects with BPD, subjects with MDD, and UC subjects to address two questions: 1) Relative to UC subjects, what transcriptome alterations are present in DLPFC L3 and L5 PCs from subjects with each diagnosis? 2) Are these alterations shared or distinctive for each diagnosis?

 ISSN: 0006-3223

METHODS AND MATERIALS

Subjects

Brain specimens (N = 76) 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. An independent committee of research clinicians made consensus DSM-IV diagnoses using information obtained from medical records and structured diagnostic interviews conducted with the decedent's family members (16). The same approach was used to confirm the absence of psychiatric diagnoses in the UC subjects. Subjects with SZ (n = 19; 6 had schizoaffective [SA] disorder), BPD (n = 19; all had bipolar I disorder), or MDD (n = 19) and UC subjects (n = 19) were matched as tetrads for sex and as closely as possible for age (Table 1 and Supplemental Table S1). All procedures were approved by the University of Pittsburgh's Committee for the Oversight of Research and Clinical Training Involving the Dead and Institutional Review Board for Biomedical Research.

Laser Microdissection

Brain tissue was prepared and PC bodies were dissected as described previously (21). Sample collection is described in Supplemental Methods. Given the limited RNA quantity obtained from each pool of microdissected neurons, RNA integrity number (RIN) values were not assessed in the samples of neurons used for microarray profiling. However, we have previously demonstrated that the Nissl staining and laser microdissection approach used here results in RIN >7 in all samples, values nearly identical to those obtained in tissue homogenates from the same subjects (21).

Table 1. Summary of Subject Characteristics

Characteristic	Subject Groups[a]			
	UC	SZ	BPD	MDD
Number	19	19	19	19
Sex	10 M, 9 F	10 M, 9 F	10 M, 9 F	10 M, 9 F
Race	18 W, 1 B	13 W, 6 B	19 W	18 W, 1 B
Age, Years	47.8 (10.4)	45.1 (8.5)	46.3 (9.5)	45.2 (10.1)
PMI, Hours	19.3 (5.3)	20.1 (6.9)	21.3 (6.6)	20.1 (6.0)
Brain pH	6.6 (0.2)	6.6 (0.3)	6.6 (0.2)	6.6 (0.2)
RIN, Frontal Pole	8.0 (0.6)	7.9 (0.7)	8.0 (0.4)	8.0 (0.5)
Storage Time, Months at −80°C	110 (43)	111 (27)	128 (26)	126 (29)
Tobacco[b]	6	12	13[c]	7
Antidepressants[b]	0	10	12	7
Benzodiazepines and/or Anticonvulsants[b]	0	8	11	4
Antipsychotics[b]	0	16	7	2

Values are presented as number or mean (SD).

B, black; BPD, bipolar disorder; F, female; M, male; MDD, major depressive disorder; PMI, postmortem interval; RIN, RNA integrity number (obtained from a tissue block near the location of the tissue sections used for pyramidal cell capture); SZ, schizophrenia; UC, unaffected comparison; W, white.

[a]Subject groups did not differ in mean age, PMI, brain pH, RIN, or tissue storage time at −80°C (all $t_{71} < 2.30$; all $p > .14$) or in race ($\chi^2 < 3.2$; $p > .07$).

[b]Number of subjects with known use at time of death.

[c]In 4 BPD subjects, tobacco use at time of death was unknown; however, given the high frequency of smoking among BPD subjects, they were included as smokers in all analyses.

Microarray

For each sample, RNA was extracted using the RNeasy Plus Micro Kit (QIAGEN, Valencia, CA). All RNA samples from the same tetrad were processed together. Complementary DNA was synthesized and amplified using the Ovation Pico WTA System (NuGEN, San Carlos, CA), labeled using the Encore Biotin Module (NuGEN), and loaded on an Affymetrix GeneChip U219 Array Plate (Affymetrix, Santa Clara, CA), which contains approximately 49,000 probe sets designed to assess the expression level of more than 20,000 transcripts in the human genome. Replicate samples were synthesized and loaded on microarrays independently, with the loading order randomized. No batch correction was performed, as principal component analysis did not detect any variance attributable to plate. Samples from 2 subjects with SZ (Supplemental Table S1) did not pass quality control following array processing; the reported data are from the remaining 17 subjects. For each of the 74 unique subject samples in each layer, expression intensities were extracted from Affymetrix Expression Console (Affymetrix) and normalized using Robust Multi-array Average Express (23). The data were deposited in GEO (GSE87610). The correlations between replicates were high ($.80 < r < .95$), and thus the expression values of the replicate samples from each layer were averaged for data analysis.

Statistical Analysis

Data Filtering and Detection of Differentially Expressed Transcripts Within Each Diagnostic Group. The Affymetrix control probe sets were removed, as they have no biological relevance. To eliminate low-expressing and noninformative probe sets, we used a modification of a previously described filtering procedure based on a threshold determined by the contrast in expression levels of Y chromosome genes (Supplemental Methods, Supplemental Figure S1, and Supplemental Table S2) between male and female subjects (24). To detect differentially expressed transcripts, we followed a previously reported procedure (21) to fit a random intercept model (25) for each diagnosis separately (Supplemental Methods) to account for the matched design and the potential impact of covariates, including sex; age; RIN; brain pH; postmortem interval; death by suicide; presence of psychosis; presence of mood diagnosis; use of antidepressant, antipsychotic, or benzodiazepines and/or anticonvulsant medications at time of death; and tobacco use at time of death (Supplemental Methods). The best model was determined through the Bayesian information criterion, and the p value of diagnosis effect was assessed via likelihood ratio test. We randomly permutated samples 500 times to correct the p value owing to bias from model selection. The corrected p value was adjusted for multiple comparisons using the Storey procedure (26).

Effects of Clinical Covariates. To explore the effects of suicide or psychosis, we used a linear regression model with