SUPPLEMENTAL METHODS AND MATERIALS

Human subjects

Data were analyzed from the publicly-available RNA sequencing (RNASeq) analyses (1) completed as part of the CommonMind Consortium. Specifically, we analyzed gene expression in DLPFC gray matter samples from schizophrenia (N=57), bipolar disorder (N=35), and unaffected comparison (N=82) subjects. In order to eliminate confounding effects of donation site (1), analyses were restricted to samples provided by the University of Pittsburgh Brain Tissue Donation Program as most samples from bipolar disorder subjects in the CommonMind Consortium were provided by that source. (Supplemental Methods Table 1). All schizophrenia and bipolar disorder subjects were matched to an unaffected comparison subject based on sex, age and race (Supplemental Methods Table 4). Subject mean values for age and postmortem interval (PMI) did not differ by diagnosis (all $F_{2,171}<0.7$, all $p>0.5$). Mean RNA Integrity Number (RIN) was significantly ($F_{2,171}=3.6$, $p=0.03$) lower in schizophrenia subjects (8.0 ± 0.6) than in unaffected comparison (8.2 ± 0.6) and bipolar disorder (8.2 ± 0.5) subjects. Mean brain pH was significantly ($F_{2,171}=4.3$, $p=0.02$) lower in schizophrenia (6.6 ± 0.3) and bipolar disorder (6.6 ± 0.3) subjects than in unaffected comparison (6.7 ± 0.2) subjects. However, the biological significance of a mean difference of 0.2 RIN units or 0.1 pH units is unknown, especially given the variance intrinsic to these assays.

We also re-analyzed data from two previously published microarray studies of DLPFC L3PNs and L5PNs (2,3). The first study included 36 pairs of unaffected comparison and schizophrenia subjects matched perfectly for sex and as closely as possible for age and PMI (Supplemental Methods Tables 2 and 4). Subject mean values for age, PMI, brain pH, and RIN did not differ by diagnosis (all $t_{71}<3.36$, all $p>0.07$). In two pairs of subjects, the L5PN data did not pass microarray quality control; therefore, the analysis of L5PNs was conducted using 34 pairs of subjects (2,3). The second study (4) included a largely unique cohort of 19 matched triads of

unaffected comparison, schizophrenia and bipolar disorder subjects (Supplemental Methods Tables 3 - 4). Samples from two schizophrenia subjects did not pass quality control and were excluded from analysis. Subject mean values for age, PMI, brain pH and RIN did not differ by diagnosis (all $F_{2,52}<0.5$, all $p>0.5$).

RNASeq and microarray differential gene expression analysis and functional pathway enrichment in human subjects

All schizophrenia and bipolar disorder subjects were matched to an unaffected comparison subject based on sex, age and race. Matched pairs were processed together to mitigate the influence of library batch. RNASeq was conducted on samples from 50 mg of DLPFC gray matter using a ribosomal depletion approach (1). To capture the diversity of mitochondrial functional domains, we analyzed a specific gene set (n=1,033 genes) defined by Gene Ontology (GO) as 'mitochondria' (GOMito). For RNASeq analysis in gray matter, the limma package in R was used for voom normalization and count per million determination for each of 56,632 ENSEMBL genes. Genes with at least 1 count per million in 50% of the samples were retained for downstream analysis, resulting in 16,113 unique genes. Of these unique genes, 871 were present in GOMito. Determination of differential expression relative to unaffected comparison subjects was performed using a basic linear regression model along with the precision weights obtained during voom normalization.

Pairs of subjects (unaffected comparison with either a matched schizophrenia or bipolar disorder subject) were processed in the same batches for RNASeq. As these samples were processed as part of a larger study, several batches included only one pair of University of Pittsburgh samples. Principal component analyses did not identify batch as a primary source of variation. Therefore, correction for library batch (using statistical approaches such as Combat) was not performed. Previous covariate analyses of the CommonMind Consortium data showed that pH did not contribute to significant sources of variance in gene expression (1). Thus,

covariates included in the final model were RIN, PMI, age and sex. Application of an alternative analytical procedure developed by Jaffe and colleagues (5) to this dataset produced similar results, with test-statistics of each analytical method strongly correlated for the schizophrenia ($r=0.8$, $p=2.3x10^{-181}$) and moderately correlated for the bipolar disorder ($r=0.6$, $p=8.8x10^{-95}$) datasets (6-8).

Analysis of microarray data from L3PNs and L5PNs in 36 pairs of unaffected comparison and schizophrenia subjects was performed as previously described (3). Probes on these microarray platforms were commonly biased towards the 3' end of transcripts. Of the GOMito genes present on the microarray platform, 662 survived data filtering in both data sets and were included for analysis. Analysis of L3PNs and L5PNs in 19 triads of unaffected comparison, schizophrenia and bipolar disorder subjects was performed as previously described, including identification of DEGs at 20% FDR due to the smaller sample size (4). Of the GOMito genes present on this microarray platform, 634 GOMito genes survived data filtering and were included for analysis. Previous analyses of the pyramidal cell data using cell type-specific microarray profiling determined RIN to be a covariate often driving some of the overall variance (2,3). Other covariates tested, such as presence of antidepressants, anticonvulsants or nicotine at time of death, death by suicide, PMI and age did not account for the effects of diagnosis. Those microarray analyses also determined that pH (which is often slightly lower in schizophrenia relative to unaffected comparison subjects) behaved as a mediating variable (3); therefore, it was not included as a covariate.

Weighted Gene Co-expression Network Analysis

The weighted network for unaffected comparison subjects was constructed using the following WGCNA method (9). An unsigned correlation network was first obtained by creating a pairwise Pearson correlation between all pairs of genes in the GOMito pathway across all unaffected comparison subjects. Then an adjacency matrix was calculated by raising unsigned correlation

matrix to a certain power such that the resulting adjacency matrix was approximately scale-free. A hierarchical tree structure of all genes was obtained by performing hierarchical clustering with average linkage on the adjacency matrix. Gene modules with similar co-expression relationship were obtained by further applying dynamic hybrid tree cut algorithm (10) and restricting the minimum module size to 50. The resulting modules were assigned with a unique color as the identifier for further analysis. To compare network structures across diagnoses, a module preservation algorithm utilizing the Zsummary method was implemented (11). This analytical technique accounts for multiple components of module preservation, including module density, separability and connectivity.

RNASeq and microarray differential gene expression analysis in monkeys exposed to antipsychotic drugs

Analysis of DLPFC L3PNs and L5PNs was performed in a cohort (N=18) of young adult male monkeys (*Macaca fasicularis*) that received twice-daily oral doses of haloperidol, olanzapine, or sham (N=6 monkeys per group) for 17–27 months. All procedures regarding drug administration and euthanasia are described in detail in (12), and were conducted in accordance with NIH guidelines and with the approval of the University of Pittsburgh's IACUC. From fresh-frozen DLPFC, cryostat-cut sections (12 μm) were mounted on slides and stained with thionin for Nissl substance. For each layer within each monkey, 200 PNs were individually collected by laser microdissection and pooled into a single sample (2). For each sample, RNA was extracted using the QIAGEN Micro RNeasy kit Plus (Qiagen, Valencia, CA). cDNA was synthesized and amplified using the Ovation Pico WTA System (Nugene, San Carlos, CA), labeled using the Encore Biotin module and loaded on an Affymetrix Rhesus Array Plate (Santa Clara, CA), which is designed to assess expression levels of transcripts in the macaque monkey genome. For each of the samples, expression intensities were extracted from Affymetrix Expression Console using the RMA method (13) and transformed to log-scale (base 2). Differential expression statistics were determined

using the same approach applied to the human microarray dataset, except no covariates were included in the statistical model.

Supplemental Methods Table S1. Summary of Characteristics for RNASeq Subject Cohort

	Control	Schizophrenia	Bipolar Disorder
Number	82	57	35
Sex	59 M, 23 F	44 M, 13 F	20 M, 15 F
Age (years)	48.2 (14.2)	48.1 (13.0)	45.5 (12.2)
PMI (hours)	19.0 (5.4)	20.0 (8.4)	20.5 (7.0)
Brain pH	6.7 (0.2)	6.6 (0.3)	6.6 (0.3)
RIN	8.2 (0.6)	8.0 (0.6)	8.2 (0.5)
Race	71 W, 11 B	41 W, 16 B	34 W, 1 B

Values are mean (SD). PMI- Postmortem interval; RIN- RNA integrity number

Supplemental Methods Table S2. Summary of Characteristics for Microarray Subject Cohort of 36 Pairs

	Control	Schizophrenia
Number	36	36
Sex	27 M, 9 F	27 M, 9 F
Age (years)	48.1 (13.0)	46.9 (12.4)
PMI (hours)	17.6 (6.1)	18.0 (8.8)
Brain pH	6.7 (0.2)	6.6 (0.4)
RIN	8.3 (0.6)	8.2 (0.6)
Race	30 W, 6 B	24 W, 12 B

Values are mean (SD). PMI- Postmortem interval; RIN- RNA integrity number

Supplemental Methods Table S3. Summary of Characteristics for Microarray Subject Cohort of 19 Triads

	Control	Schizophrenia	Bipolar Disorder
Number	19	19	19
Sex	10 M, 9 F	10 M, 9 F	10 M, 9 F
Age (years)	47.8 (10.4)	45.1 (8.5)	46.3 (9.5)
PMI (hours)	19.3 (5.3)	20.1 (6.9)	21.3 (6.6)
Brain pH	6.6 (0.2)	6.6 (0.3)	6.6 (0.2)
RIN	8.0 (0.6)	7.9 (0.7)	8.0 (0.4)
Race	18 W, 1 B	13 W, 6 B	19 W

Values are mean (SD). PMI- Postmortem interval; RIN- RNA integrity number

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