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Critical period plasticity-related transcriptional aberrations in schizophrenia and bipolar disorder

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ABSTRACT

Childhood critical periods of experience-dependent plasticity are essential for the development of environmentally appropriate behavior and cognition. Disruption of critical periods can alter development of normal function and confer risk for neurodevelopmental disorders. While genes and their expression relevant to neurodevelopment are associated with schizophrenia, the molecular relationship between schizophrenia and critical periods has not been assessed systematically. Here, we apply a transcriptome-based bioinformatics approach to assess whether genes associated with the human critical period for visual cortex plasticity, a well-studied model of cortical critical periods, are aberrantly expressed in schizophrenia and bipolar disorder. Across two dozen datasets encompassing 522 cases and 374 controls, we find that the majority show aberrations in expression of genes associated with the critical period. We observed both hyper- and hypo-critical period plasticity phenotypes at the transcriptome level, which partially mapped to drug candidates that reverse the disorder signatures in silico. Our findings indicate plasticity aberrations in schizophrenia and their treatment may need to be considered in the context of subpopulations with elevated and others reduced plasticity. Future work should leverage ongoing consortia RNA-sequencing efforts to tease out the sources of plasticity-related transcriptional aberrations seen in schizophrenia, including true biological heterogeneity, interaction between normal development/aging and the disorder, and medication history. Our study also urges innovation towards direct assessment of visual cortex plasticity in humans with schizophrenia to precisely deconstruct the role of plasticity in this disorder.

1. Introduction

Critical periods of experience-dependent plasticity are discrete neurodevelopmental windows wherein brain circuitry is remodeled to produce environmentally relevant behavior and cognition. Disruption of critical periods can alter the neurodevelopmental trajectory and confer risk for neurodevelopmental disorder (Takesian and Hensch, 2013). While genes and their expression relevant to neurodevelopment are associated with schizophrenia (Birnbaum and Weinberger, 2017; Gandal et al., 2012; Hagihara et al., 2014; Smith et al., 2018) and a role for critical periods in schizophrenia has been hypothesized (Do et al., 2015), the molecular relationship between schizophrenia and critical periods has not been systematically assessed.

There are likely one or more critical periods for functions relevant to brain regions important in schizophrenia (such as prefrontal cortex; PFC). However, they have yet to be precisely defined. In contrast, the critical period for visual cortex plasticity as assessed by ocular dominance, the reduction of visual response in primary visual cortex (V1) following monocular deprivation, is one of the the best characterized critical periods across species from mouse to higher mammals (Morishita and Hensch, 2008). This positions the critical period for ocular dominance plasticity as a relevant model to effectively ask whether gene expression related to critical periods are impacted in schizophrenia. Moreover, there is growing evidence of shared mechanisms between the ocular dominance plasticity critical period and other

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cortical areas and their plasticity-related functions (Nabel and Morishita, 2013), suggesting the ocular dominance plasticity model as a useful starting point to generally understand the role of critical period mechanisms in schizophrenia.

Over the past decade, critical period mechanisms have been elucidated in the rodent visual system that may be relevant to schizophrenia and other neurodevelopmental disorders (Do et al., 2015; LeBlanc and Fagiolini, 2011). For example, a mouse model of Rett syndrome shows early opening and closure of the critical period (Krishnan et al., 2015), while a mouse model of elevated oxidative stress in parvalbumin interneurons relevant to schizophrenia shows a prolonged critical period into adulthood (Morishita et al., 2015a; Morishita et al., 2015b). These discoveries have led to the preclinical implementation of several pharmacological and behavioral interventions and provide a general conceptual framework for therapeutic intervention for recovery of function in adulthood (Bavelier et al., 2010; Morishita et al., 2010). However, these findings are necessarily restricted to animal models. Translating mechanistic insights from animal models to humans is a key next step in understanding critical period plasticity in human schizophrenia. Previous studies in individuals with schizophrenia detected deficits in cortical long-term potentiation/long-term depression (LTP/LTD)-like plasticity (Daskalakis et al., 2008; Hasan et al., 2011; Mears and Spencer, 2012; Pajonk et al., 2010), including in the visual system (Çavuş et al., 2012), but not by using the monocular deprivation paradigm used to map the critical period for ocular dominance plasticity in rodent models. Importantly, LTP/LTD-based plasticity mechanisms only partially correlate with deprivation-induced critical period plasticity (Hensch, 2005). Therefore, identifying biological correlates of the critical period for ocular dominance plasticity in individuals with schizophrenia and relating this back to existing knowledge about critical period biology in animal models is an essential step to understanding the role of critical period plasticity in schizophrenia.

We developed an integrative informatics approach to address the challenges of assessing the relationship between critical period plasticity and schizophrenia. Enabled by the growth of publicly available transcriptomic data (i.e. >70 K studies available at Gene Expression Omnibus; GEO), we previously showed that molecular matching between transcriptional signatures of diseases or cells and drugs yields high-quality preclinical targets, validated in vitro and in animal models (Dudley et al., 2011; Jahchan et al., 2013; Kidd et al., 2016; Sirota et al., 2011). In the context of schizophrenia, transcriptome-based approaches have effectively revealed biology (Fromer et al., 2016; Hoffman et al., 2017; Torkamani et al., 2010). Previously, we used knowledge of critical period biology in mice to assess the impact of over 400 diseases on a transcriptional signature of the mouse critical period for ocular dominance plasticity, a correlate of critical period plasticity (Smith et al., 2016). This analysis revealed a molecular relationship between schizophrenia and critical period gene expression, though a limited number of schizophrenia datasets were available, and the analysis was based on mouse biology.

Here, we interrogated the relationship between schizophrenia and critical period biology in human by applying an informatics approach to leverage knowledge of critical period biology in both mouse and non-human primate to generate a human transcriptional signature of the ocular dominance critical period in V1 and then systematically assessing the expression of these genes across all publicly available microarray experiments of schizophrenia and bipolar disorder (n = 24 datasets). We calculated a "critical period index" in each of 24 transcriptome-wide signatures of schizophrenia or bipolar disorder from PFC, parietal cortex, and temporal cortex to measure the relative enhancement or suppression of V1 critical period-associated gene expression in a given schizophrenia or bipolar disorder sample tissue. We found an elevated critical period index in the majority of schizophrenia and bipolar disorder datasets, indicating a hyper-critical period phenotype even in late adulthood and suggestive of elevated neuroplasticity. Computational drug repurposing analysis to discover drugs that reverse the transcriptional profile of a given disorder signature in silico identified distinct clusters of schizophrenia and bipolar disorder cohorts who do not share drug candidates, highlighting opportunities to identify precision therapies.

2. Experimental/materials and methods

2.1. Critical period signatures

A juvenile mouse critical period signature from primary visual cortex (V1) was generated from GSE89757 (Smith et al., 2016). Rhesus and human critical period V1 signatures were generated from the NIH Blueprint Non-Human Primate and BrainSpan data (www.blueprintnhpatlas.org and www.brainspan.org). Raw microarray probe-level data was quantile normalized using Limma (Smyth, 2005) and probes were mapped to corresponding genes. If more than one probe mapped to the same gene, we retained genes with the highest average expression across all samples. On gene-level data, we used RankProd (Hong et al., 2006) to compute a rank-based differential expression of genes. For the mouse critical period signature this was the differential expression of juvenile at postnatal day (P) 26 versus adult (>P60) (N = 3 both groups). For rhesus, the tissue was captured via laser microdissection, so we collapsed gene-level expression data by taking the average expression for each gene across all V1 substructures. Then, we computed the differential expression of 0 month versus 48 month (N = 3 both groups). For human, we generated differential expression signatures using N = 3 minimum number of samples across 4 sliding childhood windows plus adolescence where each window was a shifted up one available sample time point. These time points were 4 mo–1 yr (N = 3), 10 mo–2 yr (N = 3), 1 yr–3 yr (N = 4), and 2 yr–4 yr (N = 4). Adolescence was 13 yr–18 yr (N = 3). All were compared to adult 21 yr–40 yr (N = 3). For all differential expression analyses, genes were considered significant if the adjusted p value (see below) was <0.05 for a given gene and those that passed the threshold were included in that critical period or sliding window signature.

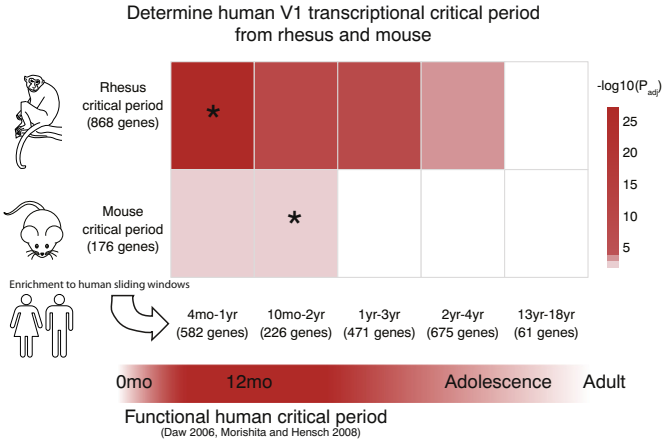


Fig. 1. Determining a human transcriptional signature associated with the critical period for V1 plasticity. The estimated peak functional critical period for ocular dominance plasticity in human is 6 months to 2 years old (Daw, 2006; Morishita and Hensch, 2008). To determine the transcriptional critical period in human, sliding windows across childhood were applied to compare differentially expressed genes at five time points relative to adult. Fisher Exact test was used to quantify the overlap of the sliding human windows with rhesus critical period genes differentially expressed in V1 of neonatal monkeys relative to adult, or mouse critical period genes differentially expressed in V1 of P26 vs > P60 mice. 4 mo–1 yr was most enriched when comparing human to rhesus and 10 mo–2 yr was most enriched when comparing human to mouse (*). Backgrounds used were the intersection of all human genes detected on the array with orthologous monkey or mouse genes detected by their respective arrays. Multiple tests were corrected using the Benjamini and Hochberg approach and depicted as the $-\log_{10}$ of the P_{adj}. Null comparisons with $P_{adj} > 0.05$ are colored white.

For comparing the overlap of critical period genes (Fig. 1 and Supplementary Table 1), we mapped signature genes to the closest orthologous gene using human as the reference species (i.e. if comparison was human to rhesus signature both would be mapped to human). Relatedly, to compute a background for Fisher's Exact tests to compare overlaps, each transcriptome was calculated (the genes detectable on the array after quality control), mapped to the closest orthologous gene for a given overlap comparison, and background taken as the genes shared by the two comparison species. The size of these backgrounds is as follows: human to rhesus: 12204 genes, human to mouse: 8957 genes.

2.2. Schizophrenia, bipolar disorder, and reference transcriptome signatures

Schizophrenia and bipolar disorder data were curated from public data by searching the Gene Expression Omnibus (GEO) with the stargeo.org (Hadley et al., 2017) and pubmed.gov tools searching the terms "schizophrenia" and "bipolar disorder" and identifying relevant datasets. All microarray datasets that had appropriate control groups were retained. A reference database of 495 disease signatures was previously curated from public data (Dudley et al., 2009). All data was normalized and differential transcriptomes calculated as previously published (Dudley et al., 2009). Briefly, raw data were downloaded from GEO, normalized by a rank-based approach (RankNorm) by ordering the expression values from highest to lowest and applying a rank where the highest expressed gene was N = total number of genes and lowest ranked gene was 1. Ranks were normalized to the range 0–1 inclusive by dividing all ranks by N. In cases where multiple probes mapped to the same gene the gene with highest average rank across samples was retained. A consensus rank was calculated per group (case or control) as the average rank across samples. Differential expression across the entire transcriptome was computed as the difference in consensus rank between case and control (SubDiff), yielding a differential expression transcriptome signature containing SubDiff gene expression values ranging from −1 to +1. For individual-level signatures, the process above was followed except that for each study a signature for each individual case was calculated by computing the difference in rank between a given case and the consensus rank of the controls.

2.3. Molecular matching of human critical period to schizophrenia, bipolar disorder, and reference signatures

A critical period index was calculated as the molecular match score of the human critical period signature to a given schizophrenia or other transcriptome signature. Briefly, the molecular match score was calculated by summing the SubDiff expression values in a given transcriptome signature that were shared with downregulated human critical period genes and subtracted from the sum of SubDiff values shared with upregulated human critical period genes to yield a summary measure of concordance between critical period and transcriptome signature. High critical period indexes indicate a given transcriptome signature expresses critical period genes in a manner similar to that in the human critical period. Low critical period indexes indicate a given transcriptome signature expresses critical period genes in a manner that opposes the human critical period signature. Therefore, a high critical period index suggests a hyper-critical period transcriptional phenotype, whereas a low critical period index suggests a hypo-critical period transcriptional phenotype. To compare match scores (M) across transcriptome signatures (e.g. schizophrenia, bipolar disorder, or reference), we normalized the scores with n = 10,000 permutations of scores using $\frac{M_{actual}-\overline{M}_{perm}}{\sqrt{\frac{\sum_{i=1}^{n}(M_{perm_i}-\overline{M}_{perm})}{n-1}^2}}$. p values were estimated from M using the Generalized Pareto Distribution (Knijnenburg et al., 2009) on n permutations and were adjusted for multiple tests (see below). M is computed similar to the approach by Zhang and Gant (2008).

We assessed the relationship of study-level covariates to the critical period index in order to determine if any known study factors explained the distribution of critical period indexes. To do so, we first identified covariates available for some or all studies (for categorical variables the number of levels are noted in brackets): case (schizophrenia or bipolar disorder) [2], N case, N control, tissue (collapsed to brain lobe to reduce the degrees of freedom required) [3], average age of case, average age of control, proportion of male participants in cases, proportion of male participants in controls, proportion of participants white in cases, and proportion of participants white in controls. Next, we set a base model with case, N case, and N control as covariates and critical period index as the outcome variable. We then assessed all other covariates asking if the Bayesian Information Criterion (BIC) was improved with the addition of covariate(s) to the linear regression model containing only the base covariates. Because some covariates were missing for some studies, covariates were assessed in subsets of the data starting from covariates that were included for all studies. The base model $R^2 = -0.039$ ($p = 0.56$) had a superior BIC than when adding tissue as a covariate ($N = 24$ studies with these covariates). Using the base model computed on a subset of data that included the average age covariates showed a $R^2 = -0.05$ ($p = 0.54$) and had a superior BIC to models that included average age of case, average age of control, or both ($N = 16$ studies). Using the base model computed on a subset of data that included the proportion male participants covariates showed a $R^2 = -0.074$ ($p = 0.58$) and had a superior BIC to models that included proportion male participants in case, proportion male participants in control, or both ($N = 15$ studies). Because these covariates were proportions, which do not follow a normal distribution, we transformed the proportions using the arcsine transformation. The subset of data that included the proportion of participants Caucasian covariates were not assessed due to low sample size ($N = 6$ studies), though it should be noted that in these 6 studies the average proportion white in cases was 91.7% and the average proportion white in controls was 96.5%, in line with the lamentable underrepresentation of non-white individuals in research studies. In all models assessed, none of the covariates explained the critical period index distribution, indicating other unmeasured covariates account for the distribution of critical period indexes across studies. Similar to above, we assessed the relationship of the base model covariates to the variance of individually-calculated critical period indexes ($R^2 = 0.31$, $p = 0.015$) to find a superior BIC than to a model that also included the study-level critical period index and the mean individual-level critical period index ($N = 24$ studies). N case in the base model drove the association ($\beta = 1.45$, $p = 0.052$) indicating that larger case sample size may correspond to increased variation in individual-level critical period indexes due to sampling a larger distribution of individuals with these disorders; in contrast critical period index itself (either study-level or mean individual-level) did not correspond to variance in individual-level critical period index.

2.4. Gene set enrichment analysis

To identify biological pathways associated with schizophrenia and bipolar disorder signatures, we assessed the overlap of genes shared between the human critical period signature and those aggregated from hyper-, null-, and hypo-critical period transcriptional subsets (respectively: critical period indexes >0 and $P_{adj} < 0.05$, critical period indexes with $P_{adj} > 0.05$, or critical period indexes <0 and $P_{adj} < 0.05$) with 5192 Gene Ontology Biological Process gene sets [Enrichr build (Chen et al., 2013)] using a hypergeometric test implemented in the HTSanalyzer (Wang et al., 2011) R package. Genes most differentially expressed (absolute Z-score > 1.5) in a given schizophrenia or bipolar disorder signature were used for aggregation into parent hyper-, null-, or hypo-critical period groups, to yield shared critical period-disorder aggregate gene sets of 311, 177, and 269 genes. For each of these three gene sets we ran Gene Ontology Biological Process enrichments separately using a background 17,260 genes, which included genes expressed on the human V1 brain microarray shared with the unique aggregate of all

genes expressed on the microarrays corresponding to the 24 schizophrenia and bipolar datasets. For each of the hyper-, null-, or hypo-critical period groups, we assessed the top 10 gene set enrichments ordered by p-value.

2.5. Computational drug repurposing

We identified repurposed drug candidates by matching the genes most differentially expressed in schizophrenia and bipolar signatures (absolute Z-score>2) to a database of drug-induced transcriptional profiles (Lamb et al., 2006) We merged 6100 individual experiments into drug-level representative signatures for each of 1309 small molecule compounds, according to the prototype-ranked list method (Iorio et al., 2010). For each unique compound, a modified Kolmogorov-Smirnov (KS) score was calculated (Lamb et al., 2006), summarizing each drug's transcriptional relationship to a given schizophrenia or bipolar disorder signature and quantifying the tendency for those genes to be concordantly up- or downregulated in the presence of a given compound. Standardization of individual scores were computed by generating an empirical KS score distribution from the query schizophrenia or bipolar signature to 1000 permuted drug signatures and the top 25 compounds with a negative score (anti-correlated to the schizophrenia or bipolar signature) were considered repurposed drug candidates for a given schizophrenia or bipolar signature. To assess similarity of drug repurposing results between disorder signatures, we calculated pairwise Pearson correlations between each schizophrenia or bipolar disorder signature's 1309 scores and performed hierarchical clustering of the resultant correlation coefficients using the Wald D approach, implementing Ward's criterion method (Murtagh and Legendre, 2014) (method = Wald.D2 in R), which squares the distances prior to updating the clusters. We assessed drug sharing within clusters by calculating the pairwise overlap of drugs for each disorder signature in a given cluster and calculated the average number of drugs shared across all signatures in a cluster. We then tabulated the number of times a given compound appeared across each disorder signature in a cluster to determine which drugs were most often shared within a cluster. If a drug was shared ≥1 times in a given cluster, it was considered a high confidence drug candidate for that cluster.

2.6. Chemogenomic enrichment analysis

We employed chemogenomic enrichment analysis (CGEA) to identify enrichments of drug targets within clusters. To do so, we calculated for each of 2378 known [DrugBank (Wishart et al., 2018)] and predicted [Similarity Ensemble Approach (Keiser et al., 2007)] drug targets a Fisher's Exact test to determine whether a given target was more likely than chance to be a target of the high confidence drug candidates in a given cluster relative to the background of the remainder of 1309 drugs available in the Connectivity Map less those in given cluster.

2.7. Statistical analysis

To address multiple comparisons, we computed an adjusted p value (P_{adj}) for all statistical tests according to the False Discovery Rate (Benjamini and Hochberg, 1995). All statistical analyses were conducted in the R programming language (v 3.2.2).

3. Results

3.1. Human transcriptional signature of critical period determined from monkey and mouse signatures

The peak of the human critical period for V1 ocular dominance plasticity is expected to be in the range of 6 months to 2 years based on clinical cases of idiopathic childhood aberrations (e.g. amblyopia) (Daw, 2006; Morishita and Hensch, 2008) (Fig. 1). To facilitate asking questions about critical period plasticity-related phenotypes in schizophrenia and bipolar disorder at the transcriptome level, we sought to calculate a transcriptional signature of human critical period in V1 by assessing the overlap of genes differentially expressed in V1 during human childhood relative to genes differentially expressed in V1 during the respective ocular dominance critical periods of rhesus macaque or mouse, which are systematically mapped by electrophysiological, anatomical, and behavioral experiments (Daw, 2006; Morishita and Hensch, 2008). To do so, we used a Fisher Exact test to compare the overlap of genes differentially expressed in 4 sliding windows of postnatal human V1 (BrainSpan data: 4 months–1 years, 10 months–2 years, 1–3 years, or 2–4 years) as well as adolescence (13–18 years) all relative to adult (21–40 years) to genes differentially expressed in rhesus V1 (NIH Blueprint Non-Human Primate data: neonatal vs. 48 months) or mouse V1 (from GSE89757: postnatal day 26 [P26] vs. >P60), during these species' critical periods for ocular dominance plasticity. We found that the greatest overlap with the rhesus critical period signature was that of the human 4 month to 1 year time point and the greatest overlap with the mouse critical period signature was that of human 10 month to 2 year time point (Fig. 1 and Supplementary Table 1). Given that both of these human juvenile signatures significantly overlapped with both rhesus and mouse critical period signatures, we calculated differential expression using human samples from both time points relative to adult to yield a summary human critical period transcriptional signature of 434 genes (171 upregulated and 263 downregulated at an adjusted p value (P_{adj}) < 0.05) for downstream analysis.

3.2. Human critical period signature is aberrantly expressed in schizophrenia and bipolar disorder

To assess the impact of schizophrenia and bipolar disorder on human critical period gene expression, we quantified a “critical period index” across dozens of schizophrenia and bipolar disorder transcriptome signatures. Critical period indexes are a measure of the coordinated expression of the human critical period signature genes derived from V1 in a given tissue-specific (prefrontal, parietal, or temporal cortex) schizophrenia or bipolar disorder signature and were calculated with a signed rank based molecular matching algorithm adapted from Zhang and Gant (2008). We scanned the Gene Expression Omnibus to identify 24 microarray datasets from 14 studies that included 522 participants diagnosed with schizophrenia or bipolar disorder (average case sample size per study = 21.8) with 374 matching control participants without these illnesses (average control sample size per study = 25.9). We normalized each dataset with RankNorm and computed differential transcriptomes via SubDiff (Dudley et al., 2009) (Fig. 2a and Supplementary Table 2; see Supplementary Fig. 1 for workflow). Next we calculated critical period indexes; a critical period index >0 indicates that human critical period genes in a given schizophrenia or bipolar disorder signature are differentially expressed in the sampled tissue similarly as in the human critical period signature. Conversely, a critical period index <0 indicates that human critical period genes in a given schizophrenia or bipolar disorder signature are differentially expressed in the opposite direction as in the human critical period signature (Fig. 2b). Using this approach, we observed that 70.8% (17 of 24) schizophrenia and bipolar disorder signatures had significant critical period indexes at a threshold of $P_{adj} < 0.05$ (Fig. 2c). This is a nearly 10-fold enrichment in significant critical period indexes compared to a reference database of critical period indexes calculated for a diverse set of 495 diseases, of which only 7.5% were significant at $P_{adj} < 0.05$ (Supplementary Table 3). Moreover, the enrichment of critical period indexes called significant among the schizophrenia and bipolar disorder signatures was 2.25-fold when considering only disease signatures with nervous system as the sampled tissue within the reference database (31.4% significant at $P_{adj} < 0.05$), indicating the magnitude of critical period indexes are particularly large in schizophrenia and bipolar disorder relative to background sampling (see Supplementary Table 3 for reference critical period indexes). Among the 17 schizophrenia and bipolar

signatures called significant, 70.6% (12 of 17) had critical period indexes >0 while 29.4% (5 of 17) had critical period indexes <0 (binomial test: $p = 0.07$; $p = 0.18$ when conditioned on probability of critical period index sign (− or +) among reference 495 signatures), suggestive that the majority of publicly available cohorts of adult schizophrenia and bipolar disorder participants may have a hyper-critical period phenotype at the transcriptome level in brain areas considered important in these illnesses. The variance and distribution of critical period indexes was similar for schizophrenia and bipolar disorder (F-test for equality of variances: $F = 1.39$, $p = 0.56$; Kolmogorov-Smirnov test of equality of distributions: $D = 0.24$, $p = 0.51$; Supplementary Fig. 2). To assess variation within studies, we calculated the critical period index at the level of individual participant and found that all studies had partially overlapping distributions (Fig. 2c), but the mean individual-level and study-level critical period indexes closely corresponded (Linear regression: $R^2 = 0.88$, $p = 9.53 \times 10^{-12}$). Individual-level critical period index variance was not explained by the study-level critical period index (Linear regression: $R^2 = -0.037$, $p = 0.67$), while a larger sample size for cases partially explained increased variance (Linear regression: $R^2 = 0.31, \beta = 1.45, p = 0.052$), indicating that null indexes are not the result of greater within-study variation (i.e. bimodal distributions of hyper and hypo together averaging to null) and instead suggest broader distributions may simply be the result of larger sample sizes.

Gene Ontology enrichment of critical period signature genes aggregated from schizophrenia and bipolar disorder signatures with hyper-critical period phenotypes (critical period indexes >0 and $P_{adj} < 0.05$) revealed myelination and synapse-related associations whereas genes aggregated from hypo-critical period signatures (critical period indexes <0 and $P_{adj} < 0.05$) identified ion transport and neuropeptide signaling pathways while null critical period signatures ($P_{adj} > 0.05$) were enriched for a mix of pathways seen in the other two phenotypes (Supplementary Table 4), suggestive of unique underlying biology depending on the critical period transcriptional phenotype.

Disorder type (schizophrenia or bipolar disorder), sample size, tissue type, age, and sex did not explain the distribution of critical period indexes (all $p > 0.05$, linear regression models; race was not assessed due to low inclusion in studies [n = 6], though for studies with race the average percent white was 91.7% for cases and 96.5% for controls), indicating other unmeasured covariates account for the impact on the critical period index.

3.3. Computational drug repurposing reveals unique drug candidates across disorder cohorts

Given heterogeneity in critical period transcriptome phenotypes across 24 schizophrenia and bipolar disorder signatures, we wondered if computational drug repurposing would yield unique drug candidates per cohort subpopulation. We employed a repurposing approach that matches transcriptional profiles of drugs to those of schizophrenia or bipolar disorder signatures to discover drugs that reverse the transcriptional profile of a given disorder signature in silico as candidate therapeutic drugs. Specifically, we calculated connectivity scores between the gene expression induced by 1309 drugs [Connectivity Map (Lamb et al., 2006)] to those genes most differentially expressed in each schizophrenia and bipolar disorder signature. The connectivity score provides a measure of how correlated or anticorrelated a given drug signature is to a given schizophrenia or bipolar disorder signature, with the expectation that drugs with anticorrelated signatures are putative drug candidates (Dudley et al., 2011; Jahchan et al., 2013; Sirota et al., 2011) (Fig. 3a–b). To determine a global similarity between each schizophrenia or bipolar disorder signature's list of 1309 drug connectivity scores, we calculated the Pearson correlation between the 1309 connectivity scores for each pair of schizophrenia/bipolar disorder signatures. Using hierarchical clustering, we identified 2 major clusters of signatures whose 1309 connectivity scores positively correlated to themselves, but anti-correlated to members of the other cluster (Fig. 3c), suggesting a unique drug candidate space in these two clusters of cohorts. We mapped the critical period indexes for each schizophrenia and bipolar disorder signature to the correlation heatmap (see annotation bar in Fig. 2c) and found that Cluster 1 contained only significant critical period indexes >0, while Cluster 2 contained mixed indexes of both >0 and <0 (Fig. 3c). Cluster 1 contained 8 schizophrenia/bipolar disorder signatures that share among their top 25 repurposed candidates (drugs with connectivity scores most anticorrelated to a given schizophrenia or bipolar disorder signature) an average 1.64 drugs with their Cluster 1 neighbors, with 8 compounds shared ≥3 times (cephaeline, chenodeoxycholic acid, cinchonine, naringenin, Prestwick-1082, thapsigargin, trifluprom azine, trihexyphenidyl) (Supplementary Table 5). Similarly, Cluster 2 contained 16 schizophrenia/bipolar disorder signatures that share among their top 25 repurposed candidates an average 1.4 drugs with their Cluster 2 neighbors, with 28 compounds shared ≥3 times, (alcuronium chloride shared 6 times, irinotecan shared 5 times, 6-azathymine, cantharidin, ebselen, milrinone, sulindac sulfide, tyrphostin AG-825 each shared 4 times, and 19 compounds shared 3 times) (Supplementary Table 5). In contrast, *zero* drug candidates were shared between Clusters 1 and 2 when considering high confidence candidates that are shared at least once within a given cluster (Supplementary Fig. 3a) and shared significantly *less* candidates than expected by chance when considering all unique candidates per cluster (Fisher's Exact test: $OR = 0.5$, $p = 0.002$) (Supplementary Fig. 3b), highlighting a distinct drug candidate space for schizophrenia/bipolar disorder subpopulations with high critical period indexes versus low.

3.4. Chemogenomic enrichment analysis of candidate repurposed drugs identifies unique drug targets per cluster

Schizophrenia genomics have been leveraged to identify drug targets that converge on schizophrenia risk loci (Ruderfer et al., 2016). Here, we leveraged transcriptomics to identify drug targets that converge on gene expression associated to schizophrenia. We employed a chemogenomic enrichment analysis (CGEA) to identify enrichments of drug targets among high confidence candidates (drug candidates shared at least once in a given cluster) relative to the background set of 1309 drugs available in Connectivity Map. For each of 2378 known and predicted drug targets we calculated a Fisher's Exact test to determine whether a given target was more likely than chance to be a target of high confidence drugs in a given cluster (Fig. 3d). For Cluster 1 (hyper-critical period transcriptional phenotypes), 127 targets were enriched at $p < 0.05$ (Supplementary Table 6) and top targets included cannabinoid receptor 1 (CNR1, also known as CB1) (rank #1, Fisher's Exact test: $OR = 5.7$, $p = 0.00042$) and monoamine oxidase A (MAOA) (rank #5, Fisher's Exact test: $OR = 5.1$, $p = 0.0064$). Of note, cannabis use is a risk factor for psychoses (Moore et al., 2007) and the main psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol, targets cannabinoid receptor 1 (Mechoulam et al., 2014). Additionally, the monoaminergic neurotransmitter systems, particularly norepinephrine, are implicated in visual cortex plasticity (Kasamatsu and Pettigrew, 1976; Kasamatsu et al., 1979), suggesting monoamine oxidase A as a target related to plasticity in schizophrenia. For Cluster 2 (mixed hyper- and hypo-critical period phenotypes), 90 targets were enriched at $p < 0.05$ (Supplementary Table 6) and top targets included signal transducer and activator of transcription 1 (STAT1) (rank #3, Fisher's Exact test: $OR = 4.4$, $p = 0.0042$), which has been implicated in visual cortex plasticity (Nagakura et al., 2014). *Zero* targets were shared between Clusters 1 and 2 (Fisher's Exact test: $OR = 0$, $p = 0.0065$), suggesting a distinct drug target space among clusters of schizophrenia and bipolar disorder cohorts. Together, these results demonstrate that independent cohorts of individuals with schizophrenia and bipolar disorder have diverse critical period plasticity-related transcriptional phenotypes and this heterogeneity is magnified when assessing drug repurposing candidates and their targets.

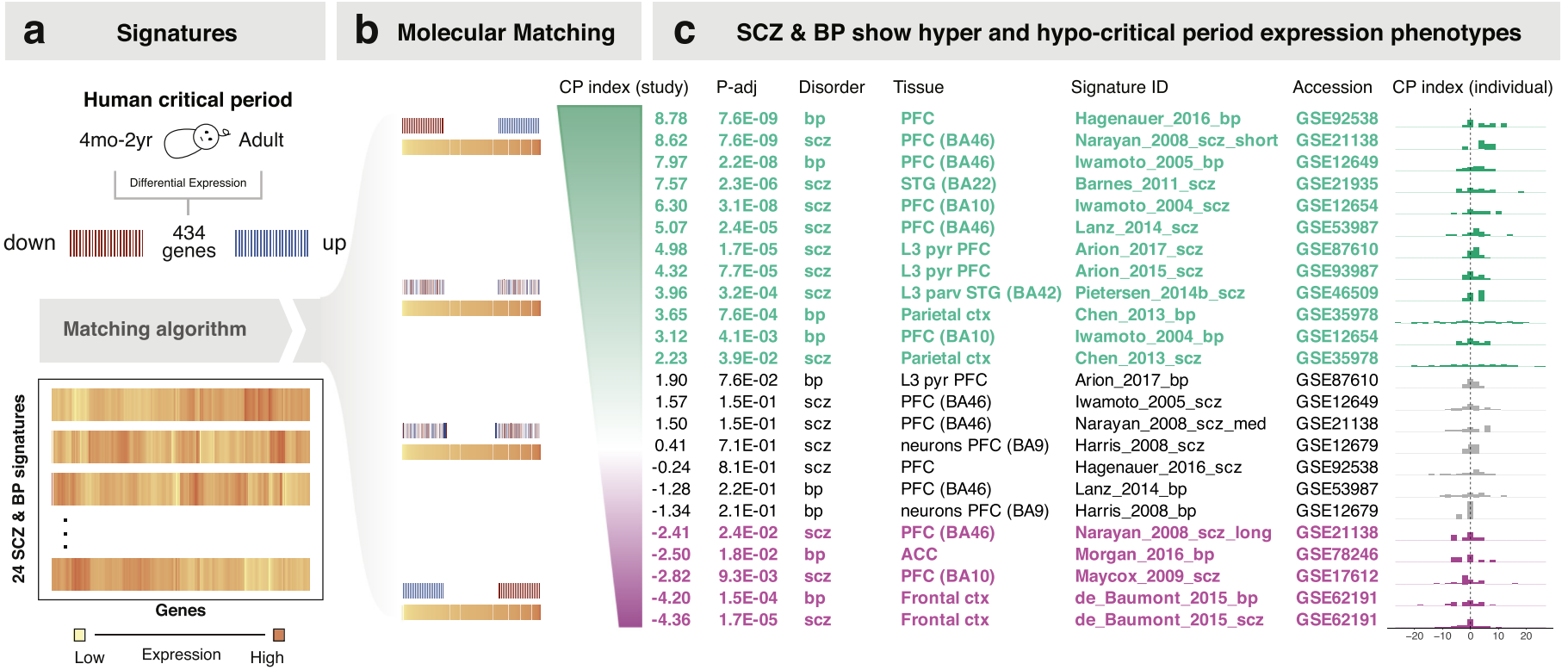
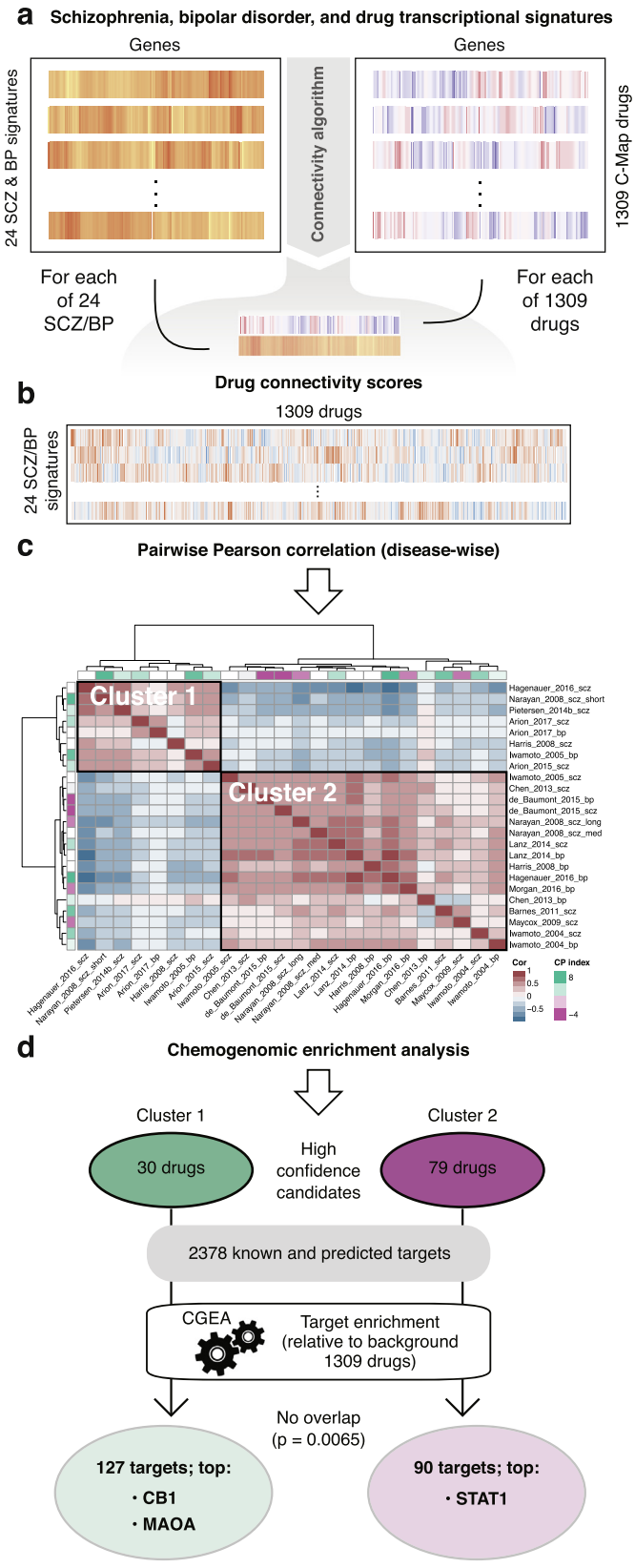


Fig. 2. Human transcriptional signature of V1 critical period matched to 24 schizophrenia and bipolar disorder transcriptomes reveals hyper- and hypo-critical period gene expression phenotypes. (**a**) Human critical period signature was calculated using RankProd as the differential expression of BrainSpan samples from V1 between 4 months and 2 years old relative to adult (21 to 40 years old). Schizophrenia and bipolar disorder microarray datasets were curated from the Gene Expression Omnibus (GEO), normalized using RankNorm and differential transcriptomes computed via SubDiff (Dudley et al., 2009). (**b**) A signed rank based molecular matching algorithm calculated a critical period index between a given schizophrenia/bipolar disorder signature and the human critical period signature. (**c**) High critical period indexes (>0) indicate that a given disorder signature transcriptionally mimics the critical period signature. Low critical period indexes (<0) indicate a given disorder signature transcriptionally opposes the critical period signature. Far right column: distribution of critical period indexes calculated at the level of individual participant depicted as proportion histograms (y-axes: 0.0–0.5). CP index = critical period index. To correct for multiple tests, we calculated an adjusted p value (P_{adj}) using the False Discovery Rate method of Benjamini and Hochberg. CP indexes with $P_{adj} < 0.05$ are highlighted in green or purple. scz = schizophrenia. bp = bipolar disorder.

4. Discussion

Using a systematic bioinformatics approach across all publicly-available microarray datasets of schizophrenia and bipolar disorder (N = 24 datasets), we observed considerable heterogeneity of critical period-related transcriptional phenotypes across cohorts, mirroring long-recognized clinical heterogeneity in these disorders. We assessed a single biological dimension, human critical period-related gene expression reflecting the V1 critical period for ocular dominance plasticity, and identified study cohorts with hyper and others with hypo-critical period transcriptional phenotypes. We utilized the V1 critical period for ocular dominance plasticity because it



is one of the best characterized models from rodents to human (Morishita and Hensch, 2008), allowing the generation of corresponding transcriptional signatures in these species. Additionally, fundamental principles underlying visual critical period plasticity are shared with other critical periods (Hensch, 2004) as well as specific molecules, such as Lynx1 in auditory and visual critical periods (Morishita et al., 2010; Takesian et al., 2018). However, there are also molecular differences between critical periods and these have not been systematically assessed. Therefore, given that changes in auditory processing in schizophrenia may portend auditory plasticity differences relative to controls (Javitt and Freedman, 2014; Javitt and Sweet, 2015; Voss et al., 2018), future studies should assess the role of diverse critical period signatures including auditory and visual may be fruitful to better understand common and unique critical period plasticity aberrations in schizophrenia.

The heterogeneity observed at the study-level was reflected in critical period transcriptional phenotypes calculated at the individual level, which also revealed considerable within-study variation of critical period transcriptional phenotypes. Overall, the heterogeneity in critical period-related phenotypes indicates plasticity aberrations in schizophrenia and their treatment may need to be considered in the context of subpopulations with elevated and others reduced functional plasticity. Computational drug repurposing and chemogenomic enrichment analysis confirmed this heterogeneity and revealed two major clusters of schizophrenia and bipolar disorder signatures whose repurposed drug lists correlated with each other, but anti-correlated to the opposing cluster, indicating unique underlying biology and identifying mutually-exclusive drug candidates and their targets. The critical period index partially mapped to these clusters and suggests critical period transcriptional phenotypes and related functional plasticity may explain some of the underlying biological heterogeneity seen across these cohorts.

What is the source of the variation that explains the heterogeneity in critical period phenotypes we observed? By assessing the role of critical period gene expression in schizophrenia and bipolar disorder, we assessed a single dimension of potential biological diversity in these conditions. By doing so, we discovered hyper-, null-, and hypo-critical period transcriptional phenotypes that were not immediately explained by the available study-level covariates, including disorder type (schizophrenia or bipolar disorder), sample size, tissue type, age, and sex. We explore three additional potential sources of variation in this Discussion: (1) clinical and biological diversity (including critical period phenotypes), (2) natural trajectory of development and aging, and (3) medication history.

Heterogeneity in critical period phenotypes may reflect genuine clinical subpopulations of schizophrenia that are selected for by local sampling biases at the study locations. In our analysis, we observed schizophrenia and bipolar disorder signatures with hyper-, null- (i.e. “normal”), and hypo-critical period transcriptional phenotypes, which mirrors clinical and experimental observations. For example, individuals with schizophrenia have reduced perineuronal nets (Enwright et al., 2016) and elimination if perineuronal nets in mice leads to protracted critical period plasticity (potentially hyper-critical period) (Carulli et al., 2010; Miyata et al., 2012). In addition, reductions of plasticity (potentially hypo-critical period) have been observed across a variety of functional modalities (Çavuş et al., 2012; Daskalakis et al., 2008; Hasan et al., 2011; Mears and Spencer, 2012; Pajonk et al., 2010), while some individuals (particularly recent onset schizophrenia) have normal plasticity (Hasan et al., 2011). However, clinical approaches to understanding the plasticity phenotype in schizophrenia are limited and the development of clinical biomarkers of plasticity will be a fruitful direction. Doing so may reveal plasticity as a quantitative dimension of these disorders that can be leveraged to inform clinicians and researchers alike as to subpopulation status and treatment modalities.

The interaction of illness mechanisms with normal developmental and aging processes across the lifespan, producing a waxing and then waning plasticity phenotype across the course of the illness, might also explain observed heterogeneity. Individuals with recent onset schizophrenia (<2 years) were no different than healthy controls on a transcranial direct current stimulation (tDCS)-induced motor plasticity assay, while individuals with a longer course of illness (>2 years) had reduced plasticity (Hasan et al., 2011). The pattern of plasticity observed by Hasan et al., 2011 is reminiscent of our analysis, which found that individuals with a short duration of illness (Signature ID: Narayan_2008_schizophrenia_ short) have a hyper-critical period transcriptional phenotype, individuals with an intermediate duration of illness (Signature ID: Narayan_2008_schizophrenia_med) have no impact on critical period gene expression, and individuals with a long duration of illness (Signature ID: Narayan_2008_ schizophrenia_long) have a hypo-critical period transcriptional phenotype (see Fig. 2c), suggesting illness duration impacts critical period gene expression in a dose-dependent manner. A similar pattern has been observed in Huntington's Disease, where pre-symptomatic CAG repeat carriers have elevated perceptual plasticity relative to controls (Beste et al., 2012), indicating in Huntington's Disease, and perhaps other psychiatric disorders, a hyper and then later hypo-plasticity phenotype may be part of the natural history of the disorder. Moreover, mouse models suggest similar environmental insults can have opposing effects on critical period plasticity depending on the animal's age and duration of intervention: Smith et al. found that acute inflammation reduced critical period plasticity at its peak at P26 (2016) while Morishita et al. found that chronic elevated oxidative stress (which also produces inflammation) led to open ended-plasticity into adult (>P50) (2015a).

Consistent with our observations of aberrations in critical period gene expression in schizophrenia, others have found evidence of transcriptional immaturity in schizophrenia. Gandal et al. found that a gene expression measure of fast spiking neuron (i.e. putative parvalbumin interneuron) maturity was disrupted in schizophrenia and bipolar disorder (2012) and Hagihara et al. found evidence of transcriptional immaturity of PFC-expressed genes in individuals with schizophrenia (2014). Together with our work these results indicate aberrations in juvenile and developmentally-regulated gene expression may be common in schizophrenia and suggest that aberrant developmental and aging trajectories should be explored as possible sources of plasticity aberrations in schizophrenia.

Antipsychotics are prescribed to virtually all individuals diagnosed with schizophrenia and differing drug regimens may lead to heterogeneous cumulative effects, including on critical period plasticity-related

Fig. 3. Transcriptional drug repurposing of 1309 compounds to 24 schizophrenia and bipolar disorder signatures reveals distinct clusters of signatures with unique candidate drugs. (**a**) Transcriptional drug signatures were derived from Connectivity Map data (Lamb et al., 2006). The most extreme genes in schizophrenia/bipolar disorder transcriptome signatures from Fig. 2a were defined as any gene whose SubDiff expression value was ≥2 standard deviations from the mean and used as input to downstream connectivity mapping. C-Map = Connectivity Map. (**b**) Connectivity scores between each of 24 schizophrenia/bipolar disorder signatures and 1309 drug signatures were calculated using a modified Kolmogorov Smirnov test, where high scores indicate correlated and low scores indicate anti-correlated expression of schizophrenia/bipolar disorder signature to a given drug signature. (**c**) By calculating the pairwise Pearson correlation coefficient of 1309 drug connectivity scores between each pair of schizophrenia/bipolar disorder signatures, we identified multiple clusters of schizophrenia/bipolar disorder cohorts whose drug repurposing candidates are similar to each other (as indicated by a high correlation coefficient, cor), but oppose the rival cluster. This effect is partially explained by the critical period index as Cluster 1 maps only to schizophrenia/bipolar disorder signatures with critical period indexes >0 and Cluster 2 maps to schizophrenia/bipolar disorder signatures with critical period indexes both >0 and <0 (only signatures with $P_{adj} < 0.05$ considered). Critical period index annotation is colored the same as in Fig. 2c. CP index = critical period index. (**d**) Chemogenomic enrichment analysis (CGEA) of Cluster 1 and 2 high confidence candidates (drug repurposing candidates shared at least once in a given cluster) across 2378 known and predicted drug targets revealed 127 and 90 enriched drug targets at a threshold of $p < 0.05$ (Fisher's Exact test) relative to the background of the remainder of 1309 drugs in the Connectivity Map. Enriched drug targets did not overlap between clusters (Fisher Exact test: OR = 0, p = 0.0065).

mechanisms. In fact, the primary mechanism of action of typical antipsychotics, dopamine D2-receptor antagonism, reduces motor plasticity in healthy human controls (Nitsche et al., 2006). Moreover, due to challenges in collecting high-quality drug regimen information (including drug type, drug dose, cumulative dose, and lapses) for individuals with schizophrenia, even well-constructed studies are unable to adequately model the role of medication on gene expression in schizophrenia. Therefore, given the important cumulative biological effects of antipsychotics (Murray, 2017), the impact of antipsychotics on critical period plasticity-related mechanisms should be explored. One possible approach to explore the basic functions of critical period mechanisms by antipsychotics is to use induced pluripotent stem cells (iPSCs) from individuals with schizophrenia that have been differentiated into brain cells and then exposed to antipsychotics. Interpretations of changes in gene expression in such experiments must be taken in the light that the estimated age of iPSC-derived neurons is fetal (Brennand et al., 2015) though there is some concordance between iPSC-derived neurons from individuals with schizophrenia and adult post-mortem gene expression of brains from individuals with schizophrenia (Hoffman et al., 2017). Acute and chronic treatment of non-human primate with antipsychotics may also be useful, but concordance between non-human primate and human critical period transcriptional phenotypes in the context of schizophrenia must first be assessed.

Other possible explanations for the heterogeneity in critical period phenotype among the datasets examined include but are not limited to: race and ethnicity, which is best assessed using population genetics approaches, environmental variables (McDonald and Murray, 2000), and technical features related to the processing of sample tissue and assessment of gene expression. To answer questions related to critical period plasticity in schizophrenia in future studies, essential covariates beyond basic demographics (i.e. sex, age, genetic ancestry) and technical features (i.e. batches, postmortem interval, RNA integrity) should include symptomatology, disorder subtype, age at onset, duration of illness, smoking status, and medication regimen and history (medication types, medication doses, cumulative duration of medications, and medication lapses). In addition to covariates, sample size may be a source of variation; the sample sizes for the 24 datasets used in this study were on average N = 21.8 for cases and N = 25.9 for controls. Small sample sizes increase the risk of over-estimates of effect size of fold change expression and increased false positives (Fromer et al., 2016), though we did not see an affect of sample size on critical period index in our analyses. Additionally, we believe it is expedient to point out that in the 6 of 24 studies that published participant's race, the average proportion white was >90%. This racial and ethnic disparity weakens the generalizability of these and previous findings and, given that non-white populations have a higher incidence of schizophrenia (Bresnahan et al., 2007) calls for researchers to fervently address this bias in future studies. Ultimately, to understand the impact of clinical and biological diversity, development/aging, medication, and other environmental or technical contributions to critical period gene expression phenotypes in schizophrenia and bipolar disorder, it will be important to leverage large gene expression studies (CommonMind Consortium, BrainSeq), strive toward deeper clinical phenotyping of representative participants, and capture a comprehensive set of covariates in future sample collection.

Our study supports the need for future studies to define more direct mechanistically-relevant behavioral or biomarker profiles in human in the context of monocular deprivation-induced ocular dominance plasticity of the visual cortex, which is well-characterized in pre-clinical models. Binocular rivalry plasticity is one phenomenon that may be such a link from pre-clinical models to clinical assessment of visual plasticity. Short term (150 min) monocular deprivation strongly affects the dynamics of binocular rivalry, causing the deprived eye to prevail in conscious perception twice as much as the non-deprived eye, as well as potentiates non-deprived eye V1 response (Lunghi et al., 2011; Lunghi et al., 2015; Zhou et al., 2013). While the relationship between binocular rivalry plasticity and ocular dominance plasticity needs to be fully assessed, brief assays such as binocular rivalry may be useful when applied throughout the course of illness to determine plasticity endophenotypes towards improved therapeutic strategy. Moreover, direct measurement of cortical plasticity will facilitate translation of pre-clinical findings of critical period mechanisms from rodent models to humans with schizophrenia and help delineate mechanisms underlying plasticity aberrations in schizophrenia, including true biological heterogeneity, interaction between normal development/aging and the disorder, and medication history. Together, we expect these approaches will move the field towards a fuller picture of the contributions of critical period plasticity in schizophrenia and enable the development of more rational and precise therapies.

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Contributions

M.R.S. and H.M. designed study, M.R.S. and B.R. performed analyses, M.R.S., B.R., J.T.D., and H.M. contributed to and approved the final manuscript.

Conflict of interest

Authors declare no conflicts of interest.

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