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Schizophrenia and bipolar disorder show both common and distinct changes in cortical interneuron markers

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ABSTRACT

Schizophrenia and bipolar disorder are often viewed as distinct clinical disorders, however there is substantial overlap in their neuropathologies. While compromised cortical interneurons are implicated in both diseases, few studies have examined the relative contribution of the distinct interneuron populations to each psychotic disorder. We report reductions in somatostatin and vasoactive intestinal peptide mRNAs in prefrontal and orbitofrontal cortices in bipolar disorder (n = 31) and schizophrenia (n = 35) compared to controls (n = 34) and increased calbindin mRNA in schizophrenia. We show, at the molecular level, shared deficits in interneuron markers in schizophrenia and bipolar disorder, and a unique interneuron marker increase in schizophrenia.

1. Introduction

Schizophrenia and bipolar disorder may be biologically distinct or, as suggested by overlap in symptoms, genetic risk factors, and affected signaling pathways, may be on a continuum of underlying neuropathology (Craddock and Owen, 2010). Indeed, cortical interneuron pathology may be shared between individuals with schizophrenia and bipolar disorder, as GABAergic dysregulation is thought to contribute to altered gamma band oscillations and cognitive deficits in both (Hall et al., 2011; Lewis et al., 2012). Abnormalities in several cortical transcripts and proteins, including reduced glutamic acid decarboxylase 67 kDa (Akbarian et al., 1995; Guidotti et al., 2000; Volk et al., 2000; Knable et al., 2002; Torrey et al., 2005; Woo et al., 2008; Thompson et al., 2009) and various interneuron biochemical markers are found, suggesting that inhibitory neurotransmission is diminished in both schizophrenia and bipolar disorder (Benes et al., 1991; Beasley and Reynolds, 1997; Caberlotto and Hurd, 1999; Cotter et al., 2002; Hashimoto et al., 2003; Pantazopoulos et al., 2007; Hashimoto et al., 2008; Morris et al., 2008; Morris et al., 2009; Fung et al., 2010; Sibille et al., 2011; Wang et al., 2011). However, before we conclude the degree of overlap in the cortical interneuron deficit across these two clinically distinct diseases, more comparative studies using similar techniques across diagnostic groups with a wider panel of interneuron makers are needed.

Interneurons are heterogeneous cells with different subtypes inhibiting pyramidal neurons by primarily targeting the cell body and/or axon initial segment (cholecystokinin, parvalbumin), or by targeting the dendrites [e.g. somatostatin, neuropeptide Y (NPY)] (Markram et al., 2004). Interneurons may also directly inhibit other interneurons [e.g. vasoactive intestinal peptide (VIP)] (Pi et al., 2013). Thus, examining these interneuron subtypes more specifically and comparatively will determine the degree and nature of the cortical interneuron pathology in bipolar disorder and schizophrenia and will determine if they can be considered different manifestations of a similar underlying neurobiological deficit, or if they are qualitatively different.

Several previous studies have examined the expression of interneuron markers in both schizophrenia and bipolar disorder (Cotter et al., 2002; Sakai et al., 2008; Wang et al., 2011). However, whether a unique profile of interneuron deficits can be distinguished for each disorder across two functionally distinct cortical regions is unknown. We examined the relative change in expression of seven interneuron biochemical marker mRNAs (parvalbumin, cholecystokinin, somatostatin, NPY, calbindin, VIP, and calretinin) in two brain regions: the dorsolateral prefrontal cortex (DLPFC) and orbitofrontal cortex (OFC). We hypothesized there may be both shared and distinct alterations in interneuron mRNA expression in the two diseases.

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2. Experimental/materials and methods

2.1. Post-mortem brain samples

Studies were carried out with approval of the University of New South Wales Human Research Ethics Committee, (#HREC07261). RNA from postmortem DLPFC (BA46) and lateral OFC cases was obtained from the Stanley Medical Research Institute Array Cohort (Table 1).

2.2. qPCR analysis

Due to availability of RNA, the examined cohort consisted of 34 controls, 31 bipolar disorder, and 35 (DLPFC) or 34 (OFC) schizophrenia subjects. cDNA was synthesized as previously described (Weickert et al., 2010). Transcript levels were measured by qPCR on ABI Prism 7900HT system using TaqMan Gene Expression Assays (Table 2) as previously described (Fung et al., 2010). The geometric mean for 4 housekeeper control mRNAs (TATA box binding protein, β-actin, ubiquitin C, β-2-microglobulin) used for normalization did not vary according to diagnostic group (DLPFC: $F = 1.51$, $df = 2, 99$, $p = 0.23$; OFC: $F = 0.53$, $df = 2, 98$, $p = 0.59$).

2.3. Analysis

Where data were not normally distributed, quantities were transformed (square root: cholecystokinin and calretinin in DLPFC, calretinin and NPY in the OFC; log: calbindin in OFC). Group outliers of >2 SD from the mean were removed (0–4 per group, average 1.57 outliers per group, 4.67%, Supplementary Table 1). Demographic variables that correlated with gene expression across the cohort (Pearson's correlation on normally distributed data, Supplementary Table 2) were used as covariates in ANCOVA analyses of differential gene expression between diagnostic groups where warranted, otherwise one-way ANOVAs were used to determine diagnostic differences (LSD post-hocs). Statistical analyses were performed using IBM SPSS Statistics, Version 20.

3. Results

3.1. Interneuron mRNAs are altered in the DLPFC of schizophrenia and bipolar disorder subjects

Several interneuron transcripts were changed in the DLPFC according to diagnostic group (Fig. 1). Somatostatin mRNA was reduced in schizophrenia (20.9%, $p = 0.021$) and in bipolar disorder (34.7%, $p < 0.001$) DLPFC relative to healthy controls (overall ANOVA, $F = 7.17$, $df = 2, 93$, $p = 0.001$). VIP was reduced 17.8% in schizophrenia subjects ($p = 0.018$) compared to controls and 32.6% in bipolar patients compared to controls ($p < 0.001$, overall ANOVA $F = 9.10$, $d = 2, 92$, $p < 0.001$). Conversely, calbindin mRNA expression was increased in people with schizophrenia relative to both controls (22.7%, $p = 0.001$) and bipolar subjects (22.5%, $p = 0.002$), and unchanged between bipolar and control subjects ($p > 0.05$, overall ANCOVA covarying for age, $F = 7.40$, $df = 2, 89$, $p = 0.001$). Parvalbumin, cholecystokinin and calretinin mRNAs did not show significant changes across diagnostic groups by ANOVA (all $F < 1.05$, $p > 0.05$). As there was a trend for overall change in NPY mRNA (ANOVA $F = 2.85$, $df = 2, 94$, $p = 0.063$) combined with our prior findings of reduced NPY mRNA in schizophrenia (Fung et al., 2010), we conducted post-hoc analysis, revealing a reduction in NPY mRNA in schizophrenia compared to controls (18.8%, $p = 0.035$), and reduced NPY mRNA in bipolar disorder relative to controls at the level of significance (17.6%, $p = 0.05$).

Table 2
TaqMan gene expression assays.

Gene name	Gene symbol	TaqMan assay ID
Interneuron marker		
Parvalbumin	PV	Hs161045_m1
Somatostatin	SST	Hs356144_m1
Calbindin	CB	Hs1077197_m1
Calretinin	CR	Hs242372_m1
Neuropeptide Y	NPY	Hs173470_m1
Cholecystokinin	CCK	Hs174937_m1
Vasoactive intestinal peptide	VIP	Hs929575_m1
Housekeeper		
TATA box binding protein	TBP	Hs00427620_m1
β-actin	ACTB	Hs99999903_m1
Ubiquitin C	UBC	Hs00824723_m1
β-2-microglobulin	B2M	Hs99999907_m1

3.2. Interneuron mRNAs are altered in the OFC of schizophrenia and bipolar disorder subjects

Somatostatin mRNA was reduced in schizophrenia (23.9%, $p = 0.004$), and in bipolar disorder relative to controls (29.9%, $p = 0.001$; overall ANOVA $F = 7.216$, $df = 2, 90$, $p = 0.001$) (Fig. 2). There was a trend for a diagnostic group difference in VIP mRNA ($F = 2.54$, $df = 2, 89$, $p = 0.085$), with reduction in bipolar relative to controls (16.2%, $p = 0.043$) and a trend toward reduction in schizophrenia relative to controls (13.6%, $p = 0.079$). Calbindin mRNA was increased in schizophrenia relative to bipolar disorder OFC (27.4%, $p = 0.016$, overall ANOVA $F = 3.33$, $df = 2, 91$, $p = 0.040$), and tended to decrease in bipolar relative to controls, but this did not quite reach statistical significance (15.3%, $p = 0.06$). No other interneuron marker mRNAs were significantly changed between groups ($F < 1.15$, $p > 0.05$).

4. Discussion

Our results indicate that bipolar disorder and schizophrenia share significant interneuron pathology, with the largest and most consistent

Table 1
Stanley array cohort demographics (based on DLPFC).

	Control group n = 34	Bipolar disorder group n = 31	Schizophrenia group n = 35
Age (years) (range)	43.8 (31–60)	44.9 (19–64)	42.6 (19–59)
Gender	9F/25M	16F/15M	9F/26M
Hemisphere	16L/18R	17L/14R	17L/18R
pH (±SD)	6.61 ± 0.27	6.46 ± 0.28	6.47 ± 0.24
PMI (hours) (±SD)	29.5 ± 13.0	36.6 ± 18.1	31.4 ± 15.4
RIN (±SD)	8.30 ± 0.69	8.32 ± 0.84	8.47 ± 0.56
Manner of death	Natural = 34	Natural = 17, suicide = 14	Natural = 28, suicide = 7
Age of onset (years) (±SD)	–	24.8 ± 8.95	21.3 ± 6.07
Duration of illness (years) (±SD)	–	20.2 ± 9.89	21.3 ± 10.1
Lifetime antipsychotics (fluphenazine equivalents, mg)	–	10,296.8 ± 23,865	85004.3 ± 100,335
Antidepressant use	Yes = 0, no = 34	Yes = 18, no = 13	Yes = 9, no = 26