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Abnormal Activity of the MAPK- and cAMP-Associated Signaling Pathways in Frontal Cortical Areas in Postmortem Brain in Schizophrenia

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Recent evidence suggests that schizophrenia may result from alterations of integration of signaling mediated by multiple neurotransmitter systems. Abnormalities of associated intracellular signaling pathways may contribute to the pathophysiology of schizophrenia. Proteins and phospho-proteins comprising mitogen activated protein kinase (MAPK) and 3′–5′-cyclic adenosine monophosphate (cAMP)-associated signaling pathways may be abnormally expressed in the anterior cingulate (ACC) and dorsolateral prefrontal cortex (DLPFC) in schizophrenia. Using western blot analysis we examined proteins of the MAPK- and cAMP-associated pathways in these two brain regions. Postmortem samples were used from a well-characterized collection of elderly patients with schizophrenia (ACC = 36, DLPFC = 35) and a comparison (ACC = 33, DLPFC = 31) group. Near-infrared intensity of IR-dye labeled secondary antisera bound to targeted proteins of the MAPK- and cAMP-associated signaling pathways was measured using LiCor Odyssey imaging system. We found decreased expression of Rap2, JNK1, JNK2, PSD-95, and decreased phosphorylation of JNK1/2 at T183/Y185 and PSD-95 at S295 in the ACC in schizophrenia. In the DLPFC, we found increased expression of Rack1, Fyn, Cdk5, and increased phosphorylation of PSD-95 at S295 and NR2B at Y1336. MAPK- and cAMP-associated molecules constitute ubiquitous intracellular signaling pathways that integrate extracellular stimuli, modify receptor expression and function, and regulate cell survival and neuroplasticity. These data suggest abnormal activity of the MAPK- and cAMP-associated pathways in frontal cortical areas in schizophrenia. These alterations may underlie the hypothesized hypoglutamatergic function in this illness. Together with previous findings, these data suggest that abnormalities of intracellular signaling pathways may contribute to the pathophysiology of schizophrenia.
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INTRODUCTION

Schizophrenia is a complex psychiatric illness associated with dysregulation of multiple brain neurotransmitter systems (Fatemi and Folsom, 2009; MacDonald and Schulz, 2009; Stilo and Murray, 2010). Although alterations of these neurotransmitter systems, including dopamine, glutamate, GABA, serotonin, and acetylcholine, have led to hypotheses centered on neurotransmitter receptor expression and function as key elements of the pathophysiology of this illness, integration of signaling mediated by multiple neurotransmitter receptors is a critical step in determining the functional consequences of receptor activation (Kyosseva, 2004b; Lang *et al*, 2007; Laruelle *et al*, 2003; Lewis and Hashimoto, 2007; Lewis and Moghaddam, 2006; Lowes *et al*, 2002; Ross *et al*, 2006; Svenningsson *et al*, 2004; Sweatt, 2001, 2004; Volk *et al*, 2010). Accordingly, alterations of signal integration pathways may contribute to the pathophysiology of schizophrenia.

Many neurotransmitter receptors are functionally coupled to protein kinases and/or G-proteins, which modulate cascades of molecules that in turn regulate critical cellular functions (Gardoni *et al*, 2006; Hosokawa *et al*, 2006; Krapivinsky *et al*, 2004; Mauceri *et al*, 2007; Santucci and Raghavachari, 2008; Song *et al*, 2004). For example, the mitogen activated protein kinase (MAPK)-associated pathway activates transcription factors related to learning, memory, cell proliferation, and apoptosis. This pathway integrates extracellular stimuli through the phosphorylation of c-Jun N-terminal kinase (JNK), extracellular

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signal-regulated kinase (ERK), p38, and other kinases (Kyosseva, 2004b; Kyosseva *et al*, 1999; Sweatt, 2001). Similar to the MAPK-associated pathway, 3′–5′-cyclic adenosine monophosphate (cAMP)-associated pathways are also coupled to activation of neurotransmitter receptors, and modulate cellular functions through the activation of protein kinase A (PKA), exchange protein activated by cAMP (EPAC), and other molecules (Borland *et al*, 2009; Cheng *et al*, 2008; Hochbaum *et al*, 2008; Ma *et al*, 2009; Roberson *et al*, 1999; Sands and Palmer, 2008). Alterations of the MAPK- and cAMP- associated signaling pathways may impact intracellular Ca^{++} levels, neurotransmitter receptors, transcription factors, crosstalk between signaling pathways, and other biological functions critical for neuroplasticity (Gelinas *et al*, 2008; Reichenberg, 2010; Sands and Palmer, 2008).

In this study, we have examined the hypothesis that intracellular signaling molecules are altered in the frontal cortex in schizophrenia. Using western blot analysis, we measured expression of proteins of the MAPK- and cAMP-associated signaling pathways in the anterior cingulate (ACC) and dorsolateral prefrontal cortices (DLPFC) in samples from a well-characterized collection of postmortem brains from subjects with schizophrenia and a comparison group.

MATERIALS AND METHODS

Tissue Acquisition and Preparation

Samples from the ACC and DLPFC were obtained from the Mount Sinai Medical Center brain collection. Patients were diagnosed with schizophrenia using DSM-III-R criteria (Bauer *et al*, 2008; Davidson *et al*, 1995; Harvey *et al*, 1992; Powchik *et al*, 1998). Each patient had a documented history of psychotic symptoms before the age of 40, and at least 10 years of hospitalization with a diagnosis of schizophrenia made by two clinicians. Patients were recruited prospectively and underwent extensive antemortem diagnostic and clinical assessment. Exclusions for this study included a history of substance abuse, death by suicide, or coma for more than 6 h before death. Neuropathological examination revealed no neurodegenerative diseases including Alzheimer's disease in any subjects. Next of kin consent was obtained for each patient (Bauer *et al*, 2008, 2009, 2010; Funk *et al*, 2009; Hammond *et al*, 2010; Oni-Orisan *et al*, 2008). Schizophrenia and comparison groups were matched for sex, age, pH, and PMI (Table 1). Comparison subjects were selected using a formal blinded medical chart review instrument with no history of psychiatric or neurological disease. The assessment included the CERAD battery, the Clinical Dementia Rating Scale, and the Positive and Negative Syndrome Scale (Powchik *et al*, 1998). Comparison subjects were also evaluated for dementia and neurodegenerative diseases as well as any history of drug and alcohol abuse (Oni-Orisan *et al*, 2008). Detailed tables of comparison and schizophrenia subjects (Supplementary Tables 1 and 2) are provided as a comprehensive list of specific demographics for each group.

Samples were obtained at autopsy from the left hemisphere. The ACC was dissected at the level of the genu of the corpus callosum, the DLPFC was dissected from Brodmann areas 9 and 46. Gray matter was dissected from white, and then samples were portioned into 1 cm^3 pieces and stored at $-80^{\circ}C$ until further processing. Tissue was pulverized into a powder using a mortar and pestle with a small amount of liquid nitrogen and stored at $-80^{\circ}C$. Samples were reconstituted and homogenized in 5 mM Tris-HCl pH 7.4, 0.32 M sucrose and a protease inhibitor tablet (Complete Mini, Roche Diagnostics, Mannheim, Germany) using a Power Gen 125 homogenizer (Thermo Fisher Scientific, Rockford, IL) at speed 5 for 60 s. The homogenates were assayed for protein concentration using a BCA protein assay kit (Thermo Scientific), and stored at $-80^{\circ}C$.

Table 1 Subject Characteristics

Category	Comparison group		Schizophrenia	
Region	ACC	DLPFC	ACC	DLPFC
N	33	31	36	35
Sex	14 m/19 f	12 m/19 f	24 m/12 f	23 m/12 f
Tissue pH	6.4 ± 0.2	6.4 ± 0.2	6.4 ± 0.3	6.4 ± 0.3
PMI (h)	8.3 ± 6.8	8.1 ± 6.9	13.2 ± 8.0	12.5 ± 6.6
Age (years)	78 ± 14	78 ± 14	74 ± 11	74 ± 12
On/off Rx	0/33	0/31	25/11	24/11

Abbreviations: Off Rx, number of patients neuroleptic free at least 6 weeks before death; On Rx, number of patients taking antipsychotic medication at time of death; PMI, postmortem interval.
Values presented as means ± standard deviation.

Western Blot Analysis

Samples for western blot analyses were diluted with ultrapure (Milli-Q A10, Millipore) water and reducing buffer (6 × solution: 4.5% sodium dodecyl sulfate (SDS), 15% β-mercaptoethanol, 0.018% bromophenol blue, and 36% glycerol in 170 mM Tris-HCl pH 6.8) to a concentration of 20 μg of protein per 12 μl and heated at 70°C for 10 min. Samples were then processed in duplicate by SDS-PAGE using Invitrogen (Carlsbad, CA) 4–12% gradient gels and transferred to PVDF membranes by BioRad semi-dry transblotters (Hercules, CA). The membranes were blocked with LiCor blocking buffer (Lincoln, NE) for 1 h at room temperature, then probed with primary antisera (Supplementary Table 3) diluted in 0.1% Tween LiCor blocking buffer. The membranes were washed twice for 10 min each in 0.1% Tween phosphate buffer solution (PBST) then probed with goat anti-mouse or goat anti-rabbit IR-Dye 670 or 800cw labeled secondary antisera in 0.1% Tween, 0.01% SDS LiCor blocking buffer for 1 h at room temperature. Washes were repeated after secondary labeling, washing twice for 10 min in PBST, then placed in water.

Membranes were imaged using a LiCor Odyssey scanner. Boxes were manually placed around each band of interest, which returned near-infrared fluorescent values of raw intensity with intra-lane background subtracted using Odyssey 3.0 analytical software (LiCor, Lincoln, NE).

Data Analysis

The near-infrared fluorescence value for each protein of interest was normalized to the in-lane value of either valosin containing protein (VCP) or β-tubulin, and this normalized

ratio from duplicate lanes was averaged. β-tubulin was used for the normalization of PSD-95, pS295 PSD-95, pATF-2, and pc-Jun. VCP was used to normalize all other target proteins, to avoid any potential interference in signal because of the molecular weight of β-tubulin (55 kDa) and proteins of interest (Bauer *et al*, 2009). We found no changes in raw intensity values for either VCP or β-tubulin between the schizophrenia and comparison groups, consistent with previous reports (Bauer *et al*, 2009; Funk *et al*, 2009; Hammond *et al*, 2010).

Data were analyzed using Statistica (Statsoft, Tulsa, OK). All dependent measures were determined to have a Gaussian distribution. Correlation analyses were performed to determine associations between the dependent variables and pH, age, and PMI. Sex and antipsychotic medication status were used as grouping variables for secondary analyses. Dependent measures were considered significant when $p<0.05$. One way analysis of covariance (ANCOVA) was used to analyze the data when significant correlations with potential covariates were found, otherwise analysis of variance was used. Post-hoc power analysis of our significant findings (with $\alpha=0.05$) in the ACC revealed an average power ($1\,\beta$) of 0.67, with a range of 0.51–0.77. The average power of our significant findings in the DLPFC revealed an average power of 0.7, with a range of 0.62–0.74.

RESULTS

MAPK-Associated Signaling Pathway

ERK1/2, JNK1/2, and p38 are central MAPK signaling proteins (Bogoyevitch *et al*, 2010; Kyosseva, 2004b; Zarubin and Han, 2005). We found decreased expression in the ACC in schizophrenia of JNK1 ($F(1,62)=4.1$, $p=0.047$) and JNK2 ($F(1,62)=7.35$, $p=0.009$). We found no changes in ERK1/2 or p38 in either cortical region (Table 2). We also measured the phosphorylation states of these proteins, and found a decrease in the dual phosphorylation state of JNK1/2 at T183/Y185 ($F(1,60)=7.46$, $p=0.008$) in the ACC in schizophrenia, but no change in the DLPFC (Table 2). We did not find changes in phosphorylated ERK1/2 in either region (Table 2). We were unable to detect and quantify phosphorylated p38.

Downstream Targets of MAPKs

Dually phosphorylated JNK is a proxy for JNK activity (Davis, 1999; Ip and Davis, 1998; Minden *et al*, 1994). Our finding of decreased phosphorylation of JNK1/2 in the ACC led us to measure the phosphorylation state of three downstream targets of JNK: c-Jun, ATF-2, and PSD-95. We found no changes in phosphorylated forms of either c-Jun or ATF-2 in the ACC (Table 2). However, phosphorylation of PSD-95 at S295 was decreased in the ACC ($F(1,64)=6.8$, $p=0.01$) and increased in the DLPFC ($F(1,48)=7.09$, $p=0.01$). We also found decreased levels of total PSD-95 protein expression in the ACC (Table 2).

cAMP- Associated Signaling Pathway

EPAC1 and EPAC2 are activated by cAMP and are guanine exchange factors of Rap (Borland *et al*, 2009; Cheng *et al*, 2008). Therefore, we examined EPAC1 and EPAC2 expression in the ACC and found no changes in schizophrenia (Table 2). Rap2 expression was decreased ($F(1,61)=4.64$, $p=0.035$) in the ACC in schizophrenia, with no change in the DLPFC (Table 2). There was no change in Rap1 in schizophrenia in either region (Table 2). We also assayed the catalytic forms of PKA, which are activated by cAMP. We found no change in the catalytic forms of PKA in schizophrenia (Table 2).

Signaling Downstream of PKA Activation

We next examined the expression of two downstream targets of PKA activation, Rack1 and Fyn, which converge

Table 2 Statistical Analyses of Dependent Variables

Protein	F	d.f.	p
ACC			
JNK1	**4.10**	**1,62**	**0.05**
JNK2	**7.35**	**1,62**	**0.01**
ERK1	0.38	1,58	0.54
ERK2	0.14	1,56	0.71
p38	1.16	1,67	0.28
pT183/Y185 JNK1/2	**7.46**	**1,60**	**0.01**
pT202/Y204 ERK1/2	1.77	1,59	0.19
Rap1	0.48	1,60	0.49
Rap2	**4.64**	**1,61**	**0.04**
EPAC1	0.01	1,67	0.95
EPAC2	0.48	1,46	0.49
PSD-95	**6.10**	**1,64**	**0.02**
pS295 PSD-95	**6.80**	**1,64**	**0.01**
pATF-2	2.17	1,50	0.15
pc-Jun	0.01	1,61	0.98
Cdk5	0.41	1,65	0.52
Rack1	0.80	1,60	0.37
Rac1	0.85	1,67	0.36
Fyn	0.48	1,65	0.49
DLPFC			
ERK1/2	0.03	1,54	0.87
p38	0.39	1,52	0.53
pT183/Y185 JNK1/2	1.39	1,54	0.24
pT202/Y204 ERK1/2	1.15	1,52	0.29
Rap1	0.53	1,60	0.47
Rap2	0.24	1,54	0.63
pS295 PSD-95	**7.09**	**1,48**	**0.01**
pY1336 NR2B	**4.50**	**1,57**	**0.04**
Cdk5	**6.70**	**1,47**	**0.01**
Rack1	**5.93**	**1,53**	**0.02**
Rac1	0.71	1,53	0.40
Fyn	**5.04**	**1,55**	**0.03**
PKA	0.07	1,60	0.80

Bold lines indicate significant differences between patients with schizophrenia and comparison subjects ($\alpha=0.05$). ANOVA was used for statistical analyses except for pT202/Y204 ERK1/2, pY1336 NR2B, Rap1, and PKA of the DLPFC, where ANCOVA was used.

upon conserved intracellular signaling molecules mentioned above (Thornton *et al*, 2004; Yaka *et al*, 2002). Rack1 ($F(1,53) = 5.9$, $p = 0.018$) and Fyn ($F(1,54) = 4.1$, $p = 0.049$) were increased in the DLPFC in schizophrenia, but not in the ACC (Table 2). NR2 subunits are phosphorylated by Fyn and other Src family kinases (Dunah *et al*, 2004; Goebel-Goody *et al*, 2009; Yaka *et al*, 2002). We therefore examined the phosphorylation of NR2B at Y1336 ($F(1,57) = 4.5$, $p = 0.038$), which was increased in the DLPFC in schizophrenia (Table 2). Additional protein-protein interactions can modulate PSD-95–NR2B association and localization (Hawasli *et al*, 2007; Kim *et al*, 2007; Morabito *et al*, 2004; Xie *et al*, 2007a, b; Zhang *et al*, 2008). Accordingly, we determined the expression of two candidate proteins that modulate such associations, Rac1 and Cdk5. We found increased Cdk5 in the DLPFC ($F(1,47) = 6.7$, $p = 0.013$), but not in the ACC. There was no change in Rac1 in either region (Table 2).

Secondary Analyses

Sex and antipsychotic medication status were used as grouping variables for secondary analyses. This cohort contains both male and female subjects, and in an effort to control for sex differences we performed an ANOVA with sex as the grouping variable when analyzing our dependent measures. No significant changes were detected between male and female subjects in either comparison or schizophrenia groups (data not shown). Additionally, we analyzed the effect of antipsychotic medication on each of our dependent measures by using medication status (ON *vs* OFF antipsychotics >6 weeks before death) as a grouping variable. Post-hoc analyses showed no significant changes because of the medication status for any dependent measure (data not shown).

DISCUSSION

In this study, we examined the expression of a series of proteins in the MAPK- and cAMP-associated signaling pathways in the ACC and DLPFC in schizophrenia. Decreased expression of Rap2, JNK1, JNK2, pT183/Y185 JNK1/2, and pS295 PSD-95 was found in ACC (Figure 1). These proteins comprise a MAPK-associated signaling pathway that integrates information from many neurotransmitter receptors implicated in schizophrenia (Figure 3). On the other hand, in the DLPFC we found increased expression of Cdk5, Rack1, Fyn, pS295 PSD-95, and pY1336 NR2B (Figure 2). These cAMP-associated signaling proteins and targets integrate information from neurotransmitter receptors (Figure 3). The proteins in these two pathways are critical for the integration of neurotransmission from diverse systems often implicated in the pathophysiology of schizophrenia. These findings may be representative of widespread signaling deficits, which universally affect all CNS cell types and regions.

Signal integration from multiple neurotransmitter receptors is essential for the modulation of learning, memory, and complex behavior (Svenningsson *et al*, 2004; Sweatt, 2001, 2004). Perhaps the most well-characterized central regulatory molecule known to influence these processes is dopamine-and-cAMP-regulated-neuronal-phosphoprotein

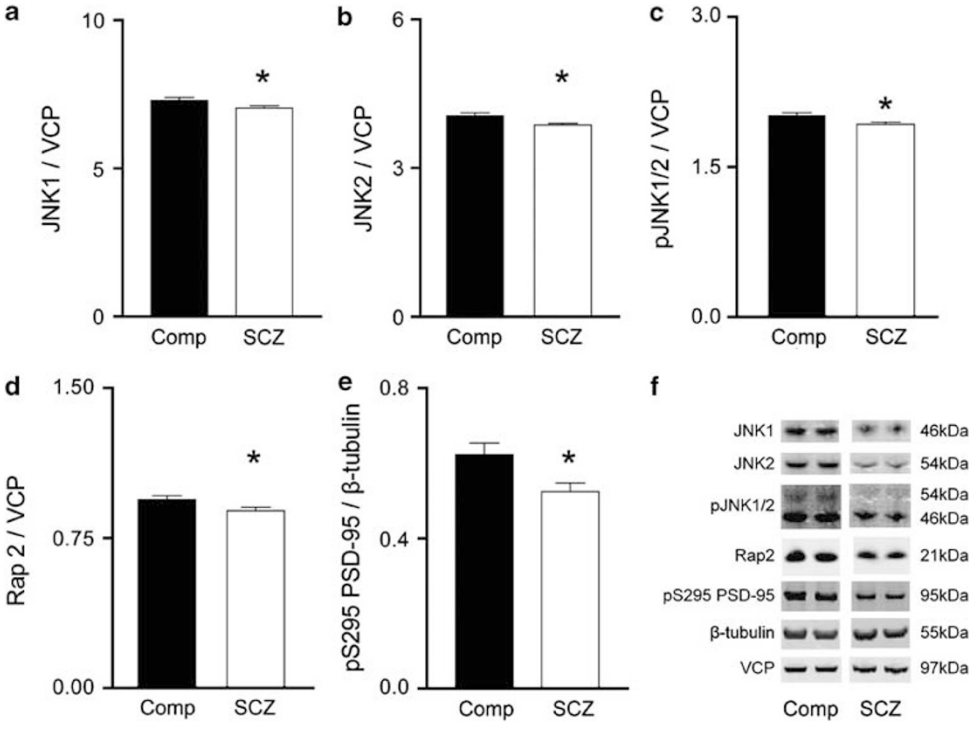

Figure 1 Intracellular signaling abnormalities of the ACC in schizophrenia. Decreased JNK1 and JNK2 expression were found in schizophrenia (a, b). Dual phosphorylation of JNK1/2 at T183/Y185 (c), Rap2 expression (d), and phosphorylation of PSD-95 at S295 (e) were all decreased in schizophrenia. Representative immunoblots (f). Data are means ± SEM. *$p < 0.05$.

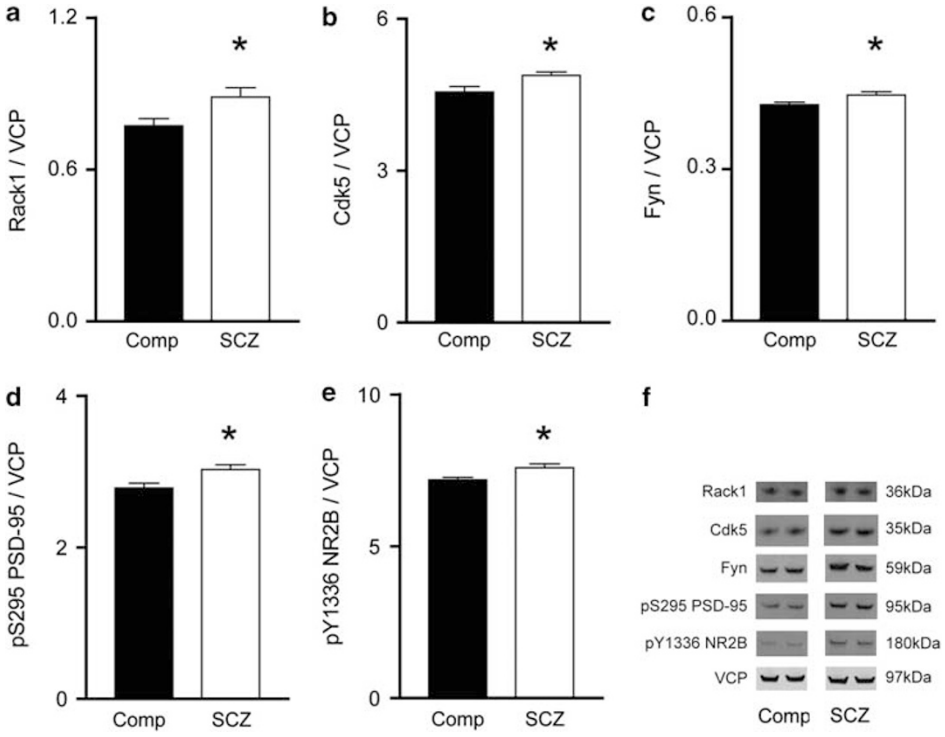

Figure 2 Intracellular signaling abnormalities of the DLPFC in schizophrenia. Increased expression of Rack1 and Fyn (a, c), Cdk5 (b), and phosphorylation of PSD-95 at serine 295 and NR2B at tyrosine 1336 (d, e) were seen in schizophrenia. Representative immunoblots (f). Data are means ± SEM. $*p < 0.05$.

(DARPP-32), which integrates signaling of glutamate, dopamine, serotonin, GABA, and other neurotransmitter families (Svenningsson *et al*, 2004). DARPP-32 knockout mice exhibit abnormalities of neurotransmitter receptor conductance and phosphorylation, synaptic plasticity, and immediate early gene and transcription factor activation (Svenningsson *et al*, 2004). These findings have been interpreted to suggest that integration of receptor signaling is critical for learning, memory, and executive functioning, which are often impaired in psychiatric illness. Although DARPP-32 is particularly well studied, there are many other kinases and effector molecules that finely tune input, output, and crosstalk between multiple neurotransmitter systems. In this study, we found region-specific changes in multiple intracellular pathways that integrate signaling of neurotransmitter systems previously reported to be dysregulated in schizophrenia, supporting a hypothesis that neuronal signal integration may be altered in this illness (Figure 3).

A growing literature implicates intracellular signaling abnormalities in schizophrenia (Kyosseva *et al*, 1999). Previous studies of the MAPK-associated pathway found changes in several constituent proteins. For example, both protein and transcript levels of ERK2, c-fos, and c-Jun were increased in the thalamus, whereas c-Jun protein and Elk-1, CREB, and ATF-2 protein levels and transcripts were noted to be increased in the cerebellar vermis (Kyosseva, 2004a; Kyosseva *et al*, 2000; Todorova *et al*, 2003). Other proteins of the MAPK pathway including MEK1, MEK2, RSK1, B-Raf, and CREB were decreased in the frontal cortex (Yuan *et al*, 2010). Although these earlier data support a hypothesis that MAPK-associated signaling is altered in schizophrenia, functional phosphorylation states and downstream phosphorylation targets were not assessed in these studies. In our study, we found a decrease in the dual phosphorylation state of JNK in the ACC in schizophrenia, which is a well-characterized proxy for JNK activity (Davis, 1999; Ip and Davis, 1998; Minden *et al*, 1994), suggesting that JNK activity is decreased in these subjects (Figure 1). We also evaluated several targets of JNK-mediated phosphorylation, to assess the potential functional relevance of decreased JNK activation. We found decreased pS295 PSD-95 in the ACC (Figure 1), consistent with decreased JNK activity (Figure 3). Decreased phosphorylation at S295 of PSD-95 suggests less PSD-95 targeted to synaptic sites (Kim *et al*, 2007), indicating impairment of glutamate neurotransmission in schizophrenia.

Abnormalities have been reported in cAMP-associated pathways in schizophrenia as well. For example, decreased DARPP-32 was detected in the frontal cortex and thalamus (Albert *et al*, 2002; Feldcamp *et al*, 2008; Ishikawa *et al*, 2007; Torres *et al*, 2009; Zhan *et al*, 2011), whereas transcripts for calcyon and spinophilin, mediators of dopaminergic signal integration, were increased in the thalamus in schizophrenia (Baracskay *et al*, 2006; Clinton *et al*, 2005). Additionally, phosphorylation of the N-methyl-D-aspartic acid (NMDA) receptor type 1 subunit at S897, a target of PKA, is decreased in the frontal cortex, suggesting fewer NMDA receptors at the synapse (Emamian *et al*, 2004; Tingley *et al*, 1997).

Increasing evidence implicates NMDA receptor dysregulation in schizophrenia, initially based on clinical evidence that non-competitive antagonists of N-methyl-D-aspartic acid receptors can be psychomimetic (Allen and Young,

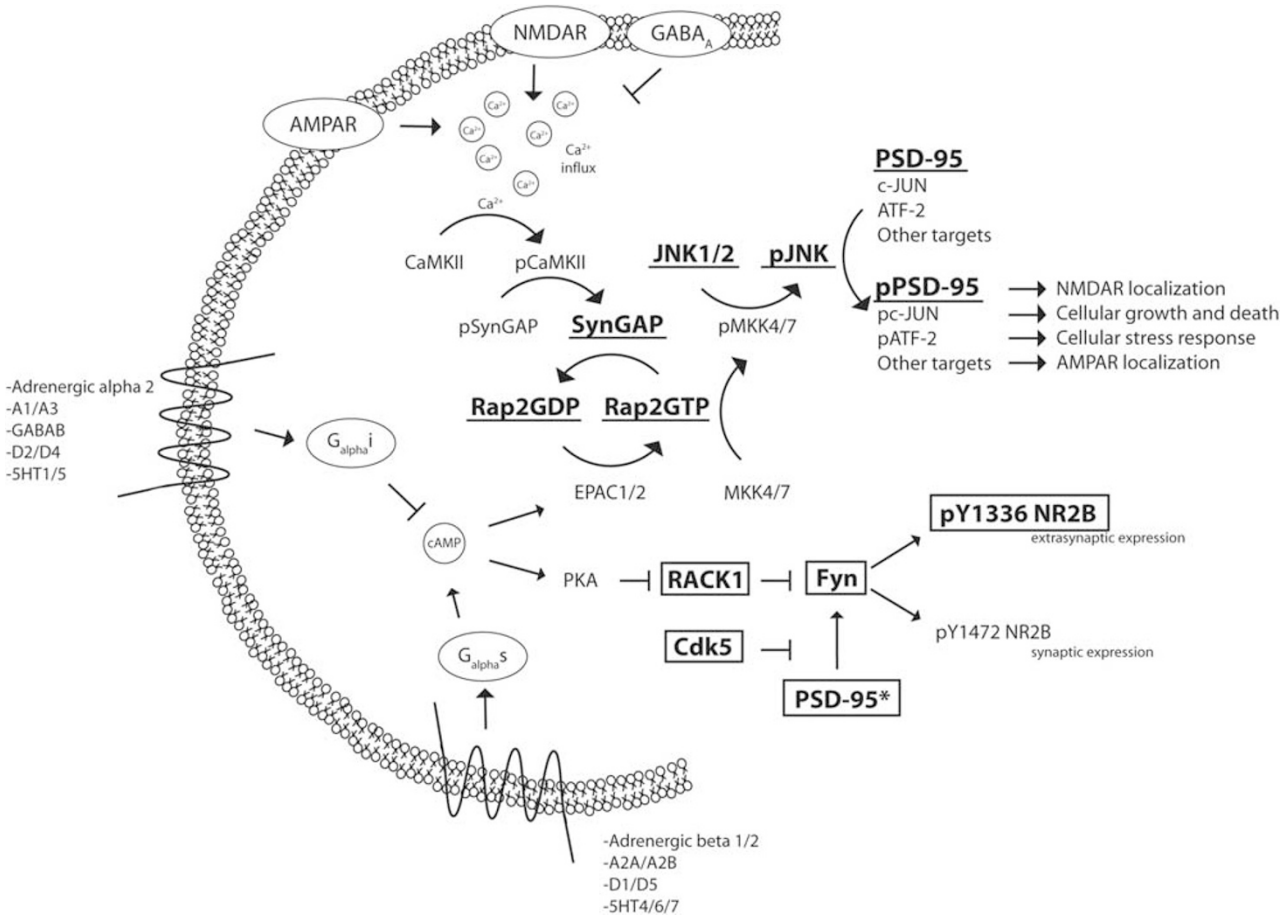

Figure 3 Dysregulation of convergent intracellular signaling pathways in frontal cortical regions in schizophrenia. Significant alterations found in the ACC and DLPFC are bolded and underlined or bolded and boxed, respectively. Downstream pathways of neurotransmitter systems known to be disturbed in schizophrenia converge upon common intracellular signaling pathways. Our data reflect abnormal protein expression and phosphorylation of key intracellular signaling molecules in both cortical regions. *A phosphorylated state of PSD-95 (pS295).

1978; Kristiansen *et al*, 2007; Lahti *et al*, 1995a, b, 2001; Luby *et al*, 1959; Meador-Woodruff and Healy, 2000; Svenningsson *et al*, 2003). Our present data suggest dysregulated localization and function of NMDA receptors. For example, increased phosphorylation of Y1336 NR2B, which targets NMDARs to extrasynaptic sites, suggests abnormal localization of NMDARs in the DLPFC (Figure 2) (Goebel-Goody *et al*, 2009; Zhu *et al*, 2005). This is consistent with the finding of decreased phospho-NR1, suggesting decreased NMDA receptors or altered subunit stoichiometry at synaptic sites (Emamian *et al*, 2004). Increased phosphorylation of NR2B at Y1336 may also confer abnormalities of LTP induction (Gardoni *et al*, 2009; Goebel-Goody *et al*, 2009).

Synaptic *vs* extrasynaptic location of NMDARs critically alters receptor function and signaling pathway activation (Groc *et al*, 2009; Hardingham and Bading, 2010; Hardingham *et al*, 2002; Ivanov *et al*, 2006; Lau and Zukin, 2007). Extrasynaptic NR2B-containing NMDARs regulate NR2A function, potentially altering synapse development and plasticity (Kollen *et al*, 2008; Zhu *et al*, 2005). Consistent with abnormal synaptic NMDAR composition and the potential impact on plasticity, subunit specific transcript studies show decreased NR2D expression in multiple regions in schizophrenia (Harney *et al*, 2008). Alterations in proteins that modulate NR2–PSD-95 interactions may also impact synaptic structure and NMDAR function. For example, Rack1 is dissociated from the C-termini of NR2 subunits via activated PKA, permitting phosphorylation by Fyn and other Srk family kinases in a PSD-95-dependent manner (Cheng *et al*, 2008; Thornton *et al*, 2004; Yaka *et al*, 2002). Cdk5 can modulate Reelin and PSD-95/Fyn interactions (Beffert *et al*, 2004; Hawasli *et al*, 2007; Morabito *et al*, 2004; Ohshima *et al*, 2007; Xie *et al*, 2007a; Zhang *et al*, 2008). Thus, increased expression of Rack1, Cdk5, and Fyn suggests altered modulation of NMDAR–PSD-95 interactions at synaptic sites (Figure 2). The net effect of altered NMDAR–PSD-95 interactions may be decreased neurotransmission and signal integration at excitatory synapses in the DLPFC, functionally consistent with the result of changes found in the ACC (Figure 3).

In addition to NMDA abnormalities in schizophrenia, the AMPA receptor has also been implicated in the pathophysiology of this disorder (Dracheva *et al*, 2005; Hammond *et al*, 2010; Meador-Woodruff *et al*, 2001; O'Connor *et al*, 2007). Previously, we reported increased expression of the AMPAR associated proteins SAP97 and GRIP1 in the brain, as well as an increase in GluR1 AMPAR protein subunit expression in endosomes in the DLPFC (Hammond *et al*, 2010). Increased phosphorylation of PSD-95 at S295 reduces

AMPAR internalization from the synapse and results in inhibition of LTD (Kim *et al*, 2007). Thus, increased phosphorylation of PSD-95 (Figure 2), in combination with increased SAP97 and GRIP1, suggests accelerated forward trafficking of AMPA receptors to synaptic sites. In the ACC, we found decreased phosphorylation of PSD-95 at S295 (Figure 1). Serine 295 is a target of JNK, phosphorylation of which induces localization of PSD-95 to synaptic sites (Kim *et al*, 2007). Thus, our data suggest less synaptically-localized PSD-95, which may induce AMPAR internalization and induction of LTD in the ACC (Kim *et al*, 2007). Additionally, Rap2, a member of the Ras family of GTPases, indirectly activates JNK (Figure 3), and Rap2-JNK interactions traffic AMPA receptors away from the synapse during LTD, or bidirectionally in an activity dependent manner (Hussain *et al*, 2010; Thomas *et al*, 2008; Zhu *et al*, 2005). Interestingly, Rap2 function is modulated by SynGAP, a Ras and Rap GTPase-activating-protein, by its C2 domain (Funk *et al*, 2009; Pena *et al*, 2008). Recently reported decreases in SynGAP (Funk *et al*, 2009), together with decreased expression of Rap2, JNK1/2, and PSD-95, suggests abnormal AMPAR localization and trafficking in schizophrenia.

Genetic studies in schizophrenia also support a hypothesis that intracellular signaling may be involved in the pathophysiology of this illness. Genes implicated in schizophrenia include candidates associated with neurodevelopment (DISC-1, DTNBP1, and NOTCH4), synapse structure and formation (NRG1, NRXN1, PDE4B, APOE, and RELN), neurotransmitter synthesis and regulation (COMT, DAOA, and PRODH), and receptor and intracellular signaling (AKT1, DRD2, ERBB4, GABRB2, GRIN2B, HTR2A, and RGS4) (Tiwari *et al*, 2010). Polymorphisms in DLG4 (PSD-95) have recently been linked with schizophrenia, further implicating synaptic structure, neurotransmission, and intracellular signaling (Cheng *et al*, 2010). Such genetic associations increasingly support the hypothesis of dysregulated intracellular signaling pathways in schizophrenia.

In this study, we found region-specific alterations of proteins associated with the MAPK and cAMP intracellular signaling pathways. Regional differences in molecular abnormalities in schizophrenia are consistently reported in the literature (Katsel *et al*, 2005a, b; Kristiansen *et al*, 2006, 2010; Oni-Orisan *et al*, 2008), and although the sets of changes we found differed by cortical region, each set is functionally similar, resulting in decreased signal integration in both regions. This differential expression pattern may be inherent to the physiological circuits and inputs for each region. For example, connectivity deficits between the DLPFC and other neocortical structures have been well documented, where abnormal DLPFC activation significantly correlates with other cortical regions (Eisenberg and Berman, 2010). Additionally, communication between the ACC and DLPFC appears to be abnormal. ACC projections to the supragranular layers of the DLPFC are implicated because of their greater inhibition of the DLPFC (Eisenberg and Berman, 2010). Thus, the inherent connectivity and input of each region may be the mitigating factor behind the differential findings.

The changes we found are of small to moderate effect size, yet the consistent pattern of changes in sequential proteins in each pathway suggests that these abnormalities may be more physiologically relevant than the modest effect sizes for each protein might otherwise suggest. In fact, relatively small changes are perhaps not unexpected, as more substantial changes in expression of these proteins are associated with oncogenic, apoptotic, and excitotoxic events (Huang *et al*, 2009; Kim and Choi, 2010; Soriano *et al*, 2008; von Engelhardt *et al*, 2007; Wagner and Nebreda, 2009).

This study used well-characterized elderly subjects, and extrapolation of these findings to younger patients should be made with caution (Akbarian and Huang, 2006; Davidson *et al*, 1995; Deep-Soboslay *et al*, 2011; Harvey *et al*, 1992, 2010; Powchik *et al*, 1998). Additionally, we could not analyze the potential impact of differential freezer storage time among subjects in this cohort, as that data is unavailable. Other potential limitations to this work include the possibility that antipsychotic treatment can affect these signaling molecules (Bjarnadottir *et al*, 2007; Leveque *et al*, 2000). Post-hoc analysis for medication status, however, revealed no differences between subjects with schizophrenia who had been free of antipsychotic medication for at least 6 weeks before death compared with subjects with schizophrenia on medication at time of death, suggesting that these changes are associated with the illness and not because ofto the past treatment.

Although we have attempted to address a lifetime of antipsychotic use, these observations do not discount the possibility that medication may alter these signaling pathways. Indeed, animal studies have demonstrated that signaling molecules are targets of modification with antipsychotic treatment. Proteins downstream of cAMP- and MAPK-associated pathways are changed after acute and chronic antipsychotic treatment (Ahmed *et al*, 2008; Molteni *et al*, 2009). Additionally, the pharmacological activity of nicotine has been shown to act via these same signaling pathways (Mobascher and Winterer, 2008). As the majority of patients with schizophrenia smoke cigarettes, nicotine may contribute to changes in signaling pathways with respect to the comparison group (Winterer, 2010).

The proteins that we have studied occupy a unique position, linking myriad neurotransmitter systems that have been implicated in the pathophysiology of schizophrenia. Our data suggest convergent pathways common to the many neurotransmitter receptors dysregulated in schizophrenia are also disturbed in this illness. Convergence of specific neurotransmitter systems onto dysregulated intracellular signaling pathways may be a final common pathway associated with the pathophysiology of schizophrenia.

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DISCLOSURE

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)