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Dopamine Receptor Signaling Molecules Are Altered in Elderly Schizophrenic Cortex

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ABSTRACT Alterations of molecules that mediate dopaminergic signal transduction have been found in schizophrenia, supporting the hypothesis of altered dopaminergic neurotransmission in this illness. To further explore this hypothesis, the authors measured transcript expression of three proteins involved in dopamine (DA) signaling in postmortem dorsolateral prefrontal and anterior cingulate cortex of elderly schizophrenic subjects and a comparison group. The transcript encoding calcyon, a protein that potentiates crosstalk between D1 DA receptors and $G_{q/11}$-linked receptors, was increased in schizophrenic prefrontal and cingulate cortex by 25%. Transcript levels of spinophilin, a protein enriched in dendritic spines that modulates excitatory neurotransmission, were increased 22% in dorsolateral prefrontal cortex but were unchanged in anterior cingulate cortex in schizophrenia. Levels of DARPP-32 mRNA, a downstream effector of dopaminergic neurotransmission, were similar in both groups for both cortical groups. These alterations in spinophilin and calcyon mRNA levels in schizophrenic prefrontal and cingulate cortex provide further evidence of altered dopaminergic neurotransmission in this illness. **Synapse 60:271–279, 2006.**

INTRODUCTION

Numerous studies have implicated dysregulation in neurotransmitter systems in the pathophysiology of schizophrenia (Benes, 2000). Dopamine (DA) dysfunction is often implicated, and one of the most convincing pieces of evidence for dopaminergic abnormalities in schizophrenia is the near universal use of DA receptor antagonists to treat this disorder (Bennett, 1998; Emilien et al., 1999; Lidow et al., 1998). Since antipsychotic medications bind to the D2-like DA receptors, a compelling hypothesis is that schizophrenia is associated with abnormal DA receptor expression or function. Although DA receptor abnormalities have been found in schizophrenic brain (Abi-Dargham et al., 2002; Meador-Woodruff et al., 1997; Suhara et al., 2002; Tuppurainen et al., 2003), results have been inconsistent, suggesting that dysregulation may be more complex than simple abnormality in DA receptor number or expression.

An alternative hypothesis is that dysfunction in the molecules that mediate the cellular response to DA agonist binding may be associated with the pathophysiology of schizophrenia. This idea is intriguing in that many of the same proteins mediate the effects of several different neurotransmitter systems, many of which have been suggested to be dysregulated in this disease. A hypothesis of abnormal expression or regulation of DA-interacting proteins in the pathophysiology of schizophrenia is supported by several studies. Recent studies have found alterations in the levels of the DA-interacting protein, calcyon, in schizophrenic brain (Bai et al., 2004; Clinton et al., 2005; Koh et al., 2003a). Calcyon was identified via a yeast two-hybrid screen using the C-terminus of the D1 DA receptor as bait (Lezcano et al., 2000). Calcyon potentiates crosstalk between G_s-coupled D1 DA receptors and $G_{q/11}$–coupled receptors of other neurotransmitter systems, leading to increased intracellular calcium release in response to DA agonist binding (Lezcano et al., 2000). Calcyon has

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TABLE I. Demographic and clinical characteristics of schizophrenic and comparison subjects examined postmortem for cortical expression of dopamine receptor signaling molecules

Subject	Sex	Age	PMI[a]	PH	Cause of death	Medication
Control						
1	F	79	3.0	6.3	Cardiopulmonary failure	None
2	F	96	3.3	6.7	Cardio respiratory failure	None
3	F	90	4.1	6.0	Cardiopulmonary failure	None
4	M	69	4.3	6.3	Unknown	None
5	F	64	19.1	6.1	Pulmonary edema	None
6	M	93	19.0	6.4	Congestive heart failure	None
7	F	102	7.1	6.5	Acute myocardial infarction	None
8	F	73	3.4	6.3	Acute myocardial infarction	None
9	F	79	7.7	6.5	Acute myocardial infarction	None
10	F	84	18.5	6.2	Unknown	None
11	M	101	4.7	6.8	Coronary artery disease	None
Total[b]	3M/8F	85 ± 13	8.6 ± 6.8	6.4 ± 0.2		
Schizophrenia						
1	F	86	6.9	5.8	Respiratory insufficiency, renal failure	None in last 6 months
2	F	84	15.6	6.2	Unknown	None in last 6 months
3	M	84	6.2	6.5	Cardiopulmonary failure	None in last 6 months
4	M	69	4.5	6.4	Cardiac infarction, renal failure	Haloperidol, fluphenazine, trifluoperazine, chlorpromazine
5	F	65	5.8	5.9	Cardiopulmonary failure	None in last 6 months
6	F	69	13.7	6.2	Cardio respiratory failure	Haloperidol
7	M	87	11.2	6.5	Cardiopulmonary failure	Trifluoperazine, chlorpromazine
8	M	68	5.6	6.8	Cardiopulmonary failure	None in last 6 months
9	F	79	20.4	7.1	Cardiopulmonary arrest, cancer of pancreas	Thioridazine, thiothixene
10	M	85	5.3	6.3	Cardiopulmonary arrest	Fluphenazine, chlorpromazine
11	M	73	7.9	6.5	Cardio respiratory failure	Haloperidol, fluphenazine
12	M	66	12.1	6.5	Acute cardiac failure	None in last 6 months
13	F	76	21.2	6.1	Cardiogenic shock	Haloperidol
14	M	97	9.3	6.5	Cardiopulmonary arrest	Haloperidol
15	M	66	8.4	6.7	Cardiopulmonary arrest	Haloperidol, trifluorperazine, chlorpromazine, thiothixene, thioridazine
16	F	82	18.8	6.6	Cardiopulmonary arrest	None in last 6 months
17	F	79	9.9	6.8	Cardiac arrest	None in last 6 months
18	M	68	17.3	6.6	Cardiopulmonary arrest	Olanzapine, risperidone, thiothixene, fluphenazine, thioridazine
Total[b]	10M/8F	77 ± 9	11.1 ± 5.5	6.4 ± 0.3		

[a]PMI, postmortem interval in hours.
[b]Numbers expressed as mean ± standard deviation.

also been shown to regulate the affinity state of D1 DA receptors (Lidow et al., 2001a).

Another component of the DA signaling cascade, DARPP-32, has also been reported to be altered in schizophrenic brain (Albert et al., 2002). DARPP-32 (DA and cAMP-regulated phosphoprotein of 32 kDa) is phosphorylated in response to DA agonist binding to the D1 class of DA receptors and dephosphorylated in response to DA agonist binding to the D2 class of DA receptors (Greengard, 2001). Phosphorylated DARPP-32 inhibits protein phosphatase-1 (PP1), which affects changes to neurotransmitter receptors, voltage-gated ion channels, ion pumps, and transcription factors (Greengard, 2001).

Spinophilin is another molecule that may be critical to the response to DA receptor stimulation. Spinophilin interacts with the third cytoplasmic loop of the DA D2 receptor (Smith et al., 1999). Spinophilin is enriched in dendritic spines, a major site of synaptic activity within the central nervous system (Allen et al., 1997). This protein binds to both PP1 and the cytoskeleton (Allen et al., 1997; Satoh et al., 1998). By controlling the localization of PP1, spinophilin has been hypothesized to control the actions/responses of PP1 to stimulation of DA receptors, as well as other types of neurotransmitter receptors (Hsieh-Wilson et al., 2003).

To test the hypothesis of abnormalities in DA receptor-interacting proteins in schizophrenia brain, we analyzed the expression of mRNA encoding calcyon, DARPP-32, and spinophilin in schizophrenic and control brain. We undertook our study in the dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC), since substantial evidence has suggested a deficit in these regions in this illness (reviewed in Bunney and Bunney, 2000; Lewis and Lieberman, 2000).

MATERIALS AND METHODS

Subjects

Eighteen elderly patients with schizophrenia and 11 nonpsychiatrically ill individuals were studied (Table I). These subjects were all from the Mount Sinai Brain Collection, which we have studied extensively in the past (Clinton et al., 2003, 2005; Gupta et al., 2005; Ibrahim et al., 2000; Richardson-Burns et al., 2000). Fresh frozen blocks of DLPFC (Brodmann area 9) and ACC ([ACC], Brodmann area 32) were cryostat-sectioned (14 μm).

Sections were mounted on Superfrost Plus microscopic slides (Fisher Scientific, Pittsburgh, PA) and stored at −80°C until use.

In situ hybridization

To generate subclones for riboprobe synthesis, unique regions of calcyon (Genbank accession number: AF225903, nucleotides 120–509), DARPP-32 (AF233349, nucleotides 12–274), and spinophilin (AF016252, nucleotides 2423–2905) genes were amplified from a human brain cDNA library using PCR. Amplified cDNA segments were extracted (QIAquick gel extraction kit, Qiagen, Valencia, CA), subcloned (Zero Blunt TOPO PCR cloning kit, Invitrogen, Carlsbad, CA), and confirmed by nucleotide sequencing. Linearized subclones were used to synthesize [^{35}S]-UTP-labeled riboprobes. In situ hybridization was performed on dehydrated and acetylated slides, as previously described (Oakman and Meador-Woodruff, 2004). Two slides per subject were used for each probe. Hybridization was allowed to occur overnight at 55°C and posthybridization washes were performed. Dehydrated slides were apposed to film (Kodak BIOMAX MR) along with [^{14}C] standards (Amersham Biosciences, Piscataway, NJ) for either 14 days (DARPP-32 and spinophilin) or 11 days (calcyon).

Imaging and statistical analyses

Autoradiographic images were acquired from films using a CCD camera and NIH Image 1.61. Analysis of mRNA expression was undertaken on a layer-by-layer basis. To quantify mRNA expression per cortical layer, we identified bands of similar expression level (isodense bands) and determined the mean grayscale value per band. To determine the correspondence between isodense bands and cortical layer, nissl-stained sections of representative areas were examined, and the rough correspondence between each isodense band and layer are shown (Figs. 2–4). Since the ACC area we studied (Brodmann area 32) does not have a well-defined Layer IV (Stark et al., 2004), we have identified this region of the cortex as a transitional zone between Layers III and V.

Tissue background values from the underlying white matter were subtracted from the grayscale values for each isodense band. Corrected grayscale values were then converted to optical density and subsequently to femtomoles of mRNA per gram tissue (fmoles mRNA/g). The latter values were determined using [^{14}C] standards, the number of uridine nucleotides in each riboprobe, and the specific activity of the [^{35}S] UTP, as described previously (Clinton et al., 2003; Mueller et al., 2004).

For each probe and cortical region, a mean value for each isodense band was determined for each subject. Multiple linear regression and correlation analysis was performed to test for associations between gene expression and postmortem interval, age, and/or tissue pH. When significant correlations were detected, we utilized analysis of covariance as our primary statistic. Otherwise, our dependent measures were analyzed by factorial analysis of variance with diagnosis and cortical lamina as independent variables. For all statistical applications, we used the Statistica (Statsoft, Tulsa, OK) software package for Windows 2000. For all tests, $\alpha = 0.05$.

Antipsychotic-treated rat experiments

To test the effects of antipsychotic treatment on calcyon, DARPP-32, and spinophilin transcript expression, adult male Sprague-Dawley rats (250 g) were injected with the typical antipsychotic haloperidol, the atypical antipsychotic clozapine, or vehicle. Subcutaneous injections of haloperidol [(2 mg/kg in acidified dimethyl sulfoxide (DMSO)], clozapine (20 mg/kg in acidified DMSO), or vehicle (acidified DMSO) in 250 μl were performed once daily for 28 days. The pH of the solution was 4.5. The rats did not appear to experience any negative reactions to the injections, other than the transient discomfort associated with the actual injection. Rats were sacrificed 24 h after the last injection. Brains were extracted, immediately frozen, and subsequently cryosectioned into 15 μm coronal sections. Sections were mounted on Superfrost Plus microscope slides (Fisher Scientific, Pittsburgh, PA), desiccated, and stored at −80°C until use. In situ hybridization, image analysis, and statistical analyses were performed as described above, with the following exceptions: (1) film development after hybridization was 2 days for spinophilin and 5 days for calcyon and DARPP-32; (2) mRNA levels were determined for the entire thickness of frontal cortex (not per individual layer); and (3) data were analyzed via one-way ANOVA; the independent and dependent variables were treatment and mRNA concentration, respectively.

RESULTS

Calcyon mRNA was abundantly expressed in DLPFC and ACC, and both areas exhibited a band of high expression corresponding to Layer III (Fig. 1). Calcyon mRNA levels were increased in schizophrenic cortex; there was a significant main effect of diagnosis for DLPFC ($F = 10.0$, $df = 1$, $P < 0.002$) and for ACC ($F = 11.5$, $df = 1$, $P < 0.001$). No significant diagnosis-by-layer interaction was found in either cortical region. Calcyon transcript expression in schizophrenic cortex was increased by ~25% across all layers, with the highest increase (~32%) in Layer II.

Analysis of DARPP-32 transcript expression in DLPFC and ACC revealed DARPP-32 mRNA in all cortical layers, with slightly increased expression in Layers V and VI (Fig. 2a). In the DLPFC, we detected an association between DARPP-32 mRNA expression and pH ($r = 0.20$, $P < 0.02$). Schizophrenic and control cortex

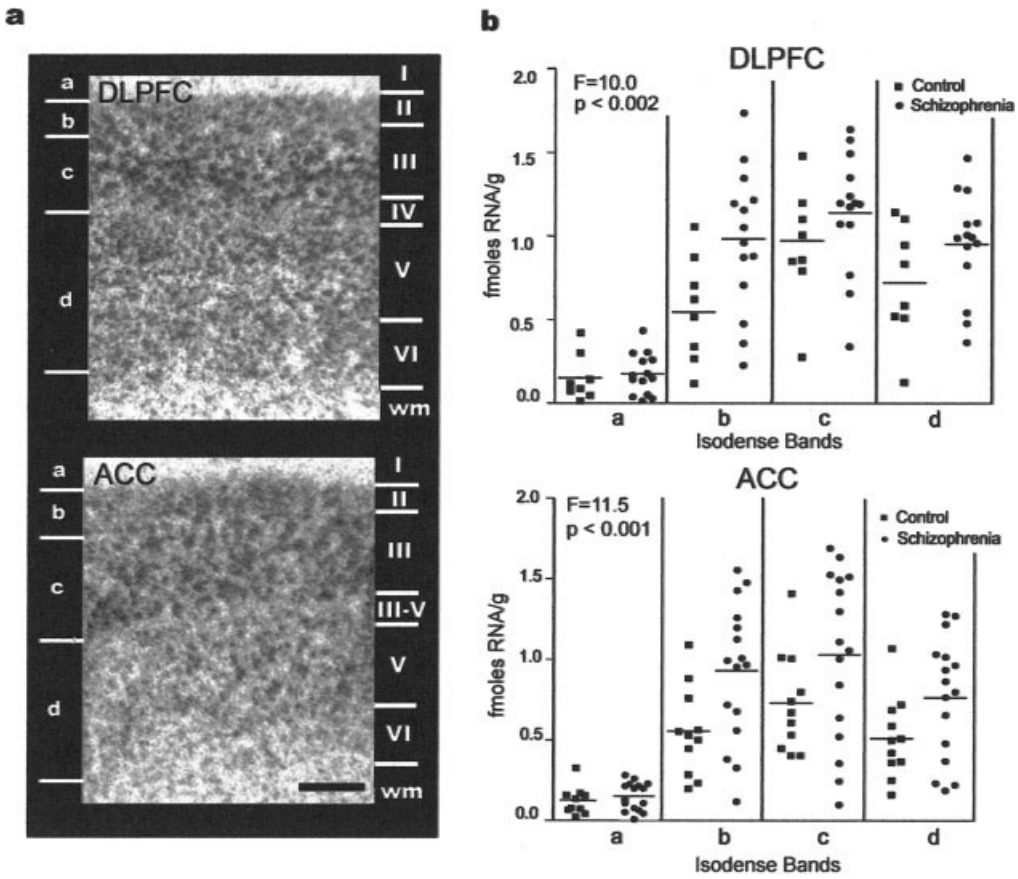

Fig. 1. Calcyon mRNA expression in DLPFC and ACC. Representative in situ hybridizations of schizophrenic cortex (**a**). mRNA expression in each cortical layer was assessed via identification of isodense bands (**a–d**). The corresponding cortical layers are shown at right. Quantification of calcyon mRNA in control and schizophrenic cortex (**b**). Calcyon transcript was upregulated in the cortex of schizophrenia subjects. Both cortical areas exhibited a significant main effect for diagnosis; schizophrenics ($n = 14$[DLPFC], $n = 16$[ACC]), controls ($n = 8$[DLPFC], $n = 11$[ACC]).

exhibited similar levels of DARPP-32 transcript expression (Fig. 2b), and no significant effect of diagnosis was found for either DLPFC or ACC.

Spinophilin mRNA was present in cortex, albeit at lower levels than that of DARPP-32 or calcyon (Fig. 3). All cortical layers expressed spinophilin transcript; Layers II, V, and VI exhibited slightly higher message levels (Fig. 3a). In DLPFC, we found an association between spinophilin mRNA expression and age ($r = 0.22$, $P < 0.006$) and pH ($r = 0.28$, $P < 0.0008$). Using ANCOVA, we detected an increase in DLPFC spinophilin mRNA in schizophrenics compared with controls ($F = 8.4$, $df = 1$, $P < 0.004$; Fig. 3b). No significant diagnosis-by-layer interaction was found. The increase in spinophilin mRNA in schizophrenic DLPFC was ~22% across all cortical layers, with the largest increase (33%) in Layer II. In ACC, we found an association between spinophilin mRNA expression and PMI ($r = 0.29$, $P < 0.0001$). In contrast to DLPFC, spinophilin transcript levels in schizophrenic and control ACC were similar (Fig. 3b).

To determine whether the observed differences between schizophrenic and control mRNA levels were possibly due to the effect of antipsychotic drugs commonly used to treat schizophrenia, we studied the effect of two antipsychotic medications, haloperidol and clozapine, on mRNA levels in cortex. Adult rats treated with these medications for 4 weeks exhibited calcyon, DARPP-32, and spinophilin transcript levels similar to vehicle-treated animals (Fig. 4). Thus, antipsychotic treatment had no effect on calcyon, DARPP-32, or spinophilin mRNA levels in adult rat frontal cortex.

DISCUSSION

The main findings of this study are increased transcript expression of calcyon and spinophilin mRNA in DLPFC of schizophrenic patients and increased expression of calcyon mRNA in ACC. These increases are not likely to be medication effects, as rats treated with the antipsychotics haloperidol or clozapine demonstrated no change in mRNA in frontal cortex for either of these molecules studied.

Our results of increased calcyon transcript in schizophrenic cortex are in agreement with previous studies

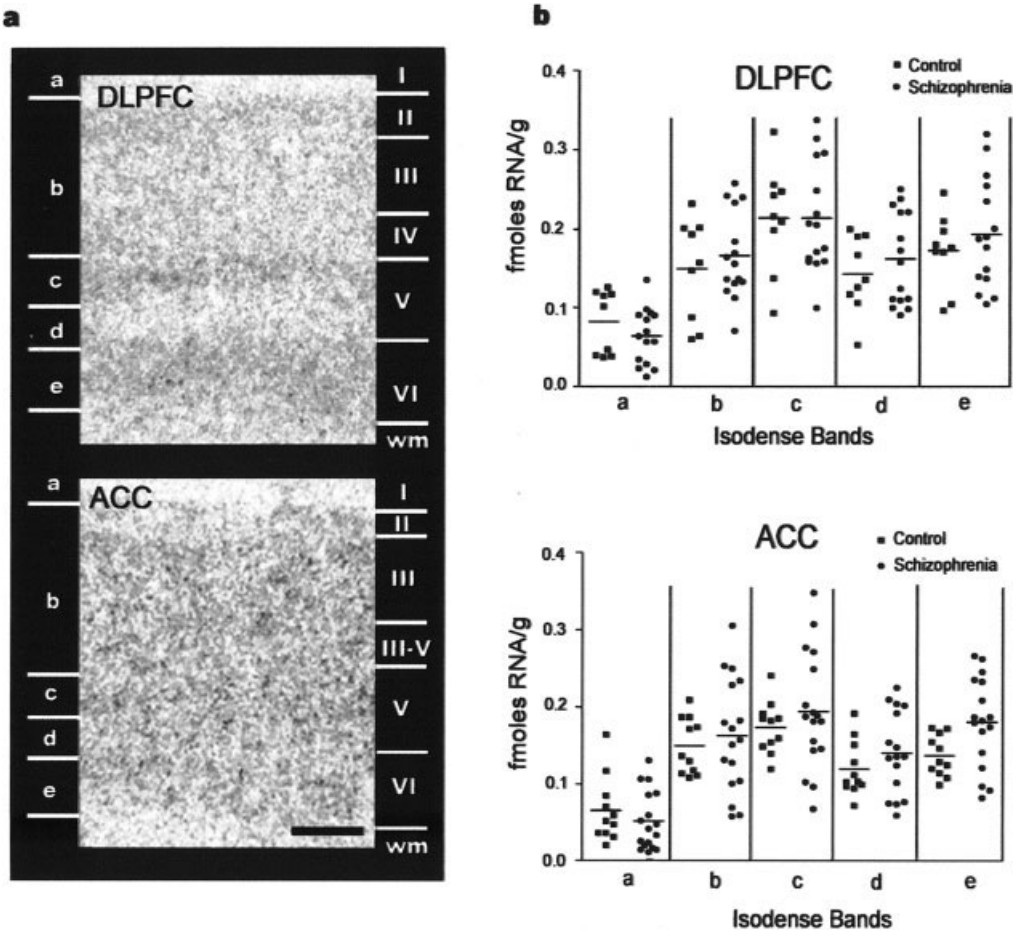

Fig. 2. DARPP-32 mRNA expression in DLPFC and ACC of schizophrenic and control patients. Representative transcript expression in the two cortical areas studied (**a**). Analysis of DARPP-32 transcript expression revealed no significant difference in DARPP-32 mRNA expression in schizophrenic vs. control cortex (**b**). In addition, there was no interaction between diagnosis and layer for either cortical region studied; schizophrenics ($n = 15$[DLPFC], $n = 16$[ACC]), controls ($n = 9$[DLPFC], $n = 11$[ACC]).

demonstrating increased calcyon in schizophrenic brain at the transcriptional and translational level. Upregulated protein levels have been found in the DLPFC of schizophrenic cases from the Stanley Foundation Collection (Koh et al., 2003a), as well as prefrontal cortex of schizophrenic cases from the Mount Sinai/Bronx V.A. Medical Center Brain Bank (Bai et al., 2004). Using real-time RT-PCR analysis, Bai et al. (2004) found significantly increased mRNA levels in patients from the Mount Sinai/Bronx V.A. Medical Center Brain Bank, the same brain collection as used in this study. Thus, using two different methodologies to examine mRNA levels, this study and that of Bai et al. (2004) found increases in prefrontal cortical calcyon mRNA expression. In addition to these results in prefrontal cortex, schizophrenic cases from the Mount Sinai/Bronx V.A. Medical Center Brain Bank demonstrate increased calcyon mRNA in ACC (this study) and thalamus (Clinton et al., 2005). Collectively, these studies provide convergent evidence of increased calcyon mRNA and protein in schizophrenic brain. Our experiments in rat and those of Lidow et al. (2001b) in primate suggest that calcyon transcript and protein are not regulated by antipsychotic treatment and that the changes in schizophrenic brain may be associated with the illness itself.

In control and schizophrenic DLPFC and ACC, calcyon mRNA was expressed throughout all cortical layers, with increased expression in layers II/III, similar to that seen in primate (Oakman and Meador-Woodruff, 2004) and in rat (Zelenin et al., 2002). Thus, our studies suggest abundant expression of calcyon in Layer III pyramidal cells, as described by Lezcano et al. (2000), but also may indicate expression in other cell types. In addition, the increased calcyon mRNA in schizophrenic cortex was throughout all cortical layers, and not confined to one cortical layer.

Since calcyon has been found to interact with the D1 DA receptor (Lezcano et al., 2000), the increased calcyon levels in schizophrenic brain may impact DA D1

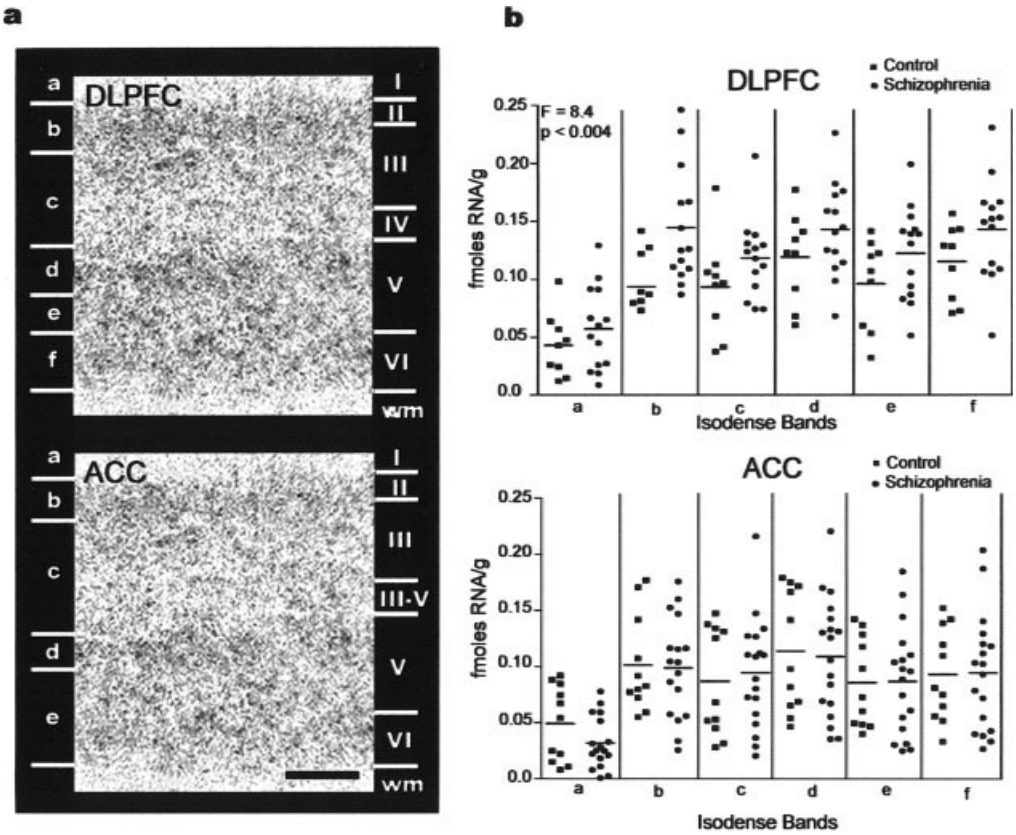

Fig. 3. Spinophilin transcript expression in schizophrenic and control cortex. Representative in situ hybridizations from DLPFC and ACC (**a**). Quantification of spinophilin mRNA revealed increased expression in schizophrenic cortex in DLPFC, but not ACC (**b**); schizophrenics (n = 14[DLPFC], n = 14[ACC]), controls (n = 9[DLPFC], n = 11[ACC]).

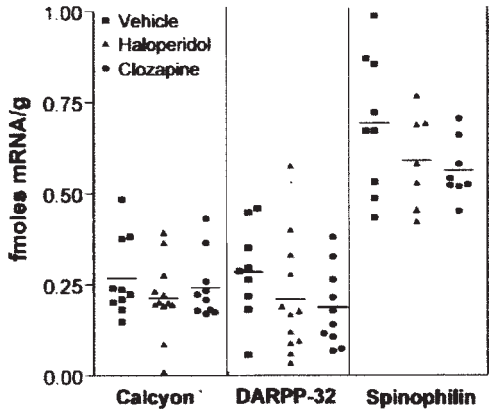

Fig. 4. Effect of antipsychotic treatment on calcyon, DARPP-32, and spinophilin mRNA. Adult rats were treated for 4 weeks with clozapine, haloperidol, or vehicle. Quantification of calcyon, DARPP-32, and spinophilin mRNAs in frontal cortex revealed no change in transcript levels after antipsychotic treatment.

receptor signaling. D1 DA receptor functioning is abnormal in the schizophrenic prefrontal cortex (reviewed in Goldman-Rakic et al., 2004; Laruelle et al., 2003), as evidenced by altered D1 DA receptor binding and impaired cognitive functioning, which is likely D1 receptor mediated (Abi-Dargham et al., 2002; Okubo et al., 1997). However, the precise function of calcyon in mediating D1 DA receptor signaling remains to be elucidated. In addition, studies in rat (Zelenin et al., 2002) and in primate (Oakman and Meador-Woodruff, 2004) have found considerable disparity in the expression patterns of calcyon and the D1 DA receptor, with calcyon exhibiting a much wider expression pattern than would be expected for a molecule solely involved in D1 DA receptor signaling. Consequently, further studies are needed to determine the exact function of calcyon in D1 receptor signaling and to determine whether calcyon plays a role in mediating the signal transduction of other neurotransmitter systems.

Analysis of DARPP-32 mRNA expression in human prefrontal cortex found low-level widespread distribution of this transcript throughout Layers II-VI of the cortex, similar to that described previously by Brené et al. (1994). DARPP-32 protein in monkey cerebral cortex has been localized to neurons, primarily in layers V–VI and also in layers II–III (Berger et al., 1990; Ouimet et al., 1984, 1992), similar to the distribution of DA receptor-positive neurons (Brené et al., 1995). Comparison of DARPP-32 transcript expression levels in schizophrenic vs. control brain found no difference between the two groups in either of the cortical regions exam-

ined. Our investigation of DARPP-32 mRNA levels in thalamus also found no difference between schizophrenics and controls (Clinton et al., 2005). In contrast, assessment of DARPP-32 protein levels in DLPFC via Western blot found schizophrenic subjects exhibited decreased DARPP-32 protein levels compared with control subjects (Albert et al., 2002). Thus, DARPP-32 may be altered in schizophrenics at a translational, but not a transcriptional, level. The present study and a previous study also in rat (Grebb et al., 1990), as well as examination of Alzheimer's subjects treated with haloperidol (Albert et al., 2002), suggest antipsychotics do not alter DARPP-32 expression.

The expression pattern of spinophilin mRNA was similar to that of DARPP-32—low levels were found throughout Layers II through VI of the cortex. Similarly, in monkey prefrontal cortex, spinophilin protein is widely expressed throughout all cortical layers (Muly et al., 2004). Spinophilin mRNA was increased by ~22% in schizophrenic DLPFC compared with control. In contrast, no difference was found between mRNA levels in ACC of schizophrenic vs. control brain.

In agreement with a previous study examining the effects of short-term antipsychotic treatment of rats on spinophilin transcript expression (Law et al., 2004a), we found no effect of antipsychotic treatment on spinophilin mRNA levels in rat frontal cortex. However, a previous study found long-term exposure (~1 year) to haloperidol resulted in a ~25–30% *decrease* in spinophilin protein in monkey prefrontal cortex (Lidow et al., 2001b). Since long-term antipsychotic treatment is likely more comparable with that received by schizophrenic patients, DLPFC mRNA levels could actually be *more* upregulated in schizophrenia than reported here.

In contrast to our finding of increased spinophilin transcript in schizophrenic cases in DLPFC, a study utilizing Northern blot to analyze spinophilin transcript expression in DLPFC found no change in spinophilin message levels compared with control (Weickert et al., 2004). Spinophilin mRNA levels in thalamus of schizophrenic patients from the Mount Sinai Medical Center Brain Bank were found to be increased (Clinton et al., 2005). In contrast, spinophilin mRNA was decreased in several regions of schizophrenic hippocampal formation (Law et al., 2004b). The only study to examine protein levels of spinophilin in schizophrenic brain found no difference between schizophrenic and control prefrontal cortex in Stanley Foundation Collection tissue (Koh et al., 2003a).

One possible explanation for the differing results between studies is that the amount of spinophilin mRNA may not reflect the amount of spinophilin protein. For example, transcription of spinophilin mRNA may be increased in an unsuccessful attempt to maintain a higher level of protein. An additional explanation for these disparate findings is that changes in spinophilin expression may be region-specific; previous studies have suggested impaired connectivity between the DLPFC and the mediodorsal nucleus of the thalamus in schizophrenia brain (reviewed in Lewis and Lieberman, 2000). Consequently, our findings of altered spinophilin expression in thalamus (Clinton et al., 2005) and in prefrontal cortex suggest abnormal spinophilin expression may occur within the context of this circuit.

The interaction of spinophilin with the D2 DA receptor has been hypothesized to link the D2 receptor to downstream signaling molecules (Smith et al., 1999). Currently, more evidence exists for D2 DA receptor abnormalities in schizophrenic striatum rather than prefrontal cortex (see Laruelle et al., 2003, for a review). However, recent studies suggest prefrontal D2 DA receptors may modulate of glutamatergic transmission (Wang and Goldman-Rakic, 2004; Wang et al., 2004), which may be abnormal in schizophrenic brain (Laruelle et al., 2003). These more recent studies suggest an additional site where D2 receptor function could be abnormal in schizophrenic prefrontal cortex. Another protein that interacts with the DA D2 receptor, NCS-1, has also been found to be upregulated in schizophrenic brain (Bai et al., 2004; Koh et al., 2003b). Thus, the increased spinophilin mRNA in schizophrenic brain found in our study and others (Clinton et al., 2005; Law et al., 2004b), as well as the alterations in NCS-1, suggest D2 receptor signaling may be altered in schizophrenic prefrontal cortex.

Another possible interpretation of altered spinophilin is that it reflects a change in the number of dendritic spines, and previous studies have used quantification of spinophilin protein as an assessment of dendritic spine number (Hao et al., 2003; Tang et al., 2004). Dendritic spine density has been reported to be altered in schizophrenic brain (Garey et al., 1998; Glantz and Lewis, 2000). Quantification of dendritic spine density of golgi-impregnated pyramidal neurons revealed a decrease in Layer III in schizophrenic brain in both the superior temporal cortex (Garey et al., 1998) and the prefrontal cortex (Glantz and Lewis, 2000). These studies on altered spinophilin mRNA and dendritic spine density suggest neurons in the DLPFC may have altered or impaired connectivity in schizophrenic brain.

In conclusion, this study provides further support for the hypothesis that proteins involved in DA receptor signaling are abnormal in schizophrenic brain, as proposed by Koh et al. (2003a). This and previous studies suggest that some of the abnormalities of dopaminergic neurotransmission in schizophrenia may be the result of aberrant expression and/or functioning of proteins such as spinophilin and/or calcyon.

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