Published in final edited form as:

Biol Psychiatry. 2010 July 1; 68(1): 25–32. doi:10.1016/j.biopsych.2010.02.016.

Altered cortical CDC42 signaling pathways in schizophrenia: Implications for dendritic spine deficits

Masayuki Ide and David A. Lewis

Departments of Psychiatry (MI, DAL) and Neuroscience (DAL), University of Pittsburgh,
Pennsylvania

Abstract

Background—Spine density on the basilar dendrites of pyramidal neurons is lower in layer 3, but not in layers 5–6, in the dorsolateral prefrontal cortex (DLPFC) of subjects with schizophrenia. The expression of CDC42 (cell division cycle 42), a RhoGTPase which regulates the outgrowth of the actin cytoskeleton and promotes spine formation, is also lower in schizophrenia; however, CDC42 mRNA is lower across layers 3–6, suggesting that other lamina-specific molecular alterations are critical for the spine deficits in the illness. The CDC42 effector proteins 3 and 4 (CDC42EP3, CDC42EP4) are preferentially expressed in DLPFC layers 2 and 3, and CDC42EP3 appears to assemble septin filaments in spine necks. Therefore, alterations in CDC42EP3 could contribute to the lamina-specific spine deficits in schizophrenia.

Methods—We measured transcript levels of CDC42, CDC42EP3, CDC42EP4, their interacting proteins [septins (SEPT2, 3, 5, 6, 7, 8 and 11), anillin], and other spine-specific proteins (spinophilin, PSD-95 and synaptopodin) in the DLPFC from 31 subjects with schizophrenia and matched normal comparison subjects.

Results—The expression of CDC42EP3 mRNA was significantly increased by 19.7%, and SEPT7 mRNA was significantly decreased by 6.9% in subjects with schizophrenia. Cortical levels of CDC42EP3 and SEPT7 mRNAs were not altered in monkeys chronically exposed to antipsychotic medications.

Conclusions—Activated CDC42 is thought to transiently disrupt septin filaments in spine necks, allowing the molecular translocations required for synaptic potentiation. Thus, altered CDC42 signaling via CDC42EP3 may perturb synaptic plasticity, and contribute to the spine deficits observed in layer 3 pyramidal neurons in schizophrenia.

Keywords

dendritic spine; CDC42; septin; schizophrenia; mRNA; prefrontal cortex

Introduction

Impaired working memory performance, a central feature of cognitive dysfunction in schizophrenia, appears to reflect functional abnormalities in the circuitry of the dorsolateral prefrontal cortex (DLPFC) (1-3). The structural basis for this functional disturbance may include a modest reduction in gray matter volume (4,5), without a decrement in neuronal number (6,7), that is attributable, at least in part, to less neuropil (8) and/or smaller neuronal cell volumes (9). For example, several groups have reported lower spine densities on the basilar dendrites of pyramidal neurons in the DLPFC, and in other cortical regions, of subjects with schizophrenia (10-12). Interestingly, within the same subjects, lower spine density was prominent in layer 3, but was not found in layers 5 or 6, of the DLPFC (13). Because dendritic spines are the principal targets of excitatory synaptic inputs to pyramidal neurons, the lower spine density prominent in layer 3 suggests that a lamina-specific impairment of excitatory connectivity may contribute to working memory dysfunction in schizophrenia. This lamina-specific impairment is consistent with findings of reduced pyramidal cell somal volumes in both the DLPFC and auditory cortex that are specific to, or are more pronounced in, layer 3 (14-17). However, the molecular mechanisms that could account for a reduction in basilar dendritic spine density selectively on layer 3 pyramidal neurons have not been identified.

We previously reported that cell division cycle 42 (CDC42), a Rho GTPase family protein that is engaged in spine morphogenesis pathways (18-20) and promotes spine formation (21-23), exhibits lower mRNA expression in the DLPFC of subjects with schizophrenia by *in situ* hybridization (24). CDC42 mRNA levels were correlated with spine density in layer 3, suggesting that reduced expression of this transcript might contribute to the spine deficits in schizophrenia. However, CDC42 mRNA expression was also lower in layer 6 where spine density was not altered. Thus, lower CDC42 mRNA expression is not a sufficient cause for the observed spine deficits in schizophrenia and additional lamina-specific factors appear to be required. Interestingly, laser microdissection of layers 2-3 versus layers 5-6 of normal human DLPFC followed by microarray analysis revealed that CDC42 effector proteins 3 (CDC42EP3) and 4 (CDC42EP4), downstream effectors of CDC42 signaling, are preferentially expressed in layers 2-3 (25). Thus, alterations in signaling pathways involving CDC42EP3 or CDC42EP4 in schizophrenia could underlie the prominence of spine density deficits in layer 3 pyramidal neurons.

The CDC42 effector protein (also known as Borg) family contains five members (26,27). All CDC42EP members except CDC42EP4 contain a Borg homology domain 3 (BD3), a binding site of septin, and ectopic expression of BD3 can reorganize septin fiber formation (28). Septins, first found in the budding neck of yeast (29), serve as a barrier that regulates the distribution of molecules between mother and daughter cells (30). In humans, 14 septins have been identified to date (31,32), and each septin has a distinctive specific cellular and subcellular localization pattern (33,34). For example, septin 7 (also known as CDC10), as well as septin 5 (also known as CDCrel-1) and septin 11, are all present in the spine necks of cultured hippocampal neurons (35,36), where they are thought to form a barrier between the spine head and dendritic shaft. Septins also bind to actin via an adaptor protein anillin (37), and can thus stabilize actin filaments (38), a major cytoskeletal component of dendritic spines (39,40). This anillin-mediated actin stabilization appears to contribute to spine morphology, as RNA interference silencing of septins results in an altered spine shape (35,36). Thus, altered expression of these septins or anillin could also contribute to CDC42-related spine abnormalities in schizophrenia.

In order to determine whether alterations in the expression of gene products associated with CDC42 signaling might contribute to the molecular basis for dendritic spine deficits in schizophrenia, we performed real-time quantitative PCR (RT-qPCR) to measure the expression

levels of these CDC42-related mRNAs in the DLPFC of schizophrenia and normal comparison subjects. To assess the association of these transcript levels with molecular markers of spine density, we also measured the mRNA levels of three proteins that are preferentially localized to spines (spinophilin)(41), the postsynaptic density (PSD-95)(42) or spine necks (synaptopodin)(43). In order to determine the specificity of any findings, we also assessed the expression of other members of the septin family that are not localized to dendritic spines.

Materials and Methods

Human subjects

With the consent of the next-of-kin, brain tissue specimens were obtained from the Allegheny County Medical Examiner's Office at the time of routine autopsy. An independent committee of experienced research clinicians made consensus DSM-IV (Diagnosis and Statistical Manual of Mental Disorders, 1994) diagnoses for each subject on the basis of medical records and the results of structured interviews conducted with family members of the deceased as previously described (11). All procedures were approved by the University of Pittsburgh's Committee for the Oversight of Research Involving the Dead and Institutional Review Board for Biomedical Research.

Brain samples from 62 subjects were used in this study (Table 1; see Tables S1 and S2 in Supplement 1 for details on individual subjects). Each subject with schizophrenia or schizoaffective disorder was matched with one normal comparison subject for sex, and as closely as possible for age and postmortem interval (PMI), to create 31 subjects pairs. Subject selection by matching was done to reduce biological variance across subject groups and to control for experimental variance by processing samples from both members of a subject pair in parallel. Diagnostic groups did not differ significantly (all $t < 1.8$; all $p > 0.08$) in mean age, PMI, RNA integrity number (RIN) or tissue storage time at -80°C . The mean value of pH was significantly ($t=2.6$; $p=0.01$) lower in the schizophrenia group; however, the difference between group means was small (0.2 pH units) and of uncertain biological significance. Every subject had $\text{RIN} \geq 7.0$, indicating an excellent quality of total RNA. Eight of the subjects had been used in a previous study of dendritic spine density in the DLPFC (11).

Tissue preparation

The right frontal cortex from each brain was blocked coronally, immediately frozen, and stored at -80°C . The location of area 9 of the DLPFC was identified cytoarchitectonically in Nissl-stained coronal sections cut at the middle rostrocaudal level of the superior frontal sulcus (44). The cortical gray matter of area 9 was dissected from cryostat sections ($40\text{ }\mu\text{m}$) in a manner that insured limited white matter contamination and excellent RNA preservation, then immediately immersed into Trizol and homogenized. RNA was extracted and purified with RNeasy column (Qiagen Valencia, CA), and was converted into complementary DNA (cDNA) with random primers and SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA). Synthesized cDNA was then stored at -20°C . To assess RNA integrity, RIN was measured using the Bioanalyzer 2100 (Agilent Technologies, Walbronn, Germany) per the manufacturer's protocol.

Real-time qPCR

The cycle threshold was determined for each transcript of interest using power SYBR Green master mix (Applied Biosystems, Foster City, CA) and ABI PRISM 7000 (Applied Biosystems) or StepOnePlus (Applied Biosystems) according to the manufacturer's instructions. Samples from both subjects in a pair were always processed together on the same 96 well plate. Each transcript of interest was amplified in quadruplicate with three internal reference transcripts, beta actin (ACTB), cyclophilin A (CYC) and glyceraldehyde-3-

phosphate dehydrogenase (GAPDH), that were previously shown to have stable expression across both subjects with schizophrenia and normal comparison subjects (44). Two RT-qPCR studies were conducted. The first study contained the seven primer sets for CDC42, CDC42EP3, CDC42EP4, septin 7 (SEPT7), anillin, PSD-95 and spinophilin mRNAs, and the second study contained the nine primer sets for SEPT2, SEPT3, SEPT5, SEPT6, SEPT7, SEPT7-R (SEPT7 and SEPT7-R amplify different sequences of SEPT7 mRNA), SEPT8, SEPT11 and synaptopodin (Table S3 in Supplement 1). Each forward and reverse primer set showed $\geq 95\%$ amplification efficiency in individual standard curve analyses, and amplified a specific single product in dissociation curve analyses. The difference in cycle threshold for each target transcript was calculated by subtracting the mean cycle threshold for the three internal reference transcripts from the cycle threshold of the target transcript. Because this difference in cycle threshold (dCT) represents the log₂-transformed expression ratio of each target transcript to the geometric mean of the three reference genes, the relative expression level of the target transcript is determined as 2^{-dCT} .

Antipsychotic-treated monkeys

In order to examine the possible effects of chronic antipsychotic medication exposure on transcripts of interest, we used tissue samples from 18 experimentally naïve, young adult, male, macaque monkeys (*Macaca fascicularis*) that had been arbitrarily divided into three groups and trained to take orally pellets containing either haloperidol, olanzapine, or sham twice a day (45). Steady-state, trough serum levels were 1.5 ng/ml for haloperidol and 15 ng/ml for olanzapine, which are in the therapeutic range for the treatment of schizophrenia in humans. After 17-27 months of drug exposure, animals (grouped into triads by body weight) were euthanized, the brains were removed, and the right hemisphere was blocked, frozen and stored at -80°C . All procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

The cortical gray matter of area 9 was dissected and extraction of RNA and synthesis of cDNA were performed using the procedures described above. CDC42EP3, SEPT7 and two internal reference transcripts (ACTB, CYC) were amplified in quadruplicate for all animals in the same triad using a 96 well plate, and then the expression ratio of each gene was determined. Primer sets were designed according to sequence databases (GenBank or Ensembl) for rhesus monkey (Table S3 in Supplement 1).

Statistical analysis

We used analysis of covariance (ANCOVA) to test the effects of diagnostic group on the relative expression level of each transcript. The model used diagnostic group as the main effect, with sex, age, PMI, tissue storage time, RIN, and brain pH as covariates. The RNA extraction and cDNA synthesis from tissue samples were conducted at separate times with different lots of reagents for two cohorts of subjects; cohort A contained the 12 pairs used in a previous qPCR study (44) and cohort B was composed of the remaining 19 pairs. Thus, we also included cohort as a covariate in the ANCOVA analyses. Correlations of the relative expression level of a transcript with dendritic spine density (11) and correlations of within-pair percent differences for two transcripts were calculated with Pearson's correlation analyses. The effect of potential confounding factors (sex; diagnosis of schizoaffective disorder; treatment with antipsychotic, benzodiazepine or sodium valproate, or antidepressant medications at time of death; history of substance dependence/abuse; tobacco use at time of death; or death by suicide) on CDC42EP3 or SEPT7 mRNA expression levels in the schizophrenia group were tested with ANCOVA models with each potential confounding factor as a main effect and with sex, age, PMI, tissue storage time, RIN, brain pH and cohort as covariates. For the antipsychotic-exposed

monkeys, analysis of variance (ANOVA) models were used with treatment group as a main effect and triad as a blocking factor.

Results

We first examined transcript levels for genes related to CDC42EP3 and CDC42EP4 signaling and dendritic spine morphology. The mean relative expression of CDC42EP3 mRNA was significantly ($F_{1,53}=10.3$, $p=0.002$) increased by 19.7% in the subjects with schizophrenia (Fig. 1A). The mean level of CDC42EP4 mRNA was also increased by 10.4% in the schizophrenia subjects, although this difference did not achieve statistical significance (Fig. 1B; $F_{1,53}=2.4$, $p=0.13$). Consistent with our previous findings by *in situ* hybridization (24), mean CDC42 mRNA expression was decreased by 7.5% in the subjects with schizophrenia, although this finding showed only a trend level of significance (Fig. 1C; $F_{1,53}=2.7$, $p=0.10$). In contrast, SEPT7 mRNA was significantly ($F_{1,53}=10.3$, $p=0.002$) decreased by 7.2% in the subjects with schizophrenia. The other transcripts in the first set of RT-qPCR analyses (anillin, PSD-95 and spinophilin) did not significantly (all $F_{1,53} < 1.5$, all $p > 0.22$) differ between subject groups, consistent with previous findings for spinophilin (46) and PSD-95 (47) mRNAs in the DLPFC of subjects with schizophrenia. Similarly, in the second set of qPCR analyses, mRNA levels for synaptopodin, another marker of spines, also did not differ ($F_{1,53}=0.1$, $p=0.74$) between subject groups.

In order to examine the validity and specificity of the group difference in SEPT7 mRNA expression level, we conducted the second RT-qPCR study. In this study, we used the original primer set for SEPT7 mRNA, and another primer set (SEPT7-R) that amplifies a different portion of the molecule. Consistent with the first RT-qPCR study, the original primer set revealed a significant ($F_{1,53}=13.9$, $p=0.0005$) 6.9% mean decrease of SEPT7 mRNA in the schizophrenia subjects (Fig. 2A), and the SEPT7-R primer set confirmed a significant ($F_{1,53}=8.5$, $p=0.005$) 10.2% mean decrease. The relative expression level of SEPT7 mRNA was significantly correlated ($r=0.71$, $p=0.048$, $n=8$) with the density of dendritic spines on layer 3 pyramidal neurons in the same subjects (11), and the within-pair percent difference of SEPT7 mRNA was significantly correlated with that of CDC42 mRNA ($r=0.68$, $p<0.0001$, $n=31$).

The correlations of SEPT7 mRNA levels with spine density and with CDC42 mRNA levels in the DLPFC of subjects with schizophrenia suggest that the three might be causally related. If the SEPT7 mRNA reduction is related to the spine deficits, SEPT5 and/or SEPT11 proteins, which are also localized to spines (35), might also altered in schizophrenia, because down-regulation of one septin protein can lead to either a parallel decrease (35,36,38) or a compensatory increase (48) of other septins. On the other hand, since SEPT7 protein is also present in other cellular components such as cell bodies and axon terminals (35), the SEPT7 decrease might reflect disturbances in those components. To clarify whether the SEPT7 reduction is related to dendritic spines or other cellular components, we examined septins present in spines (SEPT5, SEPT11), in other neuronal subcellular locations (SEPT3, SEPT6, SEPT8), or in glial cells (SEPT2) (33,35,49) in the second RT-qPCR study. Of the two other septin proteins localized to spines, SEPT11 mRNA was significantly ($F_{1,53}=6.0$, $p=0.017$) increased by 20.1% in schizophrenia (Fig. 2B), whereas SEPT5 mRNA levels were unchanged ($F_{1,53}=2.6$, $p=0.11$). This increase of SEPT11 mRNA could be a compensation for decreased SEPT7 mRNA, although the within-pair percent differences of SEPT11 and SEPT7 mRNA levels were not significantly correlated ($r=0.26$, $p=0.16$, $n=31$). In addition, the expression levels of all other septins examined (SEPT2, SEPT3, SEPT6, SEPT8), which are not localized to spines, did not significantly differ (all $F_{1,53} < 1.9$, all $p > 0.17$) between subject groups, suggesting that the alteration in SEPT7 mRNA expression might be specific to alterations in dendritic spines, and not to disturbances in other neuronal subcellular locations or in glial cells.

In order to control for possible false positive findings due to multiple comparisons, we employed a Bonferroni correction for all of the transcript comparisons reported above across both RT-qPCR analyses. Only the alterations in CDC42EP3 and SEPT7 mRNAs remained significant.

In order to determine whether the alterations of CDC42EP3 or SEPT7 mRNAs in the subjects with schizophrenia might reflect the effects of chronic treatment with antipsychotic medications, we studied monkeys chronically exposed to haloperidol, olanzapine or sham. Neither the expression of CDC42EP3 ($F_{2,10}=1.3$, $p=0.31$) nor SEPT7 ($F_{2,10}=0.08$, $p=0.93$) mRNA significantly differed across these three groups of animals (Fig. 3). We also tested the effects of potential confounding factors on the relative mRNA levels of CDC42EP3 and SEPT7 in the schizophrenia subjects (Fig. 4). None of these factors (sex; diagnosis of schizoaffective disorder; treatment with antipsychotics, benzodiazepine or sodium valproate, or antidepressants at time of death; history of substance dependence/abuse; tobacco use at time of death; or death by suicide) showed a significant effect on the relative expression level of either transcript in the schizophrenia subjects (all $F<2.5$, all $p>0.13$).

Discussion

Of the 14 transcripts examined in this study, only two mRNAs were significantly altered after corrections for multiple comparisons; CDC42EP3 mRNA was increased and SEPT7 mRNA was decreased in the DLPFC of the schizophrenia subjects. The expression levels of these mRNAs were not significantly altered by chronic haloperidol or olanzapine treatment in monkeys, and could not be attributed to potential confounding factors in the subjects with schizophrenia. Together, these findings suggest that the altered expression of CDC42EP3 and SEPT7 mRNAs in the DLPFC reflects the disease process of schizophrenia.

A molecular model of lamina-specific dendritic spine abnormalities in schizophrenia

We previously demonstrated lower mRNA levels for CDC42, a Rho GTPase family protein that promotes spine formation (21-23), in the DLPFC of the subjects with schizophrenia by *in situ* hybridization (24). The expression level of CDC42 mRNA in layer 3 was significantly correlated with spine number in that layer, but lower CDC42 mRNA was also found in layer 6 of the schizophrenia subjects where spine density was not altered (24). Thus, because CDC42EP3 mRNA is preferentially expressed in DLPFC layers 2 and 3 (25), the increased expression of this gene product in schizophrenia subjects could explain the lamina-specific spine deficits in the DLPFC according to the following model.

CDC42EP3 (also known as Borg2) protein contains a BD3 domain, which can bind to and assemble septin filaments in MDCK cells (28) (Fig. 5A). Thus, CDC42EP3 may consolidate the complex, formed by SEPT7 along with SEPT5 and 11, in the spine neck that is thought to serve as a barrier to protein movements between the spine head and parent dendrite (50,51) (Fig. 5C *top*). Activated CDC42 binds to the CRIB (Cdc42/Rac interactive binding) domain of CDC42 effector proteins (26), releasing septins from the BD3 domain and disrupting the assembly of septin filaments (28). Thus, activated CDC42 may bind to the CRIB domain of CDC42EP3 and disrupt the assembly of septin filaments (Fig. 5B). Consistent with this interpretation, the transfection of either CDC42EP3 or CDC42EP5 in NIH 3T3 fibroblasts extends long processes in which septins localize (34), whereas the co-expression of CDC42 with either CDC42EP blocks this effect (26).

The transient activation of CDC42 which occurs in individual spines after glutamate stimulation (52) (Fig. 5C *middle*) would be predicted to transiently disrupt the septin barrier in spine necks via the inhibition of CDC42EP3, permitting the entry into the spine head of post-synaptic molecules required for synaptic plasticity (Fig. 5C *bottom*). Supporting this

prediction, a similar machinery would allow protein translocation into only potentiated spines in the induction of long-term potentiation by tetanic stimulation, which induces local transient depolymerization of F-actin, via dephosphorylation (activation) of cofilin, a downstream effector of CDC42 (53), probably triggered by the activation of NMDA receptors (54).

Our findings suggest that schizophrenia is associated with both lower levels of CDC42 and higher levels of CDC42EP3 (Fig. 5D *top*). This combination would render spines less able to transiently alter the septin barrier in response to glutamate stimulation (Fig. 5D *middle*), preventing the influx of postsynaptic molecules required for normal spine plasticity (Fig. 5D *bottom*). The preferential expression of CDC42EP3 in layers 2-3 could cause a lamina-specific impairment of spine plasticity, ultimately leading to the spine loss seen in the illness, although it remains a possibility that the increase of CDC42EP3 occurs in layers 5-6. It should be noted that this hypothesis requires that CDC42EP3 protein be present in dendritic spines in order to exert this regulatory function in protein translocation at the spine neck; however, the subcellular localization of CDC42EP3 in neurons has not been reported.

The alteration in transcript level for SEPT7 suggests that the components of the septin filament at spine necks might be defective in schizophrenia (Fig 5D *top*). The absence of alterations in the expression of septins found in other subcellular locations or glia cells (35) supports the idea of a spine-specific disturbance in septin filaments in schizophrenia. Such a basal disturbance of the septin barrier in spine necks could result in postsynaptic molecules, such as cytoskeletal proteins and/or second messengers (55), diffusing from the spine neck to the parent dendrite, resulting in altered spine morphology and function (Fig. 5D *top*).

In concert with previous findings of alterations in other mediators of spine morphology (e.g., Duo)(24) in schizophrenia, the results of the present study suggest that alterations in several molecular pathways may contribute to the spine deficit present in layer 3 pyramidal neurons in schizophrenia. However, it may be that increased expression of CDC42EP3 is an essential requirement for the apparent laminar-specificity of the spine deficit.

mRNA expression of spine markers

We expected that the deficit in spine density in schizophrenia would be accompanied by lower mRNA levels of the spine-related gene products, spinophilin, PSD-95 and synaptopodin. However, consistent with previous reports (46,47) we did not find a decrease in any of these mRNAs in the DLPFC of the subjects with schizophrenia. This discrepancy might reflect the fact that the mean decrease in spine density was only around 20% and was restricted to layer 3 (11,13); thus, any associated changes in the mRNA expression of these spine-related proteins might not be detectable by RT-qPCR in total gray matter samples. Alternatively, it is possible that the mRNA levels of these genes do not reflect the cognate protein levels. For example, spinophilin (56) and PSD-95 (57) mRNAs are found in dendrites, and their protein levels seem to be regulated by dendritic translation (58). Therefore, spinophilin and PSD-95 mRNAs in dendrites could be less translated and the protein levels decreased in schizophrenia, without a change in mRNA expression levels. Consistent with this interpretation, the density of spinophilin-immunoreactive puncta has been reported to be lower in layer 3 of the auditory cortex in subjects with schizophrenia (59). In concert, these findings suggest that spinophilin protein levels, but not mRNA levels, are decreased in association with fewer spines in the illness.

Conclusions

The altered expression of CDC42EP3 and SEPT7 mRNAs suggest a substrate for the perturbed regulation of molecular movements into and out of dendritic spines in the DLPFC of the subjects with schizophrenia. The preferential expression of CDC42EP3 in layer 3 of the human

DLPFC (25) may provide the molecular link between CDC42 signaling and septin filaments (Fig. 5D) that underlie the preferential decrement in spine density in layer 3. Since dendritic spines are the principal targets of excitatory synaptic inputs to pyramidal neurons, pharmacological modulation of CDC42EP3 activity might restore synaptic plasticity, and improve excitatory connectivity, in a cell-type or circuit-specific fashion in schizophrenia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Mary Brady, BS for her assistance in editing the graphics, Holly Bazmi, MS and Dominique Arion, PhD for their assistance in the RT-qPCR studies and the members of the Clinical Services and Diagnostics Core of the Conte Center for the Neuroscience of Mental Disorders (MH084053) for their assistance in diagnostic assessments. This work was supported by National Institutes of Health grants MH043784 and MH084053.

References

1. Manoach DS, Press DZ, Thangaraj V, Searl MM, Goff DC, Halpern E, et al. Schizophrenic subjects activate dorsolateral prefrontal cortex during a working memory task, as measured by fMRI. *Biol Psychiatry* 1999;45:1128–1137. [PubMed: 10331104]
2. Barch DM, Carter CS, Braver TS, Sabb FW, MacDonald A III, Noll DC, et al. Selective deficits in prefrontal cortex function in medication-naïve patients with schizophrenia. *Arch Gen Psychiatry* 2001;58:280–288. [PubMed: 11231835]
3. Callicott JH, Mattay VS, Verchinski BA, Marengo S, Egan MF, Weinberger DR. Complexity of prefrontal cortical dysfunction in schizophrenia: More than up or down. *Am J Psychiatry* 2003;160:2209–2215. [PubMed: 14638592]
4. Gur RE, Cowell PE, Latshaw A, Turetsky BI, Grossman RI, Arnold SE, et al. Reduced dorsal and orbital prefrontal gray matter volumes in schizophrenia. *Arch Gen Psychiatry* 2000;57:761–768. [PubMed: 10920464]
5. Cannon TD, Thompson PM, van Erp TGM, Toga AW, Poutanen V-P, Huttunen M, et al. Cortex mapping reveals regionally specific patterns of genetic and disease-specific gray-matter deficits in twins discordant for schizophrenia. *Proc Natl Acad Sci USA* 2002;99:3228–3223. [PubMed: 11867725]
6. Akbarian S, Kim JJ, Potkin SG, Hagman JO, Tafazzoli A, Bunney WE Jr, et al. Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. *Arch Gen Psychiatry* 1995;52:258–266. [PubMed: 7702443]
7. Thune JJ, Uylings HBM, Pakkenberg B. No deficit in total number of neurons in the prefrontal cortex in schizophrenics. *J Psychiatr Res* 2001;35:15–21. [PubMed: 11287052]
8. Selemon LD, Goldman-Rakic PS. The reduced neuropil hypothesis: A circuit based model of schizophrenia. *Biol Psychiatry* 1999;45:17–25. [PubMed: 9894571]
9. Lewis DA, Sweet RA. Schizophrenia from a neural circuitry perspective: advancing toward rational pharmacological therapies. *J Clin Invest* 2009;119:706–716. [PubMed: 19339762]
10. Garey LJ, Ong WY, Patel TS, Kanani M, Davis A, Mortimer AM, et al. Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. *J Neurol Neurosurg Psychiatry* 1998;65:446–453. [PubMed: 9771764]
11. Glantz LA, Lewis DA. Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch Gen Psychiatry* 2000;57:65–73. [PubMed: 10632234]
12. Broadbelt K, Byne W, Jones LB. Evidence for a decrease in basilar dendrites of pyramidal cells in schizophrenic medial prefrontal cortex. *Schizophr Res* 2002;58:75–81. [PubMed: 12363393]
13. Kolluri N, Sun Z, Sampson AR, Lewis DA. Lamina-specific reductions in dendritic spine density in the prefrontal cortex of subjects with schizophrenia. *Am J Psychiatry* 2005;162:1200–1202. [PubMed: 15930070]

14. Rajkowska G, Selemon LD, Goldman-Rakic PS. Neuronal and glial somal size in the prefrontal cortex: A postmortem morphometric study of schizophrenia and Huntington disease. *Arch Gen Psychiatry* 1998;55:215–224. [PubMed: 9510215]
15. Pierri JN, Volk CLE, Auh S, Sampson A, Lewis DA. Decreased somal size of deep layer 3 pyramidal neurons in the prefrontal cortex of subjects with schizophrenia. *Arch Gen Psychiatry* 2001;58:466–473. [PubMed: 11343526]
16. Sweet RA, Pierri JN, Auh S, Sampson AR, Lewis DA. Reduced pyramidal cell somal volume in auditory association cortex of subjects with schizophrenia. *Neuropsychopharm* 2003;28:599–609.
17. Sweet RA, Bergen SE, Sun Z, Sampson AR, Pierri JN, Lewis DA. Pyramidal cell size reduction in schizophrenia: Evidence for involvement of auditory feedforward circuits. *Biol Psychiatry* 2004;55:1128–1137. [PubMed: 15184031]
18. Negishi M, Katoh H. Rho family GTPases and dendrite plasticity. *Neuroscientist* 2005;11:187–191. [PubMed: 15911868]
19. Newey SE, Velamoor V, Govek EE, Van Aelst L. Rho GTPases, dendritic structure, and mental retardation. *J Neurobiol* 2005;64:58–74. [PubMed: 15884002]
20. Calabrese B, Wilson MS, Halpain S. Development and regulation of dendritic spine synapses. *Physiology (Bethesda)* 2006;21:38–47. [PubMed: 16443821]
21. Irie F, Yamaguchi Y. EphB receptors regulate dendritic spine development via intersectin, Cdc42 and N-WASP. *Nat Neurosci* 2002;5:1117–1118. [PubMed: 12389031]
22. Tada T, Sheng M. Molecular mechanisms of dendritic spine morphogenesis. *Curr Opin Neurobiol* 2006;16:95–101. [PubMed: 16361095]
23. Wegner AM, Nebhan CA, Hu L, Majumdar D, Meier KM, Weaver AM, et al. N-wasp and the arp2/3 complex are critical regulators of actin in the development of dendritic spines and synapses. *J Biol Chem* 2008;283:15912–15920. [PubMed: 18430734]
24. Hill JJ, Hashimoto T, Lewis DA. Molecular mechanisms contributing to dendritic spine alterations in the prefrontal cortex of subjects with schizophrenia. *Mol Psychiatry* 2006;11:557–566. [PubMed: 16402129]
25. Arion D, Unger T, Lewis DA, Mirnics K. Molecular markers distinguishing supragranular and infragranular layers in the human prefrontal cortex. *Eur J Neurosci* 2007;25:1843–1854. [PubMed: 17432970]
26. Joberty G, Perlungher RR, Macara IG. The Borgs, a new family of Cdc42 and TC10 GTPase-interacting proteins. *Mol Cell Biol* 1999;19:6585–6597. [PubMed: 10490598]
27. Hirsch DS, Pirone DM, Burbelo PD. A new family of Cdc42 effector proteins, CEPs, function in fibroblast and epithelial cell shape changes. *J Biol Chem* 2001;276:875–883. [PubMed: 11035016]
28. Joberty G, Perlungher RR, Sheffield PJ, Kinoshita M, Noda M, Haystead T, et al. Borg proteins control septin organization and are negatively regulated by Cdc42. *Nat Cell Biol* 2001;3:861–866. [PubMed: 11584266]
29. Byers B, Goetsch L. A highly ordered ring of membrane-associated filaments in budding yeast. *J Cell Biol* 1976;69:717–721. [PubMed: 773946]
30. Takizawa PA, DeRisi JL, Wilhelm JE, Vale RD. Plasma membrane compartmentalization in yeast by messenger RNA transport and a septin diffusion barrier. *Science* 2000;290:341–344. [PubMed: 11030653]
31. Hall PA, Jung K, Hillan KJ, Russell SE. Expression profiling the human septin gene family. *J Pathol* 2005;206:269–278. [PubMed: 15915442]
32. Peterson EA, Kalikin LM, Steels JD, Estey MP, Trimble WS, Petty EM. Characterization of a SEPT9 interacting protein, SEPT14, a novel testis-specific septin. *Mamm Genome* 2007;18:796–807. [PubMed: 17922164]
33. Kinoshita A, Noda M, Kinoshita M. Differential localization of septins in the mouse brain. *J Comp Neurol* 2000;428:223–239. [PubMed: 11064363]
34. Lindsey R, Momany M. Septin localization across kingdoms: three themes with variations. *Curr Opin Microbiol* 2006;9:559–565. [PubMed: 17067846]
35. Xie Y, Vessey JP, Konecna A, Dahm R, Macchi P, Kiebler MA. The GTP-binding protein Septin 7 is critical for dendrite branching and dendritic-spine morphology. *Curr Biol* 2007;17:1746–1751. [PubMed: 17935997]

36. Tada T, Simonetta A, Batterton M, Kinoshita M, Edbauer D, Sheng M. Role of Septin cytoskeleton in spine morphogenesis and dendrite development in neurons. *Curr Biol* 2007;17:1752–1758. [PubMed: 17935993]
37. Oegema K, Savoian MS, Mitchison TJ, Field CM. Functional analysis of a human homologue of the *Drosophila* actin binding protein anillin suggests a role in cytokinesis. *J Cell Biol* 2000;150:539–552. [PubMed: 10931866]
38. Kinoshita M, Field CM, Coughlin ML, Straight AF, Mitchison TJ. Self- and actintemplated assembly of Mammalian septins. *Dev Cell* 2002;3:791–802. [PubMed: 12479805]
39. Fifkova E, Delay RJ. Cytoplasmic actin in neuronal processes as a possible mediator of synaptic plasticity. *J Cell Biol* 1982;95:345–350. [PubMed: 6890558]
40. Matus A, Ackermann M, Pehling G, Byers HR, Fujiwara K. High actin concentrations in brain dendritic spines and postsynaptic densities. *Proc Natl Acad Sci U S A* 1982;79:7590–7594. [PubMed: 6760199]
41. Allen PB, Ouimet CC, Greengard P. Spinophilin, a novel protein phosphatase 1 binding protein localized to dendritic spines. *Proc Natl Acad Sci U S A* 1997;94:9956–9961. [PubMed: 9275233]
42. Hunt CA, Schenker LJ, Kennedy MB. PSD-95 is associated with the postsynaptic density and not with the presynaptic membrane at forebrain synapses. *J Neurosci* 1996;16:1380–1388. [PubMed: 8778289]
43. Deller T, Merten T, Roth SU, Mundel P, Frotscher M. Actin-associated protein synaptopodin in the rat hippocampal formation: localization in the spine neck and close association with the spine apparatus of principal neurons. *J Comp Neurol* 2000;418:164–181. [PubMed: 10701442]
44. Hashimoto T, Bazmi HH, Mirnics K, Wu Q, Sampson AR, Lewis DA. Conserved regional patterns of GABA-related transcript expression in the neocortex of subjects with schizophrenia. *Am J Psychiatry* 2008;165:479–489. [PubMed: 18281411]
45. Dorph-Petersen K-A, Pierri JN, Perel JM, Sun Z, Sampson AR, Lewis DA. The influence of chronic exposure to antipsychotic medications on brain size before and after tissue fixation: A comparison of haloperidol and olanzapine in macaque monkeys. *Neuropsychopharm* 2005;30:1649–1661.
46. Weickert CS, Straub RE, McClintock BW, Matsumoto M, Hashimoto R, Hyde TM, et al. Human dysbindin (DTNBP1) gene expression in normal brain and in schizophrenic prefrontal cortex and midbrain. *Arch Gen Psychiatry* 2004;61:544–555. [PubMed: 15184234]
47. Beneyto M, Meador-Woodruff JH. Lamina-specific abnormalities of NMDA receptor-associated postsynaptic protein transcripts in the prefrontal cortex in schizophrenia and bipolar disorder. *Neuropsychopharm* 2008;33:2175–2186.
48. Peng XR, Jia Z, Zhang Y, Ware J, Trimble WS. The septin CDCrel-1 is dispensable for normal development and neurotransmitter release. *Mol Cell Biol* 2002;22:378–387. [PubMed: 11739749]
49. Fujishima K, Kiyonari H, Kurisu J, Hirano T, Kengaku M. Targeted disruption of Sept3, a heteromeric assembly partner of Sept5 and Sept7 in axons, has no effect on developing CNS neurons. *J Neurochem* 2007;102:77–92. [PubMed: 17564677]
50. Sharma K, Fong DK, Craig AM. Postsynaptic protein mobility in dendritic spines: long-term regulation by synaptic NMDA receptor activation. *Mol Cell Neurosci* 2006;31:702–712. [PubMed: 16504537]
51. Muly EC, Nairn AC, Greengard P, Rainnie DG. Subcellular distribution of the Rho-GEF Lfc in primate prefrontal cortex: effect of neuronal activation. *J Comp Neurol* 2008;508:927–939. [PubMed: 18399541]
52. Murakoshi H, Yasuda R. Imaging Rho GTPases activation in single dendritic spines of hippocampal neurons. Society for Neuroscience. 2008 Abstract 636.18.
53. Chen TJ, Gehler S, Shaw AE, Bamberg JR, Letourneau PC. Cdc42 participates in the regulation of ADF/cofilin and retinal growth cone filopodia by brain derived neurotrophic factor. *J Neurobiol* 2006;66:103–114. [PubMed: 16215999]
54. Ouyang Y, Wong M, Capani F, Rensing N, Lee CS, Liu Q, et al. Transient decrease in F-actin may be necessary for translocation of proteins into dendritic spines. *Eur J Neurosci* 2005;22:2995–3005. [PubMed: 16367766]
55. Koch C, Zador A. The function of dendritic spines: Devices subserving biochemical rather than electrical compartmentalization. *J Neurosci* 1993;13:413–422. [PubMed: 8426220]

56. Zhong J, Zhang T, Bloch LM. Dendritic mRNAs encode diversified functionalities in hippocampal pyramidal neurons. *BMC Neurosci* 2006;7:17. [PubMed: 16503994]
57. Zalfa F, Eleuteri B, Dickson KS, Mercaldo V, De Rubeis S, di Penta A, et al. A new function for the fragile X mental retardation protein in regulation of PSD-95 mRNA stability. *Nat Neurosci* 2007;10:578–587. [PubMed: 17417632]
58. Schuman EM, Dynes JL, Steward O. Synaptic regulation of translation of dendritic mRNAs. *J Neurosci* 2006;26:7143–7146. [PubMed: 16822969]
59. Sweet RA, Henteloff RA, Zhang W, Sampson AR, Lewis DA. Reduced dendritic spine density in auditory cortex of subjects with schizophrenia. *Neuropsychopharm* 2009;34:374–389.

Figure 1.

Relative expression levels of (A) CDC42EP3, (B) CDC42EP4, and (C) CDC42 mRNAs in the DLPFC of schizophrenia and matched comparison subjects. Values for subjects with schizophrenia and their matched comparison subjects are indicated as filled circles, and those for subjects with schizoaffective disorder and matched comparison subjects are indicated as open circles. Blue and green circles represent cohorts A and B, respectively. The mean value for each diagnostic group is indicated by an X. Markers above the diagonal unity line indicate subjects pairs for which the subject with schizophrenia or schizoaffective disorder had a higher expression level than the matched comparison subject.

Figure 2.

Relative expression levels of (A) SEPT7 and (B) SEPT11 mRNAs in the DLPFC of schizophrenia and matched comparison subjects. Values for subjects with schizophrenia and their matched comparison subjects are indicated as filled circles, and those for subjects with schizoaffective disorder and matched comparison subjects are indicated as open circles. Blue and green circles represent cohorts A and B, respectively. The mean value for each diagnostic group is indicated by an X. Markers above the diagonal unity line indicate subjects pairs for which the subject with schizophrenia or schizoaffective disorder had a higher expression level than the matched comparison subject.

Figure 3. Mean (SD) relative expression levels of (A) CDC42EP3 and (B) SEPT7 mRNAs in the DLPFC of matched triads of sham-, olanzapine- and haloperidol-exposed monkeys.

Figure 4.

Relative expression levels for individual subjects (circles) and mean values for the indicated group (bars) for (A) CDC42EP3 mRNA and (B) SEPT7 mRNA for the subjects with schizophrenia grouped by potential confounding factors. Numbers in bars indicate the number of subjects with schizophrenia in each category. * Tobacco use at time of death was unknown for five schizophrenia subjects.

Figure 5.

Schematic diagrams of CDC42-CDC42EP3-septin interactions and their proposed roles in spine dysfunction in schizophrenia. (A) CDC42EP3 binds to septins via its BD3 domain, inducing the assembly of septin filaments. The inactive form of CDC42 cannot bind to the CRIB domain of CDC42EP3. (B) The activated form of CDC42 binds to CDC42EP3 via its CRIB domain and inhibits CDC42EP3, disrupting the septin filament assembly. (C *top*) In the normal state, CDC42EP3 consolidates the septin 5/7/11 complex in spine necks, providing a barrier for molecular diffusion with the parent dendrite. (C *middle*) Transient activation of CDC42 by glutamate stimulation inhibits the CDC42EP3-mediated assembly of the septin barrier (C *bottom*) enabling postsynaptic molecules to enter the spine for synaptic potentiation.

(D *top*) In schizophrenia, decreased mRNA expression of SEPT7 contributes to an impaired septin barrier function at the spine neck, limiting the retention of postsynaptic molecules, such as cytoskeletal proteins and/or second messengers, which are critical for spine structure and function in the spine head. (D *middle*) Furthermore, lower levels of CDC42 and increased levels of CDC42EP3 lead to a reduced capacity for glutamatergic stimuli to produce opening of the septin barrier, impairing synaptic plasticity and contributing to spine loss (D *bottom*).

Table 1

Characteristics of subjects

	Control	Schizophrenia
Number of subjects (SA)	31	31 (10)
Sex: M/F	24/7	24/7
Race: W/B	24/7	24/7
Age: years	47.4(12.8)	46.6(11.8)
PMI: hours	17.0(6.2)	18.3(9.1)
Brain pH	6.7(0.2)	6.5(0.3)
RIN	8.2(0.7)	8.0(0.6)
Storage time: months	63(35)	62(35)

Abbreviations: B, Black; F, Female; M, Male; PMI, Postmortem interval; RIN, RNA integrity number; SA, Schizoaffective disorder; W, White.

Age, PMI, Brain pH, RIN and storage time are represented as mean (SD).