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Altered Markers of Cortical γ -Aminobutyric Acid Neuronal Activity in Schizophrenia:

Role of the *NARP* Gene

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Abstract

IMPORTANCE—In schizophrenia, working memory deficits appear to reflect abnormalities in the generation of gamma oscillations in the dorsolateral prefrontal cortex. The generation of gamma oscillations requires the phasic excitation of inhibitory parvalbumin-containing interneurons. Thus, gamma oscillations depend, in part, on the number of synaptic glutamate receptors on parvalbumin interneurons. However, little is known about the molecular factors that regulate glutamate receptor-mediated excitation of parvalbumin interneurons in schizophrenia.

OBJECTIVE—To quantify in individuals with schizophrenia the expression of immediate early genes (*NARP*, *ARC*, and *SGKI*) regulating glutamate synaptic neurotransmission.

DESIGN, SETTING, AND PARTICIPANTS—Postmortem brain specimens ($n = 206$) were obtained from individuals with schizophrenia, bipolar disorder, or major depressive disorder and from well-matched healthy persons (controls). For a study of brain tissue, quantitative polymerase chain reaction, in situ hybridization, or microarray analyses were used to measure transcript levels in the dorsolateral prefrontal cortex at gray matter, laminar, and cellular levels of resolutions. This study was conducted between January 1, 2013, and November 30, 2014.

MAIN OUTCOMES AND MEASURES—Expression levels for *NARP*, *ARC*, and *SGKI* messenger RNA (mRNA) were compared between specimens from individuals with schizophrenia

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and controls. Diagnostic specificity was assessed by quantifying *NARP* mRNA levels in specimens from individuals with mood disorders.

RESULTS—By quantitative polymerase chain reaction, levels of *NARP* mRNA were significantly lower by 25.6% in specimens from individuals with schizophrenia compared with the controls (mean [SD], 0.036 [0.018] vs 0.049 [0.015]; $F_{1,114} = 21.0$; $P < .001$). Levels of *ARC* ($F_{1,112} = 0.93$; $P = .34$) and *SGKI* ($F_{1,110} = 2.52$; $P = .12$) were not significant. These findings were supported by in situ hybridization (*NARP*; individuals with schizophrenia vs controls: 40.1% lower [$P = .003$]) and microarray analyses (*NARP*; individuals with schizophrenia vs controls: 12.2% lower in layer 3 [$P = .11$] and 14.6% lower in layer 5 pyramidal cells [$P = .001$]). In schizophrenia specimens, *NARP* mRNA levels were positively correlated with *GAD67* mRNA ($r = 0.55$; $P < .001$); the expression of *GAD67* mRNA in parvalbumin interneurons is activity dependent. The *NARP* mRNA levels were also lower than healthy controls in bipolar disorder (−18.2%; $F_{1,60} = 11.39$; $P = .001$) and major depressive disorder (−21.7%; $F_{1,30} = 5.36$; $P = .03$) specimens, especially those from individuals with psychosis. In all 3 diagnostic groups, *NARP* mRNA levels were positively correlated (all $r > 0.53$; all $P < .02$) with somatostatin mRNA, the expression of which is activity dependent.

CONCLUSIONS AND RELEVANCE—Given the role of *NARP* in the formation of excitatory inputs to parvalbumin (and perhaps somatostatin) interneurons, our findings suggest that lower *NARP* mRNA expression contributes to lower excitatory drive onto parvalbumin interneurons in schizophrenia. This reduced excitatory drive may lead to lower synthesis of γ -aminobutyric acid in these interneurons, contributing to a reduced capacity to generate the gamma oscillations required for working memory.

The neural substrate for cognitive functions, including working memory, involves synchronization of cortical neuronal activity at gamma frequency oscillations.^{1–4} Consequently, gamma oscillation abnormalities in the dorsolateral prefrontal cortex (DLPFC) are thought to contribute to working memory deficits in schizophrenia.^{5–8} Gamma oscillations require the synchronized inhibition of neighboring populations of pyramidal neurons by the parvalbumin-containing basket cell subclass of γ -aminobutyric acid interneurons.^{9–11} Specifically, excitatory input from pyramidal neurons activates parvalbumin basket neurons, which furnish feedback inhibition to pyramidal neurons.¹² Because the axons of parvalbumin basket neurons are highly divergent,¹³ this feedback inhibition simultaneously hyperpolarizes multiple neighboring pyramidal neurons. The fast and synchronous decay of this inhibition enables the simultaneous firing of these pyramidal neurons at gamma band frequency.¹⁴ Thus, given their importance in gamma oscillations, excitatory inputs onto DLPFC parvalbumin interneurons might be a key component in the neural circuitry basis of working memory.

The strong coupling of the phasic excitation of parvalbumin interneurons with gamma oscillation frequency¹⁵ suggests that the composition of synaptic glutamate receptors on parvalbumin interneurons is critical for gamma oscillations.¹⁶ The α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/*N*-methyl-D-aspartate receptor (AMPA/NMDAR) ratio in synapses onto parvalbumin interneurons is approximately 3 times greater than onto cortical pyramidal neurons and other γ -aminobutyric acid interneurons.^{17–19} Selective knockout of the AMPAR subunits GluR1 or GluR4 from parvalbumin interneurons reduces

both their phasic excitatory drive and the power of gamma oscillations.²⁰ Furthermore, fast AMPAR-mediated excitation of parvalbumin interneurons is sufficient to support gamma oscillations.^{19,21} Collectively, these data suggest that alterations of the AMPAR-mediated excitatory inputs onto parvalbumin interneurons could contribute to impaired gamma oscillations in schizophrenia.

One potential regulator of AMPAR-mediated excitatory input onto parvalbumin interneurons is the immediate early gene, neuronal activity–regulated pentraxin (*NARP*; GenBank NM_002523), which is prominently expressed in pyramidal neurons in response to neuronal activation²² and is secreted at presynaptic axon terminals in glutamate synapses onto parvalbumin interneurons.²³ At these synapses, *NARP* binding helps to cluster GluR4-containing AMPARs (Figure 1A) and enhances excitatory input to parvalbumin interneurons.^{23,24} Consistent with these findings, mice homozygous for a *NARP* gene knockout exhibit reduced excitatory inputs onto parvalbumin interneurons.²⁴ These findings suggest that deficient *NARP* messenger RNA (mRNA) expression contributes to lower AMPAR-mediated excitation of parvalbumin interneurons in schizophrenia.

To test this hypothesis, we used quantitative polymerase chain reaction (qPCR), in situ hybridization, and microarray analyses to quantify the expression of *NARP* mRNA at the gray matter, laminar, and cellular levels in the DLPFC from a large cohort of brain tissue samples from individuals with schizophrenia as well as healthy comparison individuals (controls). To determine the molecular specificity of altered *NARP* expression, we examined them RNA levels of 2 other immediate early genes that regulate AMPAR-mediated excitatory synaptic transmission through different mechanisms: activity-regulated, cytoskeleton-associated protein (*ARC*; GenBank NM_015193) and serum/glucocorticoid-regulated kinase 1 (*SGKI*; GenBank NM_001143677). Unlike *NARP*, *ARC* regulates homeostatic scaling of AMPARs specifically on pyramidal neurons,²⁵ whereas *SGKI* affects glutamate transmission without cell-type specificity.²⁶ To test the disease process specificity of altered *NARP* expression, we examined *NARP* mRNA levels in the DLPFC in brain tissue samples from individuals with bipolar disorder or major depressive disorder and in samples from monkeys with long-term exposure to antipsychotic medications. Finally, because the expression of glutamic acid decarboxylase 67 kD (GAD67), the major enzyme of γ -aminobutyric acid synthesis in the cortex, is regulated by neuronal activity^{27,28} and is lower in parvalbumin interneurons in the DLPFC of people with schizophrenia,^{29,30} we also determined whether *NARP* expression predicted GAD67 mRNA levels in the DLPFC.

Methods

Human Tissue Samples

Brain specimens (n = 206) were obtained at the Allegheny County Office of the Medical Examiner (Pittsburgh, Pennsylvania) after consent was obtained from next of kin. Diagnoses were made using *DSM-IV-R* for each patient,³¹ and tissue was collected from DLPFC area 9, as described previously^{30,32} (eMethods in the Supplement). All procedures were approved by the University of Pittsburgh's Committee for the Oversight of Research and Clinical Trials Involving the Dead and Institutional Review Board for Biomedical Research. The study was conducted between January 1, 2013, and November 30, 2014.

To control for experimental variance and reduce biological variance between groups, each sample from a person with schizophrenia or schizoaffective disorder (n = 62), bipolar disorder (n = 35) (21 met the criteria for bipolar 1 disorder), or major depressive disorder (n = 19) was matched with a sample from 1 control (n = 90) for the donor's sex and as closely as possible for age (Table; eTable 1 in the Supplement provides details on each person); some control samples were matched to more than 1 sample from an individual with a psychiatric illness. Consequently, to compare the transcript levels between groups, individual data are presented in scatterplots that show the values for both members in a sample pair. For each set of disease and comparison samples, group means did not differ significantly for age, postmortem interval, RNA integrity number (Agilent Bioanalyzer), brain pH, or tissue storage time at -80°C with one exception: brain pH significantly differed ($t_{61} = 2.68$; $P = .01$) between groups in the schizophrenia cohort, but the mean difference was small (0.1 pH unit) and of uncertain biological relevance.

Quantitative PCR

Quantitative PCR was performed to determine relative expression levels of each target transcript (eTable 2A in the Supplement), as described previously³³ (eMethods in the Supplement). Three internal reference transcripts (β -actin, cyclophilin A, and glyceraldehyde-3-phosphate dehydrogenase), selected based on their stable expression across the subjects in this cohort regardless of diagnoses,^{34,35} were used to normalize the target transcripts (eMethods in the Supplement).

In Situ Hybridization

In situ hybridization procedures for *NARP* mRNA (eTable 2B in the Supplement) were performed with 3 tissue sections from each donor³³ (eMethods in the Supplement). The *NARP* mRNA levels in area 9 were measured at total gray matter, laminar, and cellular levels of resolution (eMethods and eFigure 1 in the Supplement).

Microarray Analyses

For each sample, 200 pyramidal cells in deep layer 3 and in layer 5 of area 9 were individually dissected and then pooled per layer per subject (LMD 6500; Leica Microsystems), as described previously.³⁶ The synthesized complementary DNA was then loaded onto an array plate (Affymetric GeneChip HT HG-U133+ PM; Affymetrix).³⁶

Antipsychotic-Exposed Monkeys

The effect of long-term exposure to antipsychotic medication on altered *NARP* mRNA levels in schizophrenia was examined using macaque monkeys exposed for a long duration to haloperidol, olanzapine, or placebo (6 monkeys per group)^{32,37} (eMethods and eTable 2A in the Supplement). All procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee.

Statistical Analysis

qPCR and In Situ Hybridization—Paired and unpaired analyses of covariance models, including covariates of sex, age, postmortem interval, brain pH, RNA integrity number, and storage time, were used to test the effects of diagnostic group on gene expression levels determined by qPCR and for total, laminar, and cellular *NARP* mRNA levels determined by in situ hybridization (eMethods in the Supplement). The results from both models are illustrated in this report, and only the results from the unpaired model are reported in the text except when the *P* values differed regarding significance. Within each group, the samples with transcript levels that were greater than 3 SDs from the group mean were considered as outliers for that measure and removed. Consequently, reported degrees of freedom differ slightly across analyses.

The influence of comorbid factors on *NARP* mRNA expression within each diagnostic group was assessed with the unpaired analysis of covariance model (eMethods in the Supplement). The relationship between each immediate early gene and *GAD67* mRNA levels was assessed by Pearson correlation. For the antipsychotic-exposed monkeys, analyses of covariance models were used with treatment group as a main effect and triad as a blocking factor. All *P* values were 2-tailed, and the significance level was set at .05.

Microarray Analysis—Probe sets from the array were filtered, and paired *t* tests were performed using the random intercept model, as described previously.³⁶ Discovery of differentially expressed genes was conducted using meta-analysis, and an adaptively weighted Fisher method was applied for each transcript. Meta-analyzed *P* values from the adaptively weighted testing were then adjusted by the Benjamini-Hochberg procedure for multiple comparisons to control false discovery rate.³⁶

Results

qPCR for *NARP*, *ARC*, and *SGK1* mRNA in Schizophrenia

The mean level of *NARP* mRNA (Figure 1B) was significantly lower by 25.6% in the schizophrenia samples compared with their matched controls ($F_{1,114} = 21.0$; $P < .001$). The *NARP* mRNA levels were lower in the schizophrenia samples for 51 of the 62 pairs (Figure 1B). However, mean mRNA levels of *ARC* and *SGK1* (Figure 1D and E) did not differ significantly between groups (*ARC*: $F_{1,112} = 0.93$; $P = .34$; and *SGK1*: $F_{1,110} = 2.52$; $P = .12$).

In the unpaired analysis of covariance model, age was a significant determinant of *NARP* mRNA expression in the gray matter ($F_{1,114} = 41.2$; $P < .001$), and *NARP* mRNA levels were similarly negatively correlated with age in the schizophrenia ($r = -0.49$; $P < .001$) and control ($r = -0.55$; $P < .001$) groups (eFigure 2A in the Supplement). Levels of *NARP* mRNA in schizophrenia samples did not differ significantly (all $F < 2.91$; all $P > .09$) as a function of sex; diagnosis of schizoaffective disorder; history of substance dependence or abuse; nicotine use at the time of death; use of antipsychotics, antidepressants or benzodiazepines, and/or sodium valproate at the time of death; or death by suicide (eFigure 2B in the Supplement). Finally, levels of *NARP* mRNA did not differ significantly ($F_{2,10} =$

0.42; $P = .67$) among monkeys with long-term exposure to haloperidol, olanzapine, or placebo (eFigure 3 in the Supplement).

We examined the relationship between changes in *NARP* mRNA expression and *GAD67* mRNA in the DLPFC using *GAD67* mRNA levels previously reported in the same 62 subject pairs.³³ Levels of *NARP* and *GAD67* mRNA as determined by qPCR were positively correlated in schizophrenia samples ($r = 0.55$; $P < .001$) but not in the control samples ($r = -0.05$; $P = .70$) (Figure 1C). In contrast, neither *ARC* nor *SGK1* mRNA levels were significantly correlated with *GAD67* mRNA levels in either the schizophrenia or control samples (all $r < 0.18$; all $P > .17$).

In Situ Hybridization for *NARP* mRNA in Schizophrenia

In situ hybridization (with a probe directed against a different portion of *NARP* mRNA than in the primer set used in the qPCR study) was performed to quantify mRNA levels in the DLPFC from 20 sample pairs with a sufficient number of available tissue sections (eTable 1 in the Supplement). Mean total gray matter levels of *NARP* mRNA were significantly ($F_{1,30} = 10.46$; $P = .003$) lower by 40.1% in schizophrenia samples compared with matched controls (mean [SD], 47.6 [35.8] vs 79.4 [24.7] nCi/g) (Figure 2A and B), a difference similar to that found by qPCR (-34.1%) in the same samples. Furthermore, levels of *NARP* mRNA quantified by qPCR or in situ hybridization were highly correlated ($r = 0.77$; $P < .001$) across all samples ($n = 38$). Analysis by cortical layers revealed that *NARP* mRNA expression was significantly lower in layers 2 (-41.7%; $F_{1,30} = 11.5$; $P = .002$), 3 (-42.0%; $F_{1,30} = 9.80$; $P = .004$), 4 (-38.2%; $F_{1,30} = 6.91$; $P = .013$), and 6 (-56.2%; $F_{1,30} = 15.9$; $P = .001$) and nearly so in layer 5 (-38.8%; paired: $F_{1,14} = 3.78$; $P = .07$; unpaired: $F_{1,30} = 6.31$; $P = .02$) (Figure 2C).

At the cellular level, the mean number of *NARP*-positive neurons per millimeters squared was significantly lower (Figure 2D and E) in both layer 2 (-30.0%; $F_{1,30} = 7.52$; $P = .01$) and layer 5 (-34.2%; $F_{1,30} = 12.48$; $P = .001$) in schizophrenia samples relative to the matched control samples. In contrast, the mean grain density per *NARP*-positive neuron did not significantly differ in either layer 2 or 5 (all $F_{1,30} < 2.62$; all $P > .11$) between groups (Figure 2F and G).

Microarray Analyses for *NARP* mRNA in Schizophrenia

Recently, Arion et al³⁶ performed microarray analyses from pools of individually dissected pyramidal neurons (eTable 1 in the Supplement) and neurons labeled with *Vicia villosa* agglutinin,³⁸ which is present in the perineuronal nets that surround parvalbumin interneurons. In controls, *NARP* mRNA expression as determined by microarray was approximately 16 times higher in pyramidal than parvalbumin cells in area 9 from the same samples. In pyramidal neurons, mean levels of *NARP* mRNA were significantly lower (Figure 3A and B) in layer 5 (-14.6%; $P = .001$) and nearly so in layer 3 (-12.2%; $P = .11$) in the schizophrenia samples compared with the matched controls; these findings show the same direction of change to the 21% reduction in mean *NARP* mRNA levels in total gray matter as determined by qPCR for the same 36 pairs of subjects. Finally, consistent with the

gray matter qPCR findings, mean levels of *ARC* and *SGK1* mRNA in layer 3 or 5 pyramidal cells did not differ significantly between schizophrenia and control samples (all $P > .58$).

qPCR for *NARP* mRNA in Mood Disorders

Mean *NARP* mRNA levels were significantly lower in samples from individuals with bipolar disorder (-18.2% ; $F_{1,60} = 11.39$; $P = .001$), bipolar 1 disorder only (-20.3% ; $F_{1,32} = 10.65$; $P = .003$), and major depressive disorder (-21.7% ; $F_{1,30} = 5.36$; $P = .03$) relative to their matched controls (Figure 4A–C). To explore the relationship between *NARP* and *GAD67* mRNA levels, we used *GAD67* mRNA levels previously reported³⁵ in matched 18 triads of bipolar disorder, major depressive disorder, and control samples included in the present study. As determined by qPCR, *NARP* and *GAD67* mRNA levels were positively correlated in the bipolar disorder and major depressive disorder samples (all $r > 0.47$; all $P < .048$) but not in their matched controls (all $r < 0.25$; all $P > .35$). In addition, 10 bipolar disorder samples (all from individuals who had bipolar I disorder) and 5 major depressive disorder samples were from individuals with a history of psychotic features (eTable 1 in the Supplement). Compared with those without a history of psychotic features, samples from those with a history of psychotic features had lower *NARP* mRNA levels (Figure 4D) for both the bipolar 1 disorder (-23.1% ; $F_{1,12} = 5.96$; $P = .03$) and major depressive disorder (-15.9% ; $F_{1,11} = 4.18$; $P = .07$) groups.

Discussion

Using total gray matter, layer, and cellular levels of resolution and different techniques (qPCR, in situ hybridization, and microarray), we found convergent evidence of lower *NARP* mRNA expression in the DLPFC of brain specimens from individuals with schizophrenia that was most prominent in a subset of pyramidal neurons. Our finding of a lower density of *NARP* mRNA-positive neurons is unlikely to represent a deficit in the number of *NARP*-expressing neurons in schizophrenia based on several findings. First, a stereologic study³⁹ reported no significant difference in the total number of neurons in the prefrontal cortex samples from individuals with schizophrenia compared with controls. Second, the density of pyramidal neurons was reported to be slightly increased across cortical layers⁴⁰ or to be unchanged in layer 3 of the DLPFC.⁴¹ In concert, these findings suggest that lower *NARP* is attributable to a downregulation of *NARP* mRNA in existing pyramidal neurons and not to a deficit in the number of these neurons.

Given the role of secreted *NARP* in regulating AMPAR clustering on parvalbumin interneurons²³ (Figure 1A), lower *NARP* mRNA expression might contribute to lower AMPAR-mediated excitatory synaptic inputs onto parvalbumin interneurons in schizophrenia. Because *GAD67* expression is activity dependent,^{27,28} disease-related alterations in expression of the activity-dependent gene *NARP* could contribute to altered levels of *GAD67* mRNA in individuals with schizophrenia. This hypothesis was supported by the significant positive correlation between *GAD67* and *NARP* mRNA levels in the schizophrenia samples, indicating that the larger range of both *GAD67* and *NARP* mRNA levels in these samples revealed the predicted positive correlation between 2 transcripts. Furthermore, although other populations of γ -aminobutyric acid interneurons may be

affected, only parvalbumin interneurons have been directly shown to have lower levels of GAD67 mRNA²⁹ and protein⁴² in schizophrenia. Together, these findings support a coupling effect of lower *NARP* expression in pyramidal cells, with a downstream, activity-dependent deficit in expression of GAD67 in parvalbumin interneurons. However, studies characterizing NARP protein levels specifically at pyramidal cell inputs to parvalbumin interneurons in individuals with schizophrenia are needed to validate this hypothesis.

The regulation of AMPAR-mediated excitatory synaptic transmission differs among *NARP*, *ARC*, and *SGKI*, with all expression being activity dependent.^{23,25,26} In contrast to our *NARP* findings, mRNA levels of *ARC* and *SGKI* did not differ significantly between groups and did not correlate with levels of GAD67 mRNA in the schizophrenia or control samples. Thus, lower expression of *NARP* mRNA in pyramidal neurons might be a specific aspect of the disease process of schizophrenia and not a general consequence of a hypoactive cortical circuit that would be expected to be associated with lower expression levels of multiple immediate early genes.

Lower levels of *NARP* mRNA in people with schizophrenia appear to be due to the disease process rather than to factors frequently associated with the illness. First, among the 62 samples from individuals with schizophrenia studied by qPCR, none of the examined comorbid factors accounted for lower *NARP* mRNA expression. Second, *NARP* mRNA expression was unaltered in the DLPFC of monkeys with long-term exposure to either haloperidol or olanzapine. Third, age and *NARP* mRNA expression were similarly negatively correlated in both the control and schizophrenia samples, with the regression line for schizophrenia samples parallel to and shifted downward from that for the controls. In a previous study⁴³ using the same tissue samples as in the present study, neither illness duration nor age accounted for lower levels of GAD67 expression in schizophrenia. Thus, the lower *NARP* and GAD67 mRNA levels in schizophrenia samples seen across specimens from individuals aged 17 to 83 years in the present study are unlikely to be a consequence of illness progression and may reflect blunting or incomplete developmental trajectories of these transcripts. However, we cannot rule out a decline in *NARP* mRNA expression during the early stages of clinical illness because we do not have data on the people immediately before and after the onset of psychosis.

Mean levels of *NARP* mRNA were also significantly lower in specimens from individuals with bipolar disorder or major depressive disorder, especially those with psychotic features, suggesting that lower *NARP* mRNA levels might contribute to the greater cognitive impairments observed in bipolar disorder or major depressive disorder with psychotic features.^{44,45} However, GAD67 mRNA levels were unchanged in the specimens from these subjects independent of the presence or absence of psychotic features.³⁵ Thus, although lower *NARP* mRNA levels could be a conserved feature across major psychiatric illnesses, achieving deficits in cortical GAD67 levels might require factors in addition to fewer *NARP* mediated glutamatergic inputs from pyramidal neurons. For example, allelic variants in the *GADI* gene associated with increased risk for schizophrenia,⁴⁶ altered chromatin structures at the *GADI* promoter region,^{47,48} or lower levels of the GAD67 transcription factor *Zif268*³³ could also be required for deficits in GAD67 expression to emerge in schizophrenia.

Alternatively, lower expression of *NARP* in schizophrenia, bipolar disorder, and major depressive disorder could be upstream of other alterations that are common to all 3 diagnoses. For example, NARP protein was recently localized to somatostatin interneurons where it is thought to regulate excitatory synapse development.⁴⁹ Because somatostatin gene expression is activity dependent,^{50,51} lower *NARP* expression could contribute to the deficits in somatostatin mRNA levels observed in schizophrenia,^{32,52–55} bipolar disorder,^{35,53} and major depressive disorder.³⁵ Consistent with this interpretation, *NARP* mRNA levels were similarly positively correlated with previously determined somatostatin levels in the specimens from individuals with schizophrenia ($r = 0.74$; $P < .01$ [n = 61]),⁵⁶ bipolar disorder, ($r = 0.76$; $P < .01$ [n = 17]),³⁵ and major depressive disorder ($r = 0.53$; $P = .02$ [n = 18])³⁵ examined in the present study.

Conclusions

The present findings suggest that lower *NARP* mRNA expression in DLPFC pyramidal neurons may lead to reduced clustering of AMPAR at excitatory synapses on parvalbumin interneurons, thus contributing to lower excitatory drive to these neurons. In individuals with schizophrenia, this reduced excitatory drive could result in lower GAD67 expression and presumably lower γ -aminobutyric acid synthesis in parvalbumin interneurons. Given the role of parvalbumin interneurons in the generation of gamma oscillations, altered *NARP* expression could contribute to the molecular basis for altered gamma oscillations and impaired cognition in schizophrenia. Across individuals with psychosis, lower *NARP* mRNA levels might also lead to less clustering of AMPAR at excitatory synapses on somatostatin interneurons, perhaps contributing to the dysfunction of these neurons seen across diagnoses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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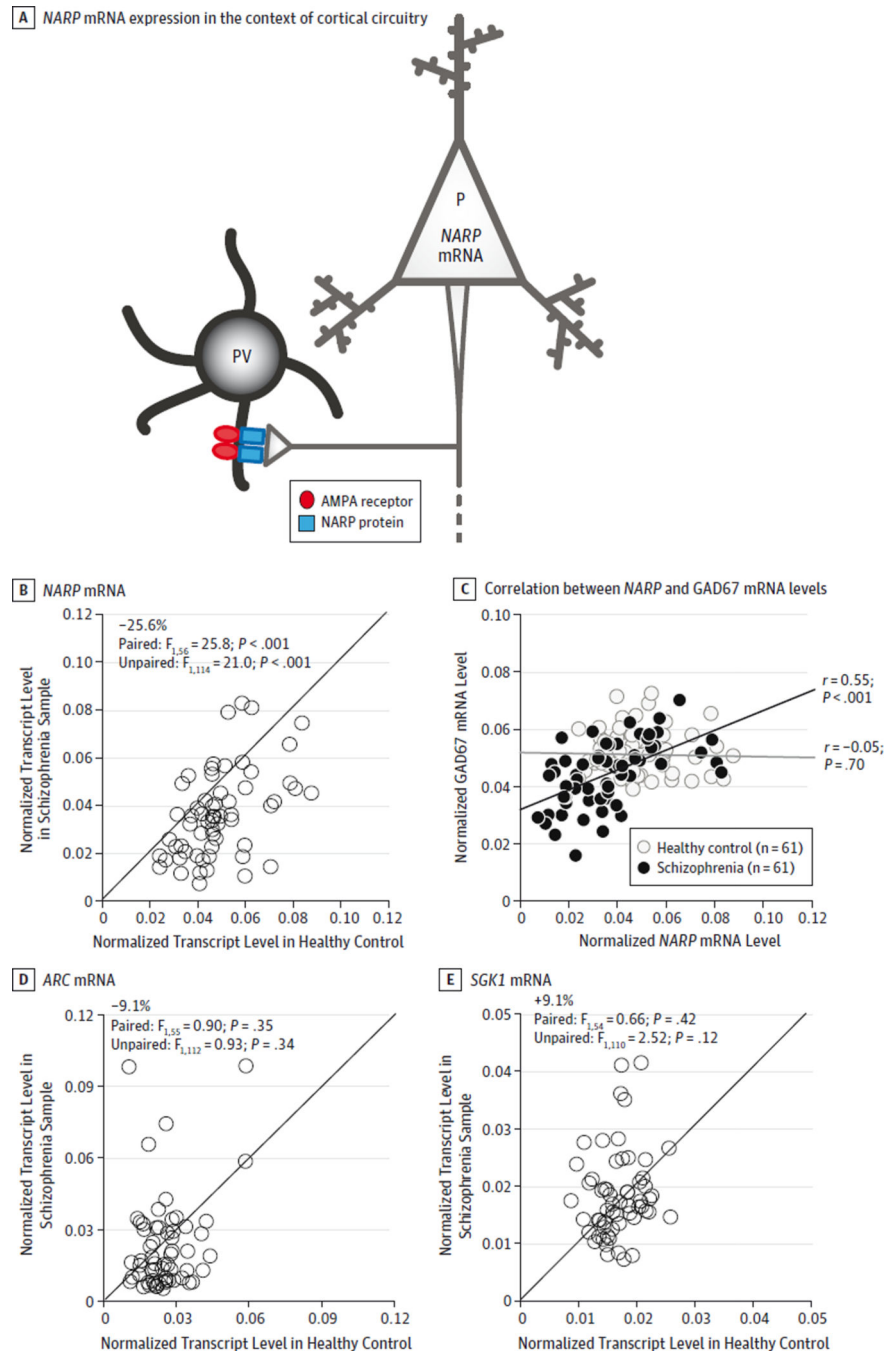


Figure 1. Polymerase Chain Reaction Determination of Relative Messenger RNA (mRNA) Levels for NARP, ARC, and SGKI in Schizophrenia and Healthy Control Samples

NARP mRNA is expressed in pyramidal cells (P), and NARP protein is secreted at excitatory synapses on the dendrites of parvalbumin interneurons (PV) (A). At these synapses, NARP contributes to the clustering of AMPA receptors. The levels of NARP (B), ARC (D), and SGKI (E) mRNA for each control and schizophrenia sample in a pair. Data points below the diagonal unity line indicate lower mRNA levels in the schizophrenia sample relative to its matched control and vice versa. B, Mean NARP mRNA level was

significantly lower in schizophrenia samples relative to matched controls. C, *NARP* mRNA levels were positively correlated with *GAD67* mRNA levels in schizophrenia samples (black line) but not in controls (gray line). D and E, Mean levels of *ARC* and *SGKI* mRNA did not differ significantly between groups.

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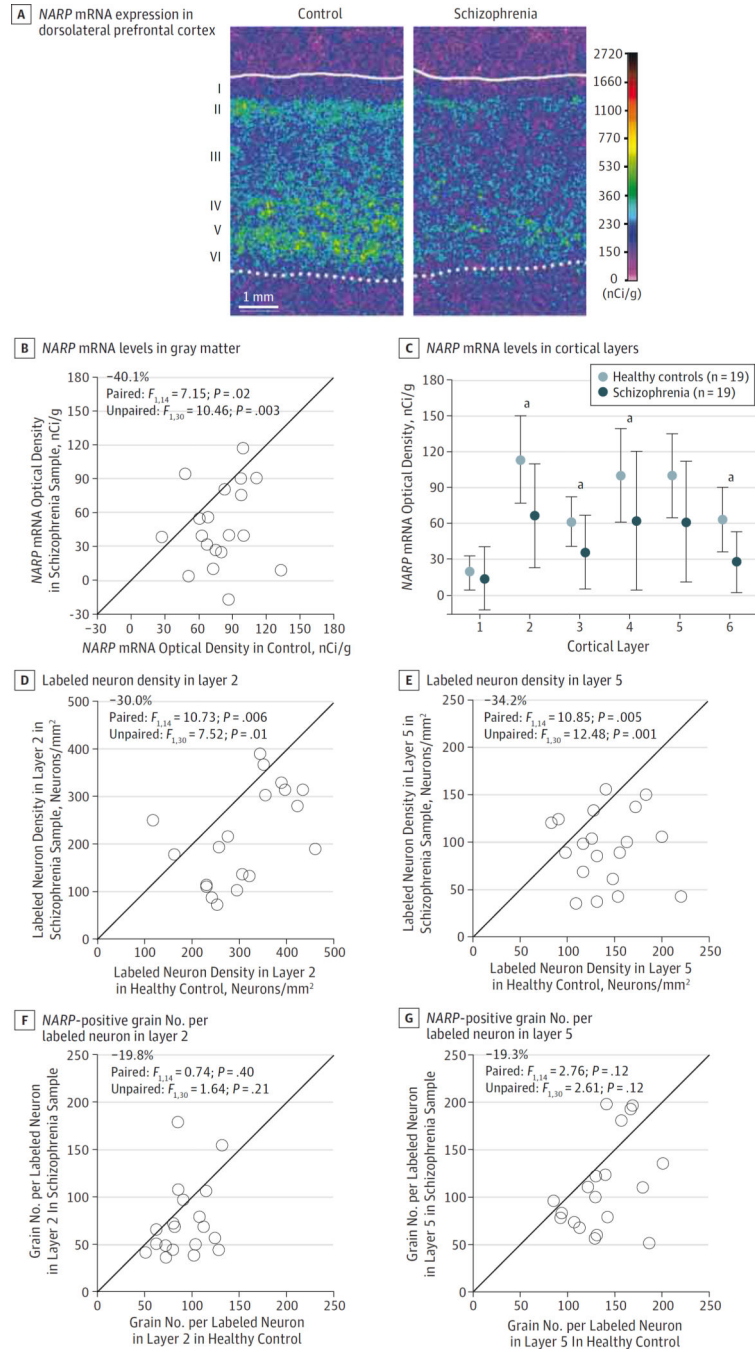


Figure 2. In Situ Hybridization Analyses for NARP Messenger RNA (mRNA) Between Schizophrenia and Healthy Control Samples

A through C, In situ hybridization film analysis. A, Representative pseudocolored film autoradiographs of dorsolateral prefrontal cortex sections illustrating NARP mRNA expression levels in 1 pair of schizophrenia and control tissue samples. The solid and dotted lines indicate the pial surface and the gray-white matter border, respectively. B, Mean NARP mRNA levels across the gray matter for each control and schizophrenia sample in a pair. Data points below the diagonal unity line indicate lower NARP mRNA levels in the

schizophrenia sample relative to the matched control and vice versa. Mean *NARP* mRNA levels in schizophrenia samples were significantly lower relative to those of the matched controls. C, Mean *NARP* mRNA levels in each cortical layer in schizophrenia and healthy comparison subjects. Mean (SD) *NARP* mRNA levels were significantly lower in layers 2, 3, 4, and 6 and nearly so in layer 5 in schizophrenia samples relative to matched controls (see the In Situ Hybridization for *NARP* mRNA in Schizophrenia subsection of the Results section for statistical results). D through G, Cellular grain counting analysis. D and F, *NARP*-positive neurons per millimeters squared. E and G, Grains per positive neuron in layers 2 and 5 for each control and schizophrenia sample in a pair. Data points below the diagonal unity lines indicate lower *NARP* mRNA levels in the schizophrenia sample relative to its matched control and vice versa. The mean number of *NARP*-positive neurons per millimeter squared in schizophrenia samples was lower in both layers 2 and 5 compared with matched controls. In contrast, the difference in mean grain density per *NARP*-positive neuron in layers 2 and 5 did not differ significantly between groups.

^a $P < .01$.

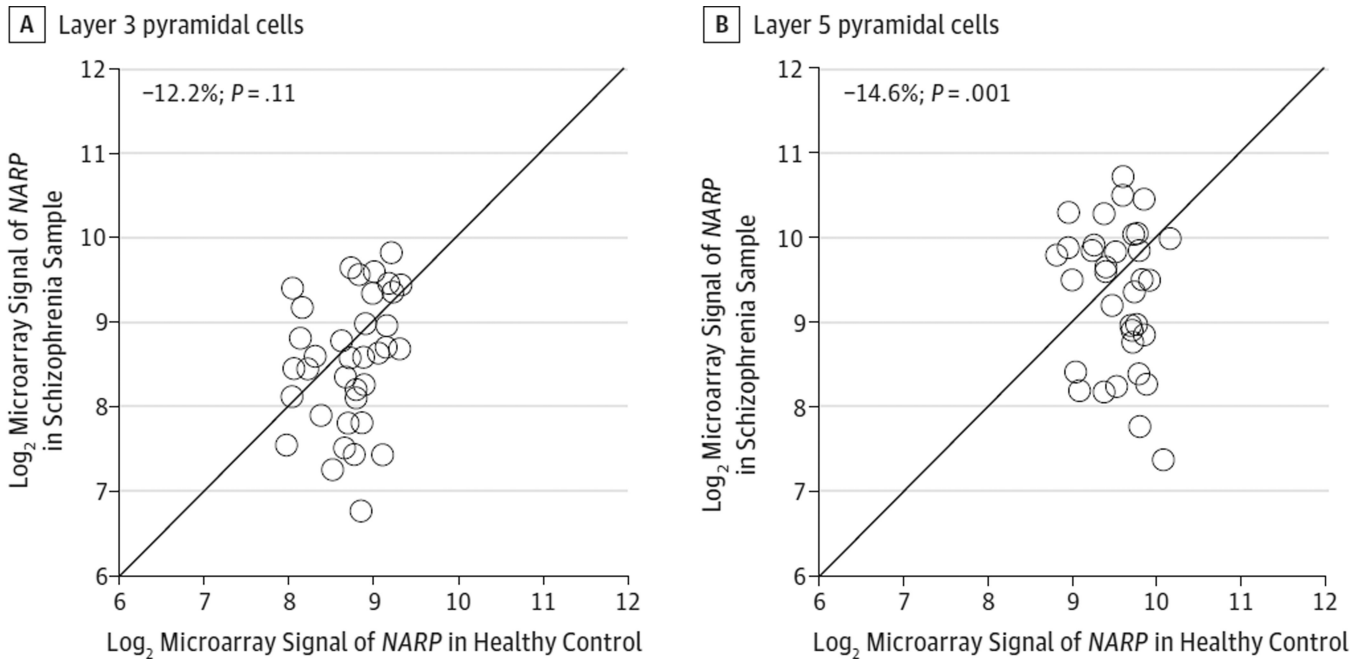


Figure 3. Microarray Analyses of *NARP* Messenger RNA (mRNA) Levels in Nissl-Stained Pyramidal Neurons in Matched Pairs of Schizophrenia and Control Samples

Log₂-transformed microarray signals of *NARP* mRNA in layer 3 (A) and layer 5 (B) for schizophrenia samples relative to matched controls are plotted for each pair. The data points below the diagonal unity line indicate lower mRNA signals in the schizophrenia sample relative to its matched control and vice versa.

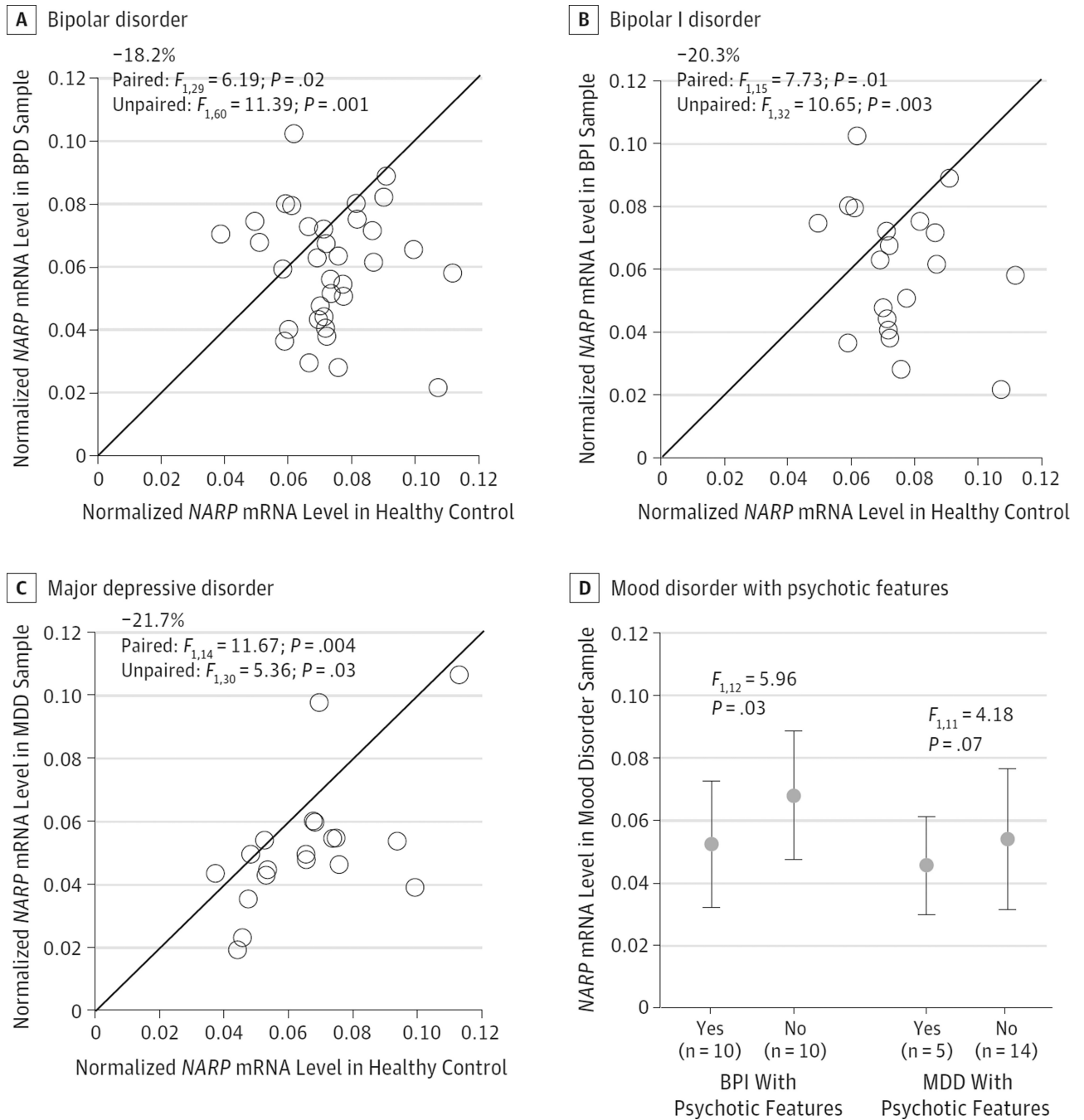


Figure 4. Quantitative Polymerase Chain Reaction Determination of Relative *NARP* Messenger RNA (mRNA) Levels in Mood Disorders and Effect of Psychotic Features on *NARP* mRNA Expression Levels in Bipolar I (BPI) Disorder and Major Depressive Disorder (MDD)

Scatterplots indicate the levels of *NARP* mRNA for each healthy control sample and bipolar disorder (BPD) (A), BPI disorder (B), or MDD (C) subject in a pair. Data points below the diagonal unity line indicate lower mRNA levels in the mood disorder subject relative to the matched healthy control and vice versa. Mean *NARP* mRNA level was significantly lower in subjects with BPD, BPI disorder, and MDD with matched healthy controls. Mean *NARP*

mRNA levels for BPI disorder and individuals with MDD are grouped by psychotic features (D).

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Summary of Demographic and Postmortem Characteristics of Individuals Contributing Tissue Samples

Table

| Characteristic | Controls (n = 62) | Schizophrenia (n = 62) | Controls (n = 35) | Bipolar Disorder (n = 35) | Controls (n = 19) | Major Depressive Disorder (n = 19) |
|--|----------------------|---------------------------|----------------------|------------------------------|----------------------|--|
| Sex, No. (%) | | | | | | |
| Male | 47 (76) | 47 (76) | 20 (57) | 20 (57) | 10 (53) | 10 (53) |
| Female | 15 (24) | 15 (24) | 15 (43) | 15 (43) | 9 (47) | 9 (47) |
| Race, No. (%) | | | | | | |
| White | 52 (84) | 46 (74) | 32 (91) | 34 (97) | 18 (95) | 18 (95) |
| Black | 10 (16) | 16 (26) | 3 (9) | 1 (3) | 1 (5) | 1 (5) |
| Age, mean (SD), y | 48.7 (13.8) | 47.7 (12.7) | 46.6 (12.7) | 45.5 (12.2) | 47.8 (10.4) | 46.3 (9.5) |
| PMI, mean (SD), h | 18.8 (5.5) | 19.2 (8.5) | 19.1 (5.1) | 20.5 (7.0) | 19.3 (5.3) | 21.3 (6.6) |
| Brain pH, mean (SD) | 6.7 (0.2) | 6.6 (0.3) | 6.7 (0.3) | 6.6 (0.3) | 6.6 (0.2) | 6.6 (0.2) |
| RIN, mean (SD) | 8.2 (0.6) | 8.1 (0.6) | 8.1 (0.6) | 8.0 (0.5) | 8.0 (0.6) | 8.0 (0.4) |
| Storage time, mean (SD), mo ^a | 125.8 (54.5) | 122.0 (58.8) | 106.8 (49.8) | 113.7 (45.1) | 124.7 (43.4) | 140.5 (28.8) |

Abbreviations: PMI, postmortem interval; RIN, RNA integrity number.

^aStorage at -80°C.