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Cognitive Impairment and Diminished Neural Responses Constitute a Biomarker Signature of Negative Symptoms in Psychosis

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The treatment of negative symptoms (NS) in psychosis represents an urgent unmet medical need given the significant functional impairment it contributes to psychosis syndromes. The lack of progress in treating NS is impacted by the lack of known pathophysiology or associated quantitative biomarkers, which could provide tools for research. This current analysis investigated potential associations between NS and an extensive battery of behavioral and brain-based biomarkers in 932 psychosis probands from the B-SNIP database. The current analyses examined associations between PANSS-defined NS and (1) cognition, (2) pro-/anti-saccades, (3) evoked and resting-state electroencephalography (EEG), (4) resting-state fMRI, and (5) tractography. Canonical correlation analyses yielded symptom-biomarker constructs separately for each biomarker modality. Biomarker modalities were integrated using canonical discriminant analysis to summarize the symptom-biomarker relationships into a "biomarker signature" for NS. Finally, distinct biomarker profiles for 2 NS domains ("diminished expression" vs "avolition/apathy") were computed using step-wise linear regression. NS were associated with cognitive impairment, diminished EEG response amplitudes, deviant resting-state activity, and oculomotor abnormalities. While a connection between NS and poor cognition has been established, association to neurophysiology is novel, suggesting directions for future mechanistic studies. Each biomarker modality was related to NS in distinct and complex ways, giving NS a rich, interconnected fingerprint and suggesting that any one biomarker modality may not adequately capture the full spectrum of symptomology.

Key words: schizophrenia/bipolar disorder/multivariate statistics/EEG/oculomotor/biotype

Introduction

Negative symptoms (NS) are one of the cardinal manifestations of schizophrenia.[1,2] They are considered to be a distinct symptom class, although conceptualizations range from being an entirely independent symptom class to extensively overlapping with positive psychosis and cognitive impairment.[3,4] Because these manifestations can be mimicked by other disorders, such as depression or intellectual disability, it is important to elucidate the characteristics of psychosis-related NS whenever possible.[5–9] NS are identified in schizophrenia, but the extent to which they appear in other psychotic disorders has been insufficiently studied.[10] Finally, the overall neurobiology of NS is not understood, and characteristic phenotypes have not been studied in depth.[11] Biological disease features are even more important to identify now that treatments directed toward NS are a focus of development.[12–22] With these points in mind, we set out to use proband characteristics from the *Bipolar-Schizophrenia Network for Intermediate Phenotypes-1* (B-SNIP1) to identify the neurobiological features of NS in psychotic disorders and to contrast these across conventional psychosis diagnoses and biologically derived psychosis subgroups.[23]

Features of brain processing which robustly differentiated psychosis from healthy were assessed in B-SNIP1, including cognitive processing, resting and evoked

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electrophysiology, structural and functional brain imaging, and oculomotor function.[24] Several psychosis disorders were included in the B-SNIP deep phenotyping protocol, including schizophrenia, schizoaffective disorder, and psychotic bipolar I disorder.[25] Clementz et al used biomarker data to classify psychosis cases into Biotypes (B1-B3), all with similar levels of psychosis. Biotype-1 has severely disordered cognition and deficient electroencephalography (EEG) amplitudes and hyporesponsivity; Biotype-2 has slightly less disordered cognition but neurophysiological hyperactivity; Biotype-3 has almost normal cognition and only slightly deviant EEG responses.[23] The broad biomarker profile of predominant NS in such a broad psychosis population, however, has not been examined.

In this article, we (1) identify individual biomarkers which separate B-SNIP1 probands with predominant NS from those without; (2) describe complex relationships between NS and a broad biomarker battery; and (3) demonstrate that 2 domains of NS ("diminished expression" vs "avolition/apathy") are characterized by distinct biomarker profiles.[8,26–31]

Methodology

Participants

A total of 932 individuals with psychosis (schizophrenia, $N = 397$; schizoaffective disorder, $N = 223$; bipolar I disorder with psychosis, $N = 312$) were recruited as a part of B-SNIP1, a 5-site deep phenotyping study of the psychosis syndrome (table 1). The SCID Interview was used for clinical diagnosis[32]; see Tamminga et al[25] for methodological details. Clinical assessments included the Global Assessment of Functioning (GAF) scale, Positive and Negative Syndrome Scale (PANSS),[33] Young Mania Rating Scale (YMRS), Montgomery-Åsberg Depression Rating Scale (MADRS),[34] and Birchwood Social Functioning Scale (BSFS).[35] Participants were excluded if they had an active substance use dependence within 3 months or abuse within 1 month, had a known brain disorder, or had a traumatic brain injury resulting in loss of consciousness lasting longer than 30 min. Participants were clinically stable outpatients 15–65 years old (49.7% female), with the majority (92.7%) on at least one psychotropic medication. This study was approved by Institutional Review Boards at all 5 B-SNIP sites, and all participants provided written informed consent.

The PANSS Negative Symptom Factor (PANSS-NSF) shows superior content validity than the original NS scale, is reliable and sensitive to treatment response, and was used throughout these analyses.[36] The PANSS-NSF, which excludes "difficulty in abstract thinking" (N5) and "stereotyped thinking" (N7) and includes "motor retardation" (G7) and "active social avoidance" (G16), has been informative in schizophrenia samples, as well as studies of persistent NS.[37–40] For a subset of the current analyses, probands were subdivided into those with (NS) vs without (non-NS) predominant NS based on a previously validated framework, where the threshold for having predominant NS was defined as having at least one of the PANSS-NSF items rated as at least moderate (≥4).[37,38] Ninety-six of the 932 B-SNIP probands lacked complete PANSS negative data and were excluded from these analyses. All analyses were performed across the psychosis syndrome, except where specifically noted.

Biomarker Assessment

The extensive B-SNIP phenotyping battery was collected on each participant, including cognitive assessments, electrophysiological measures, and structural and functional brain imaging.[24,25,41] This battery included the Brief Assessment of Cognition in Schizophrenia (BACS) battery,[42] the Stop Signal Task,[43] the Dot Probe Expectancy Task,[44,45] the Wechsler Memory Scale spatial span,[46] the Penn Conditional Exclusion Test,[47] the Penn Emotion Recognition Test,[47–49] smooth pursuit eye movements,[50–61] pro-/anti-saccades,[62–68] electroencephalography (auditory oddball, paired stimuli, resting-state),[69–71] resting-state fMRI,[72,73] and structural tractography MRI (diffusion tensor imaging; DTI).[74] Procedures and findings for each measure from B-SNIP1 are available in previous reports (also see supplemental methods).[69–84] Phenotype assessment often included multiple individual variables for each task, paradigm, or imaging modality.[23,41,85,86]

Statistical Analyses

All biomarker measures were adjusted for age and sex using models constructed from the B-SNIP1 healthy control group ($N = 459$),[24,25] an approach we have taken in previous B-SNIP publications (see supplemental methods).[70,75,80,83,85–93] Cognition measures were also adjusted for race in similar manner. To directly examine individual biomarkers differentiating NS and non-NS groups, separate one-way ANOVAs were performed with each individual biomarker as dependent variables and group (NS vs non-NS) as a fixed factor, using SPSS v25 (IBM Corporation). Multiple testing was accounted for using a false discovery rate (FDR) threshold of 5%.[94]

To examine the structure of associations between NS and biomarkers, we used canonical correlation analysis (CCA), a data-driven, multivariate approach that identifies the bidirectional relationship between 2 sets of variables. This is accomplished by weighting each variable such that the weighted sum of one set of variables (eg, NS) is maximally correlated with a second set (eg, biomarkers). The resulting constructs are interpreted based on the relative strength and polarity of these weights. CCA was performed separately for each biomarker modality, with the biomarker variables on one side of the equation and PANSS-Negative items on the other, using SAS software v9.4 (SAS Institute Inc.). Individual participants with missing data were excluded modality wise.

Table 1. Clinical Characteristics of Probands With and Without Negative Symptoms

	NS (*N* = 322)		Non-NS (*N* = 515)		Statistic	*P*-value
Sociodemographic Characteristics						
Age, years, mean (SD)	35.6	(12.4)	36.7	(12.6)	$F_{1,835} = 1.8$	.18
Gender, female, *N* (%)	156	(48.4 %)	260	(50.4 %)	$\chi^2 = 0.25$	.62
Race, *N* (%)						
Caucasian	169	(52.5 %)	326	(63.3 %)	$\chi^2 = 9.59$	.002
African American	137	(42.5 %)	183	(35.5 %)	$\chi^2 = 3.83$	.050
Education, years, mean (SD)	13.0	(2.3)	13.5	(2.4)	$F_{1,833} = 11.1$	.001
Hollingshead score	51.5	(14.7)	56.9	(15.1)	$F_{1,779} = 17.6$	< .001
Hollingshead score, maternal	42.5	(17.3)	41.2	(17.0)	$F_{1,704} = 0.96$	.33
Hollingshead score, paternal	39.2	(18.7)	37.6	(17.8)	$F_{1,612} = 1.10$	.29
Age of illness onset	20.0	(8.1)	20.4	(8.7)	$F_{1,804} = 0.30$	.59
Illness duration, years	15.5	(12.1)	16.4	(12.4)	$F_{1,804} = 1.09$	.30
Number of hospitalizations	6.1	(7.0)	5.7	(7.1)	$F_{1,667} = 0.69$	.41
At least one suicide attempt	139	(43.8 %)	193	(38.0 %)	$\chi^2 = 4.27$	.12
Lifetime ECT	23	(7.3 %)	19	(3.7 %)	$\chi^2 = 5.09$	.04
Psychosis Subgroups, *N* (%)						
Schizophrenia	167	(48.8 %)	175	(51.2 %)	$\chi^2 = 0.19$	.67
Schizoaffective disorder	93	(42.1 %)	118	(57.9 %)	$\chi^2 = 2.96$	.09
Bipolar I w/psychosis	62	(21.8 %)	222	(78.2 %)	$\chi^2 = 90.14$	< .001
Biotype-1[a]	91	(46.9 %)	103	(53.1 %)	$\chi^2 = 0.74$	.39
Biotype-2	92	(40.7 %)	134	(59.3 %)	$\chi^2 = 7.81$	.005
Biotype-3	90	(32.8 %)	184	(47.2 %)	$\chi^2 = 32.25$	< .001
Clinical Characteristics, Mean (SD)						
GAF	47.7	(11.7)	56.6	(13.4)	$F_{1,833} = 96.4$	< .001
Birchwood SFS	114.9	(25.7)	130.7	(23.4)	$F_{1,665} = 66.8$	< .001
PANSS-Positive	17.3	(6.1)	14.9	(5.2)	$F_{1,834} = 36.7$	< .001
PANSS-Negative	19.5	(5.2)	12.0	(3.4)	$F_{1,835} = 638.8$	< .001
PANSS-General	35.8	(9.2)	29.4	(8.0)	$F_{1,834} = 111.4$	< .001
MADRS	14.11	(10.9)	8.5	(7.8)	$F_{1,814} = 74.0$	< .001
Young Mania Rating Scale	7.0	(7.0)	5.9	(6.1)	$F_{1,808} = 5.7$	.02
WRAT-4 IQ	96.0	(15.6)	98.2	(14.8)	$F_{1,815} = 4.2$	.04
PANSS Negative Symptom Factor items						
N1. Blunted affect	3.3	(1.5)	1.7	(0.8)	$F_{1,835} = 413.5$	< .001
N2. Emotional withdrawal	3.0	(1.2)	1.7	(0.8)	$F_{1,835} = 358.7$	< .001
N3. Poor rapport	2.5	(1.3)	1.4	(0.6)	$F_{1,835} = 208.6$	< .001
N4. Passive/apathetic social withdrawal	3.3	(1.4)	1.8	(0.8)	$F_{1,835} = 447.1$	< .001
N6. Lack of spontaneity	2.6	(1.4)	1.5	(0.7)	$F_{1,835} = 244.8$	< .001
G7. Motor retardation	2.2	(1.1)	1.3	(0.6)	$F_{1,835} = 214.9$	< .001
G16. Active social withdrawal	2.9	(1.4)	1.8	(0.8)	$F_{1,835} = 242.3$	< .001
Concomitant Medications, *N* (%)						
Any psychotropic medication	297	(92.2 %)	479	(93.0 %)	$\chi^2 = 0.18$	.91
Antipsychotics (any)	272	(84.4 %)	411	(79.8 %)	$\chi^2 = 3.27$	.20
Antipsychotics, first generation	41	(12.7 %)	39	(7.6 %)	$\chi^2 = 6.20$	.05
Antipsychotics, second generation	230	(71.4 %)	372	(72.2 %)	$\chi^2 = 0.17$	.97
Mood stabilizers (any)	117	(36.3 %)	256	(49.7 %)	$\chi^2 = 14.46$	.001
Antidepressants (any)	150	(46.6 %)	222	(43.1 %)	$\chi^2 = 1.03$	.60
Sedatives/anxiolytics	85	(26.4 %)	146	(28.3 %)	$\chi^2 = 0.38$	.83
Stimulants	17	(5.3 %)	39	(7.6 %)	$\chi^2 = 1.67$	.43
Anticholinergics	48	(14.9 %)	64	(12.4 %)	$\chi^2 = 1.08$	.58

[a]Of the 837 probands with complete PANSS Negative data, only 694 had Biotype designations due to biomarker data requirements for the Biotype classification process. These individuals overlapped completely.

All measures were standardized before the CCA to eliminate differences in scale from contributing to the outcome. The multivariate nature of CCA does not require multiple testing within a CCA, although multiple testing across the separate CCAs was accounted for using an FDR threshold of 5%.[94] To evaluate the consistency of the models produced by the CCA solutions and latent variate pairs, we conducted delete-2 jackknife analyses with 1000 replicates constructed using random sampling without replacement, with CCAs conducted on each

replicate. No individual measure included zero in the 99% confidence interval across all jackknife outcomes for that behavioral or biological modality.

Psychosis Subgroups. Subsequent analyses evaluated the similarity of symptom-biomarker associations in NS vs non-NS groups, as well as across conventional clinical diagnoses[95] and B-SNIP Biotypes.[23] The significant CCA pairs (NS-biomarker/phenotype constructs) were each subjected to a multivariate general linear model with subgroup (either NS/non-NS, DSM diagnosis, or Biotype, respectively) as a fixed factor and the CCA variates as dependent variables. This allowed us to assess group differences across both aspects of the symptom-biomarker construct simultaneously.

Negative Symptom Biosignature in Psychosis. Finally, we performed a multivariate analysis integrating variables from all biomarker modalities. Canonical discriminant analysis (CDA) with step-wise variable introduction was used to identify those variables that maximally discriminated NS vs non-NS probands. To avoid large decreases in sample size, missing values were imputed using Markov chain Monte Carlo multiple imputation in SPSS v25.

Two-Factor Model of NS. Confirmatory factor analysis (CFA) was conducted using a 2-factor model of NS identified in previous exploratory and CFA studies, "avolition/apathy" (including anhedonia, avolition, and asociality) and "diminished expression" (including blunted affect and alogia).[26,96] The maximum likelihood method was used for estimation as the data did not show a tendency to non-normality (skewness < 2.0, Kurtosis < 3.0).[97] CFA was conducted using SAS v9.4 and evaluated using multiple indices of goodness-of-fit: chi-square, comparative fit index (CFI > 0.9), root mean square error of approximation (RMSEA < 0.1), standardized root mean square residual (SRMS < 0.08), and goodness-of-fit index (GFI > 0.9).[98–103]

The factor loadings from the CFA were used to compute individual subject scores for the 2 NS factors. A step-wise linear regression model ($P_{in} \leq .05$, $P_{out} \geq .1$) was fit for each factor, using all biomarker variables as predictors. This resulted in a biomarker profile for each NS factor.

Results

Clinical Characteristics

Probands without predominant NS were more likely to be Caucasian ($P = .002$) with more years of education ($P = .001$) compared to probands with NS, but the groups were otherwise demographically similar (table 1). NS probands exhibited more severe clinical symptoms than non-NS on the PANSS positive and general symptom scores ($P < .001$), MADRS ($P < .001$), and YMRS ($P = .02$), and more severe psychosocial impairments on GAF ($P < .001$), Birchwood SFS ($P < .001$), and WRAT-4 ($P = .04$). There was a large overlap, however, in the distributions of these characteristics between NS and non-NS (supplemental figure 1). To address the question of whether NS were largely secondary to depression in our sample, we examined PANSS Negative scores as a function of MADRS scores (supplemental table 1). Including probands with high MADRS scores showed no appreciable effect on NS scores, so we proceeded by including them in all analyses.

The distribution of NS probands was not equal across DSM diagnoses or Biotypes (supplemental figure 2). Probands diagnosed with schizophrenia (48.8%; $\chi^2 = 0.19$, $P = .67$) and schizoaffective disorder (42.1%; $\chi^2 = 42.1$, $P = .09$) were more likely to exhibit NS than those with psychotic bipolar disorder (21.8%). The Biotypes showed a step-wise NS expression, from Biotype-1 (46.9%) to Biotype-2 (40.7%) to Biotype-3 (32.8%) (B1 vs B2, $\chi^2 = 1.28$, $P = .26$; B2 vs B3, $\chi^2 = 3.81$, $P = .05$; B1 vs B3, $\chi^2 = 9.34$, $P = .002$).

Biomarker Characteristics

Individual Biomarkers. To identify biomarkers specifically associated with NS-psychosis, we examined each biomarker individually. For individual biomarkers/phenotypes, NS cases showed more extensive impairment than non-NS on the BACS (NS = −1.18; non-NS = −0.80; $P = 2.3E\text{-}4$; Cohen's $d = -0.28$), the Penn Emotion Recognition Test (NS = 83.7; non-NS = 86.3; $P = 2.8E\text{-}4$; $d = -0.28$), and the WMS Backward Score (NS = 8.7; non-NS = 9.1; $P = .001$; $d = -0.24$). No other individual biomarker differences survived FDR correction.

Modality-Wide Biomarker Associations. We examined complex relationships between NS and biomarker modalities using CCA. Each biomarker modality had a distinct and unique relationship with NS, giving NS a rich, interconnected fingerprint. For instance, poor performance on several cognitive measures associated with more severe blunted affect (N1), poor rapport (N3), lack of spontaneity (N6), and motor retardation (G7). Evoked-EEG amplitudes were positively correlated with active social avoidance (G16) severity, but negatively correlated with blunted affect (N1), poor rapport (N3), lack of spontaneity (N6), motor retardation (G7), and passive social avoidance (N7) severity. The association patterns for all NS-biomarker constructs are presented in figure 1, with CCA statistics and subgroup comparisons presented in table 2. Although subgroups often differed significantly, scatter and silhouette plots of canonical variate scores indicated large overlaps between subgroups (figure 2 and supplemental figure 3).

Negative Symptom Biosignature. To identify a multimodal biosignature of NS, imputed values for all biomarker variables were used as predictors in a CDA, with NS vs non-NS status as the criterion. The CDA returned a set of 11 biomarkers which together maximally discriminate NS vs non-NS (Wilks' $\Delta = .925$, $F_{11,781} = 5.77$, $P = 9.0E\text{-}6$). The latent biosignature of NS includes poor antisaccade performance, poor emotion recognition, slow prosaccades, low BACS, low evoked-EEG amplitudes, and decreased frontoparietal rs-fMRI activity (figure 3A).

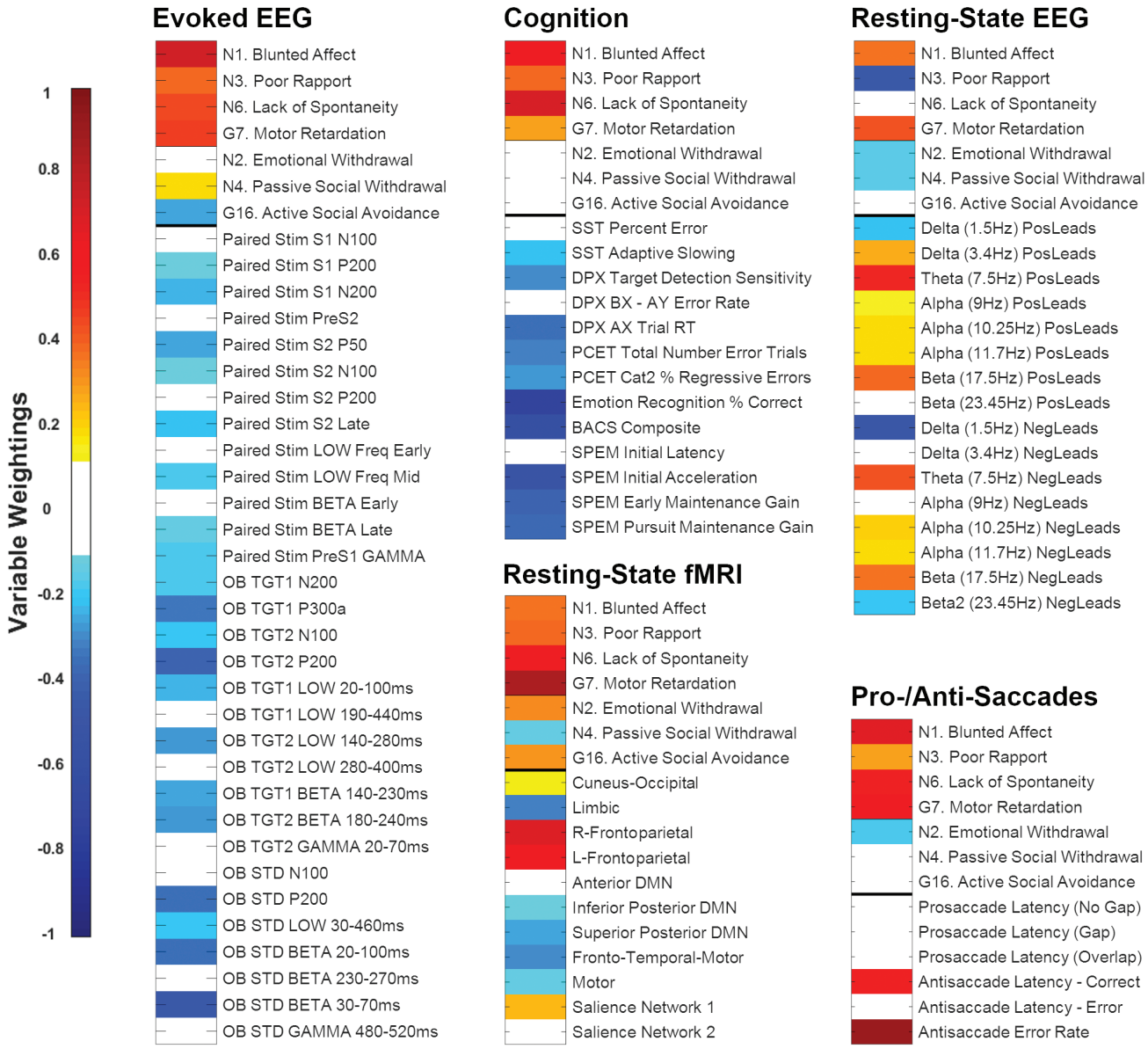

Fig. 1. Each biomarker modality shows a distinct pattern of multivariate associations with negative symptoms. *Note*: The heat maps show the loading strength of individual negative symptoms and biomarker variables on their respective latent variates for each canonical correlation analysis. Warmer colors indicate stronger positive loadings; cooler colors indicate stronger negative loadings. For clarity, loadings between −.1 and .1 are shown in white. Paired Stim, auditory paired stimulus paradigm; S1/S2, the first or second paired stimulus; OB STD, auditory oddball standard stimuli; TGT1/2, PCA component 1/2 for auditory oddball target stimuli; SST, stop signal task; DPX, dot probe expectancy task; PCET, Penn conditional exclusion task; SPEM, smooth pursuit eye movements; PosLeads, mean of spatial leads that were positively correlated with the frequency component; NegLeads, mean of spatial leads that were negatively correlated with the frequency component; DMN, default mode network.

Two-Factor Model of Negative Symptoms. Recent factor analyses have identified a 2-factor latent structure for NS, which we examined using CFA.[26,96] The 2-factor model yielded the following fit index values: $\chi^2 = 186.06$, $df = 11$ ($P < .0001$); CFI = .934; RMSEA = 0.138 [CI: 0.121–0.156, 90%]; SRMR = 0.048; and GFI=0.941, indicating good fit. Therefore, the common practice of considering 2 distinct factors of NS ("diminished expression" vs "avolition/apathy") is supported by these data.

Standardized factor loadings from the 2-factor model (supplemental table 2) were used to compute individual subject scores for the 2 NS factors. A linear regression model was fit for each factor, using the evoked-EEG, cognition, and saccade measures as predictors. "Diminished expression" was predicted by low BACS (β = −.143, $P < .001$), low evoked-EEG amplitude on oddball standards beta-band 230–270 ms (β = −.15, $P < .001$) and 30–70 ms (β = −.08, $P = .03$), poor antisaccade performance (β = −.092, $P = .01$), and poor emotion recognition (β = −.072, $P = .04$;

Table 2. Modality-Wide Associations and Subgroup Differences

Canonical Correlation Analysis Results								
	F-value	*P*-value	FDR-Adjusted *P*-value	Canonical Correlation	% Variance Accounted	Wilks' λ[a]	*N*	*N–BM*[b]
Cognition	$F_{91,2159.1} = 1.47$	.003	.009	.34	34.9	.69	362	15
Evoked-EEG	$F_{217,3838.5} = 1.29$	.003	.009	.37	30.8	.62	597	31
rs-EEG	$F_{112,2725.1} = 1.31$	.02	.04	.34	37.7	.71	443	16
rs-fMRI	$F_{77,2620.1} = 1.31$	.04	.06	.32	47.9	.80	454	11
Pro-/anti-saccades	$F_{42,2944.3} = 1.31$	.09	.10	.22	61.6	.93	640	6
DTI[c]	$F_{350,846.05} = 0.92$	.81	.81	.38	22.2	.11	177	50

Probands With vs Without Predominant Negative Symptoms				
	ANOVA		NS vs non-NS	
	F-value	*P*-value	Cohen's *d*	
Evoked-EEG	$F_{1,595} = 60.32$	3.5E-14	0.62	
Cognition	$F_{1,363} = 28.17$	1.9E-7	0.55	
rs-EEG	$F_{1,441} = 1.37$	.24	—	
rs-fMRI	$F_{1,452} = 22.98$	2.0E-6	0.46	
Pro-/anti-saccades	$F_{1,638} = 37.85$	1.4E-9	0.49	

Clinical Diagnoses								
	ANOVA		SZ vs SAD		SZ vs BDP		SAD vs BDP	
	F-value	*P*-value	*P*-value	Cohen's *d*	*P*-value	Cohen's *d*	*P*-value	Cohen's *d*
Evoked-EEG	$F_{2,594} = 18.07$	2.4E-8	< .001	0.39	< .001	0.57	*ns*	—
Cognition	$F_{2,362} = 16.41$	1.5E-7	.001	0.43	< .001	0.69	*ns*	—
rs-EEG	$F_{2,440} = 3.41$	.03	< .001	0.08	< .001	0.30	*ns*	—
rs-fMRI	$F_{2,451} = 1.13$	.32	*ns*	—	*ns*	—	*ns*	—
Pro-/anti-saccades	$F_{2,637} = 33.17$	2.0E-14	< .001	0.56	< .001	0.69	*ns*	—

Biotypes								
	ANOVA		B1 vs B2		B1 vs B3		B2 vs B3	
	F-value	*P*-value	*P*-value	Cohen's *d*	*P*-value	Cohen's *d*	*P*-value	Cohen's *d*
Evoked-EEG	$F_{2,564} = 13.92$	1.0E-6	.04	0.23	< .001	0.57	.002	0.31
Cognition	$F_{2,358} = 27.86$	5.7E-12	.04	0.31	< .001	0.97	< .001	0.68
rs-EEG	$F_{2,402} = 1.24$	.29	*ns*	—	*ns*	—	*ns*	—
rs-Fmri	$F_{2,451} = 1.45$	.23	*ns*	—	*ns*	—	*ns*	—
Pro-/anti-saccades	$F_{2,574} = 44.88$	7.8E-19	< .001	0.58	< .001	0.89	.004	0.34

[a]Wilks' λ is the product of the values (1—Canonical R^2) for the current canonical variate and all variates below it. Therefore, lower values for Wilks' λ represent a greater proportion of variance shared between the variable sets across all canonical variate functions.
[b]N-BM, number of biomarker variables included in a given modality.
[c]As the negative symptom—DTI construct did not meet even exploratory significance, subgroup comparisons were not performed.

overall model fit: $R = .266$, $F_{5,787} = 11.95$, $P = 9.63E\text{-}11$). "Avolition/apathy" severity was predicted by low evoked-EEG amplitude on oddball standards beta-band 230–270ms ($\beta = -.110$, $P = .002$; overall model fit: $R = .11$, $F_{1,791} = 9.62$, $P = .002$). These results suggest that the 2 NS domains have distinct underlying biological correlates.

Negative Symptoms and Cognitive Impairment. The results described above suggest a strong link between NS and cognitive impairment. While multiple individual NS items are significantly correlated with BACS composite scores (N1, $P < .001$; N2, $P = .09$; N2, $P < .001$; N3, $P = .002$; N4, $P = .03$; N6, $P < .001$; G7, $P < .001$; G16, $P = .25$), the strength of each correlation is low (all $r < .2$; supplemental figure 4). Further, NS and non-NS show BACS composite score distributions which are similar in shape but are slightly shifted with respect to one another, with NS showing lower race-adjusted BACS scores (NS: −1.17; non-NS: −0.80; $t_{1,762} = 3.70$; $P = 2.3E\text{-}4$; $d = -0.28$; supplemental figure 1).

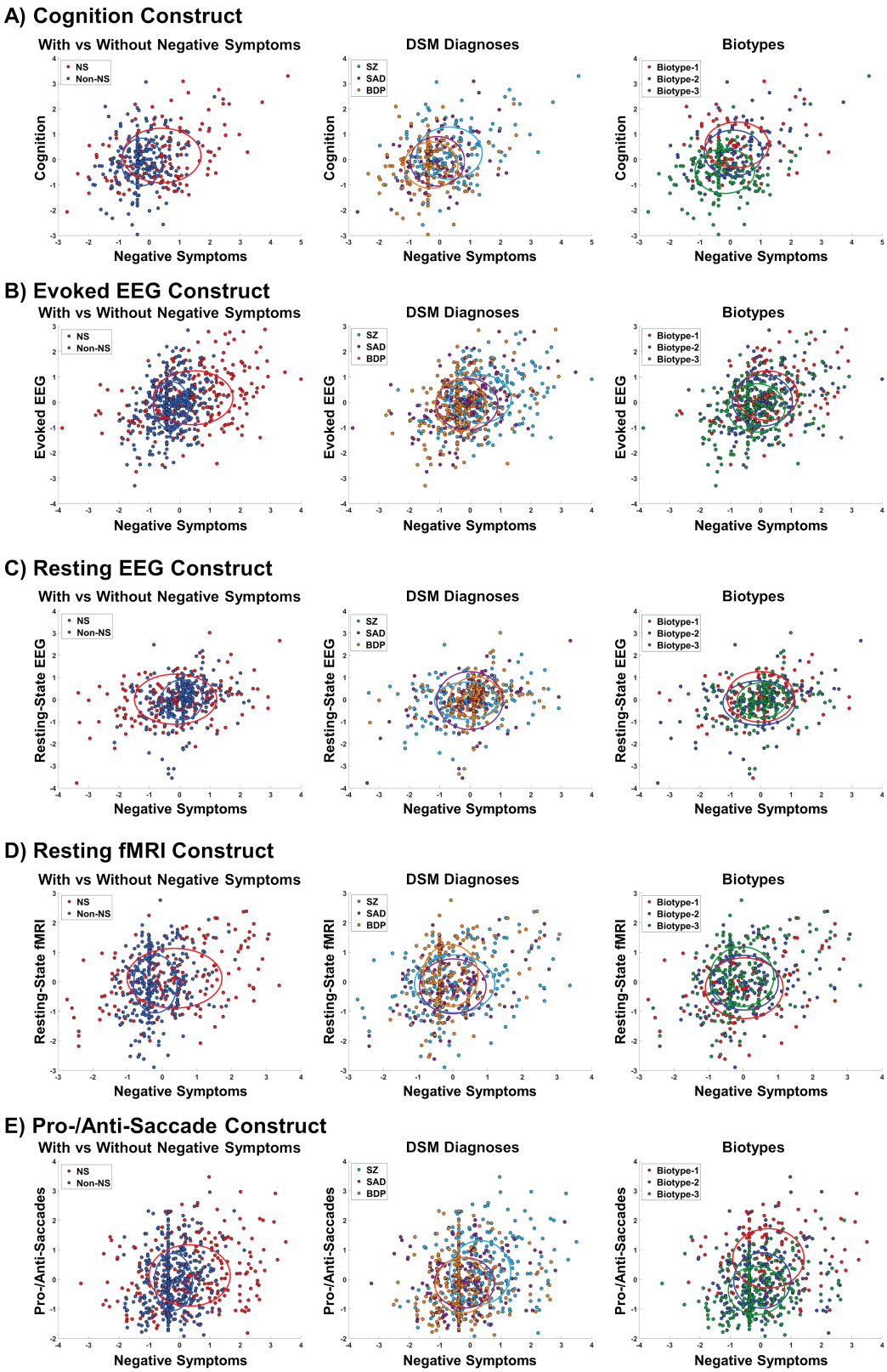

Fig. 2. Scatterplots of canonical variate scores color coded by probands with vs without predominant negative symptoms (left), clinical diagnosis (middle), and Biotype (right) indicate largely overlapping subgroups. *Note*: Scores represent the sum of the standardized data weighted by the loading strength of individual negative symptoms and biomarker variables on their respective latent variates for each canonical correlation analysis. Each dot is an individual participant. The color-coded crosses and ellipsoids show the centroids and ±1 SD for each subgroup.

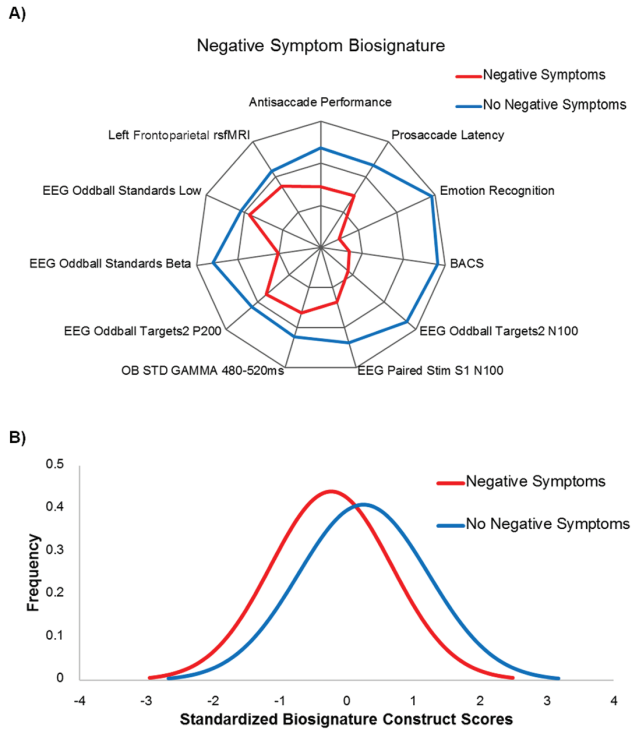

Fig. 3. Probands with negative symptoms are characterized by slow saccades, low cognitive performance, low EEG amplitudes, and deviant resting-state network activity. *Note*: The latent biosignature of negative symptoms includes poor antisaccade performance, poor emotion recognition, slow prosaccades, low BACS, low evoked-EEG amplitudes, and decreased frontoparietal rs-fMRI activity. BACS, Brief Assessment of Cognition in Schizophrenia; Paired Stim, auditory paired stimulus paradigm; S1, the first paired stimulus; OB STD, auditory oddball standard stimuli; Targets1/2, PCA component 1/2 for auditory oddball target stimuli.

Medication Effects. Of the 837 probands, 776 (92.7%) were actively treated with at least one psychotropic medication, including mood stabilizers and antidepressants in addition to antipsychotics (table 1). NS and non-NS probands had similar medication profiles, with 297 NS (92.2%) and 479 non-NS (93.0%) receiving at least one psychotropic agent. More NS (12.7%) were taking first-generation antipsychotics than non-NS (7.6%) (P = .05). NS probands (36.3%) were less likely than non-NS (49.7%) to be on mood stabilizers of any kind (P = .001). Inclusion of usage (on/off) of first-generation antipsychotics and mood stabilizers as a factor in the individual biomarker ANOVAs and CCA subgroup comparisons did not reveal significant effects related to medication status.

Discussion

We examined the associations between NS and neurobiological characteristics of brain function; identified specific biomarkers, indeed a biomarker fingerprint, differentiating individuals with predominant NS from those without; and demonstrated that two. The first goal was addressed using multivariate approaches across all psychosis probands, which found NS to have strong associations with low cognitive ability and aberrant neurophysiology, highlighting information and sensory processing as potential distinguishing alterations for NS. For the second goal, probands were divided into subgroups, NS and non-NS. The only individual phenotypes which differentiated NS from non-NS related to cognition, suggesting that NS may share a relatively direct (albeit modest) relationship with cognitive impairment in psychosis. In addition to these primary goals, we also assessed a number of outstanding issues from the NS literature, including whether NS items are individually informative or can be meaningfully combined into a NS "total" score, whether NS are secondary to depressive symptoms, and the extent of the relationship between NS and cognitive impairment.

Based on these analyses, the biomarker modalities most strongly associated with NS are cognitive measures and evoked-EEG; secondarily, resting-state EEG and fMRI; and finally, pro-/anti-saccades, emphasizing the importance of both sensory and information processing constructs for understanding negative symptoms. These biomarker associations were largely specific to NS and did not extend to positive symptoms (see supplemental materials). A consensus has formed around the main constructs to be included in the definition of NS–ie, alogia, blunted affect, anhedonia, asociality, and avolition.[4] Along with this, NS are commonly reported as an aggregate score, both in treatment trials and in clinical research. In contrast, our data show that individual NS items captured unique biomarker variance (see figure 1). As discussed below, NS are neurobiologically complex, and the individual NS constructs are distinct and informative.[104]

There is a growing consensus that, in terms of both phenomenology and co-occurrence, NS can be grouped into 2 domains, "avolition/apathy" (including avolition, asociality, and anhedonia) and "diminished expression" (including blunted affect and alogia).[8,26,28–30,104] Recent evidence suggests that the "avolition/apathy" domain may be linked specifically to goal-directed behavior and reward system dysfunction.[27,28,31] Our biomarker-based testing supports 2 separate NS domains, which were characterized by distinct biomarker profiles. "Avolition/apathy" scores were predicted by decreased delta-band rs-EEG activity, decreased evoked-EEG responses, and decreased ability to identify facial emotion, a critical aspect of social cognition. "Diminished expression" scores were predicted by poor emotion recognition, decreased evoked-EEG responses, and poor antisaccade performance. These observations suggest that battery of biomarkers will be required to fully characterize the biological underpinnings of NS, rather than any particular biomarker in isolation. For example, greater symptom severity in the "diminished expression" domain was

associated with lower evoked-EEG amplitude, lower cognitive performance, increased frontoparietal rs-fMRI activity, and poor antisaccade performance in the CCA analysis (figure 1). This was echoed in the follow-up discriminant analysis, which identified low cognitive performance, low evoked-EEG amplitudes, and deviant frontoparietal rs-fMRI as a biosignature of NS (figure 3). Conversely, the relationship between NS in the "avolition/apathy" domain was inconsistent with respect to their association with the various biomarker modalities (see figure 1). These results provide further evidence that neither one biomarker modality nor one biomarker itself adequately captures the full spectrum of symptomology. This also suggests that attempts to determine the latent structure of NS using only symptom scale information may miss important clinically relevant neurobiological information. One caveat is that recent factor analyses of the Scale for the Assessment of Negative Symptoms (SANS),[105] Brief Negative Symptom Scale (BNSS),[106] and Clinical Assessment Interview for Negative Symptoms (CAINS)[28] support a model with separate factors for the 5 domains of the NIMH consensus development conference (blunted affect, alogia, anhedonia, avolition, and asociality).[4,104,107] These scales include greater numbers of NS items than the PANSS, perhaps capturing an increased amount of symptom variance.[108] To our knowledge, there are no reports of 5-factor models using PANSS Negative data. Accordingly, the extent to which our findings would generalize if another NS scale were used is unclear. While algorithms for converting PANSS scores to SAPS/SANS have been proposed,[109] comparisons with newer scales which incorporate a more nuanced understanding of NS psychopathology (eg, CAINS or BNSS) are limited to summary scores in small samples.[106,108]

NS can be either a primary component of psychosis pathophysiology or secondary to other factors, such as depression, environmental factors, or antipsychotic medication, and they can also be either transient or enduring.[1,6,110] It is believed that while no currently available treatments are effective for primary or enduring NS, secondary symptoms, specifically those associated with depression, can be responsive to treatment.[1,111,112] Subgrouping individuals with schizophrenia based on this distinction has proven informative in a variety of contexts.[6,113–115] Three common approaches to NS study are (1) primary, enduring symptoms or "deficit symptoms"; (2) persistent symptoms, which may include treatment-resistant secondary NS; and (3) NS broadly construed, without differentiating primary from secondary symptoms.[6,38,112,113] As the PANSS does not distinguish between primary and secondary NS, the current analyses address NS broadly construed. Surprisingly, in the current study, mean PANSS Negative scores were not impacted by the inclusion of probands with different levels of depression severity (supplemental table 1). From a biological perspective, the body of NS may all converge on the same body of biological functions. Research on NS pathophysiology is surprisingly limited considering the urgent therapeutic need, although work on social cognition, reward processing, reinforcement learning, and oxytocin are promising.[116–119]

The only individual biomarkers that significantly differentiated NS from non-NS were emotion recognition, BACS, and the working memory span backward score, suggesting that overall NS severity may share a relatively direct relationship with cognitive impairment. While these NS-cognition associations were significant, the correlations were weak, indicating that poor cognition alone does not fully explain NS. The modest association between NS and cognitive impairment found here is consistent with other studies, suggesting that cognitive impairment accounts for only a small amount of the variance in NS. Nonetheless, the potential for medication effects is not easily controlled, and it is of potential relevance that a higher proportion of NS were medicated with first-generation antipsychotics and mood stabilizers, compared to non-NS. This cross-sectional study was not designed to examine medication effects in depth, and the effects of this sort cannot be ruled out completely. Future studies will be needed to examine distinctions in biomarker profiles associated with primary vs antipsychotics-related NS. The relationship between NS and white matter tract integrity also requires further study. Reports of the existence and location of effects are inconsistent, although this could potentially be due to methodological differences.[120–123] As a final caveat, the NS-cognition construct correlated with years of education (Pearson $r = .23$; $P < .001$). As NS probands had fewer years of education (see table 1), it is possible that the difference between these groups on the cognition construct might be confounded by education levels.

The findings from this investigation highlight the potential value of extensive biomarker batteries and integration across multiple levels of analysis for characterizing a neurobiologically complex clinical construct such as NS. While we observed complex relationships between NS and cognitive and neurophysiological measures, each biomarker modality was related to NS in distinct ways, suggesting that any one biomarker modality may not adequately capture the full spectrum of symptomology and that attempts to determine the latent structure of NS using only symptom scale information may be missing important clinically relevant information. While the multivariate nature of these analyses prohibited splitting our current sample, a larger independent replication sample (B-SNIP2) is in its final year of data collection. Our findings suggest that cognitive impairment, low evoked-EEG amplitude, slow saccades, and deviant resting-state activity may serve as a biosignature for the presence of NS. While the "diminished expression" and "avolition/

apathy" domains revealed distinct and condensed biomarker profiles, much more symptom variance was explained in those analyses which included NS as a complex set rather than as a summary score, highlighting the importance of multilevel integration of biomarker and clinical batteries.

Supplementary Material

Supplementary data are available at *Schizophrenia Bulletin* online.

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