Does Electroencephalogram Phase Variability Account for Reduced P3 Brain Potential in Externalizing Disorders?

A Thesis

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Scott J. Burwell

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Abstract

Background. Amplitude deficits of the P3 event-related brain potential (ERP) are associated with externalizing psychopathology but little is known about nature of the underlying brain electrical activity that accounts for this amplitude reduction. P3 amplitude is partially determined by electroencephalographic (EEG) frequencies in delta and theta bands and differences in phase-invariant stimulus-evoked energy and task-induced phase-locking associated with these frequencies may account for the P3-externalizing association.

Methods. Adult males (N = 410) completed a visual oddball task and frontal and parietal ERPs were analyzed as P3-related (300 to 600 milliseconds post-stimulus) evoked energy and inter-trial phase-locking measures in the delta (1 – 3.5 Hz) and theta-frequency (3.5 – 8 Hz) bands. We investigated how the delta and theta activity underlying P3 varies with disorders in the externalizing spectrum, including substance dependence, adult antisociality, and childhood disruptive disorders. We hypothesized that P3-related phase-locking is weaker in externalizing-diagnosed individuals and this might mediate prior findings of reduced evoked P3energy.

Results. Reductions in both evoked energy and phase-locking, in both frequency bands, at both scalp sites, were associated with greater odds of having an externalizing disorder. In most cases, adding phase-locking to evoked energy came with better prediction model fit. Moreover, reduced theta- but not delta-band phase-locking partially mediated the effects of within-frequency evoked energy on externalizing prediction.

Conclusions. These results suggest that phase-locking of inter-trial EEG during the P3 time-window is an important distinction between externalizing-prone individuals and

control subjects. This cross-trial phase-variability for externalizing-diagnosed individuals might reflect deficient top-down "tuning" by neuromodulatory systems.

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Introduction

Amplitude reduction of the P3 event-related potential (ERP) is well-documented in cases of alcoholism (1). In recent years, this deficit in P3 amplitude (P3AR) has been extended to a broader spectrum of "externalizing" psychopathology which includes substance use disorders (SUDs), antisociality, and attention-deficit hyperactivity disorder (2). A common externalizing dimension is thought to unite these disorders (3, 4) and accounts for their relationship with P3AR (5). This association between P3AR and externalizing is heritable (6, 7) and genetically mediated (8) and has been posited as an endophenotype for such disorders (9) whereby a biological measure (e.g., P3 amplitude) is thought to be closer to genetic influence than otherwise complex symptomatology (e.g., alcohol dependence). Although substantial clinical and epidemiological evidence exists demonstrating P3AR's robust association with externalizing disorders, of less focus remain the mechanics underlying this difference in brain response. The current study asks whether reduced P3-related phase-locking (arrhythmic brain responding) could account for differences between externalizing and control groups.

P3 is likely the most scrutinized ERP component but because it lacks rigorous neurobiological framework, interpretation of P3AR associated with externalizing psychopathology is complicated. Target P3 (or "P3b"; see 10) is commonly studied using a stimulus "oddball" task whereby participants are instructed to attend (e.g., button-press) to infrequent target stimuli and ignore frequent non-target stimuli. During the task, electroencephalogram (EEG) is recorded to measure the brain-generated electrical field at

numerous locations on the scalp. Fluctuations in this electrical field are driven by the synchronized activation of post-synaptic currents of pyramidal neurons in the cortex (11). Cross-trial averaging of stimulus-locked EEG produces a waveform characterized by three positive deflections; the third of these peaks (P3) occurs roughly 300 to 600 milliseconds following stimulus-onset. The augmentation of P3 is thought to reflect the arousal of neurotransmitter systems to promote attentional gain, stimulus evaluation, and response selection; therefore, decrements in P3 amplitude might mark deficiencies in these aspects of cognition (10, 12).

P3AR is classically studied in the time-domain by its trial-averaged amplitude or peak-latency (time-delay from stimulus). The convention of doing so adopts the "evoked" model of ERP generation which assumes that: 1) P3-voltage is fixed in polarity and latency relative to stimulus-onset (i.e. phase-locked), 2) there is an increase from post- relative to pre-stimulus EEG power, and 3) all non-phase-locked EEG is additive noise and suppressed by averaging (13). Indeed, evoked P3 has been tractable in the prediction of substance use and related disorders (1). Some have even extended time-domain evoked P3AR to the time-frequency (TF) domain which affords information about the ERPs energy at specific times and frequencies. For instance, evoked P3 is thought to be comprised primarily of superimposed delta (1 – 3 Hz) and theta (4 – 8 Hz) frequency oscillations (14-16). Our group and others have shown that parietal evoked delta underlying the time-domain P3 peak explains a large portion of the variance in P3AR's association with externalizing psychopathology (17-19). It is less clear how P3-related theta might contribute to this group difference. One study showed that theta-focused principal components derived from evoked ERPs are smaller for externalizing-

relative to control-subjects (20); however, others suggest that theta's contribution to P3 is more complex than what can be observed in the evoked ERP (17, 18, 21). Because the vast majority of studies investigating P3AR focus exclusively on evoked P3, the working-conclusion that can be made is that on average, externalizing-prone individuals respond with smaller-voltage P3s to the stimulus on each trial.

Temporal shifts in ERP peaks from trial-to-trial (i.e. "latency jitter" or more generally, a reduction in "phase-locking") can also diminish their trial-averaged voltage (22-24). We posit that the temporal onset and offset of P3-related delta and theta components may be more variable across trials in externalizing-diagnosed individuals relative to controls and this heightened "ERP arrhythmia" could be partly responsible for P3-reductions seen in externalizing-prone individuals. This phenomenon would be characterized by a reduction in inter-trial phase-locking for the externalizing group and is not directly measured by evoked P3. Recent work has supported this notion (25, 26) and investigating this idea more fully could lend to our understanding of the pathophysiology involved in P3AR.

Extending an earlier report (20), the present study extracted evoked energy and inter-trial phase-locking ERP components temporally-corresponding to the P3 peak in delta- and theta-frequencies to predict lifetime diagnoses of externalizing disorders. Because amplitude and frequency characteristics of evoked ERPs rely on phase-locked voltage of trial-level EEG (13), we sought to determine if a reduction in inter-trial phase-locking could account for P3-related energy reductions.

Methods and Materials

Participants

Participants were drawn from the older cohort of the Minnesota Twin Family Study, a longitudinal community-based sample studying twins and their parents (explained further in 27). This cohort was initially assessed when the twins were approximately 17 years of age and is almost entirely Caucasian (over 95%; consistent with the demographics for the state of Minnesota at the time the twins were born). At approximate ages of 17, 20, 24, and 29, participants underwent a battery of clinical interviews and psychophysiological measurements. The age-29 assessment included higher-density EEG recording than previous ones. Only participants who visited in-person and had EEG data at their age-29 visit were considered for the present study (N = 434, 199 twin pairs, 36 unmatched twins, mean age = 29.6, standard deviation = 0.6, age range = 28.5 – 31.9).

Diagnostic assessment/groups

At each assessment, each twin was interviewed individually by a trained clinical interviewer. Because P3AR has been shown to persist through adolescence and into adulthood (2, 20) and we wanted to identify all cases of externalizing psychopathology, the present study constructed diagnostic groups based on *lifetime diagnosis of an externalizing disorder*. Those who received a disorder diagnosis at any one of our four assessments were given a lifetime diagnosis for that disorder. Externalizing diagnoses of attention-deficit hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), conduct disorder (CD), adult antisocial behavior (AAB), nicotine dependence (NicD), alcohol dependence (AlcD), and illicit drug dependence (DrgD) were based on criteria from the *Diagnostic and Statistical Manual of Mental Disorders*, third edition, revised (DSM-III-R; 28). To simplify grouping, DrgD was collapsed into one group that

included a substance dependence diagnosis of any of the following psychoactive substance classes: amphetamines, cannabis, cocaine, hallucinogens, inhalants, opioids, phencyclidine, and sedatives. Childhood disruptive disorders (CDDs; ADHD, ODD, CD) were only assessed at the twins' intake assessment whereby twins and mothers (asked in pertinence to the twins' behavior) reported symptoms for these disorders in accordance with the Diagnostic Interview for Children and Adolescents (29, 30) child- and parent-versions, respectively. To ensure diagnostic certainty, a "best-estimate" approach combining twin and mother reports was adopted (31, 32). All other disorders (AAB, NicD, AlcD, DrgD) were assessed at all four visits. AAB was diagnosed if an individual met *DSM-III-R* criteria for antisocial personality disorder but did not necessarily qualify the presence of childhood history of CD (cf. 33). The presence of SUDs (NicD, AlcD, DrgD) was assessed using the expanded substance abuse module (34) as they pertained to lifetime (intake and second visits) and since-last-visit (third and fourth visits) experience. Clinical reports were independently reviewed by at least two individuals with advanced clinical training (Cohen's κ's > 0.7) (35) who whenever they did not score a symptom identically, reviewed the symptom together to achieve consensus . The comparison group for the present study consisted of those who had not met complete diagnostic criteria for any of the above disorders at any of the four assessments nor possible *DSM-III-R* substance abuse.

ERP task

The rotated-heads oddball paradigm (see Supplement 1; 36) used in the current study is a complex visuo-spatial task which has been repeatedly shown to differentiate

externalizing subjects and non-externalizing controls (2, 37). The current study focused solely on ERP data from the target condition, as consistent with prior research.

EEG data acquisition

Continuously recorded EEG (61 sensors, 10/20 placement) data were digitized at 1024 Hz with a passband of DC to 205 Hz using the BioSemi ActiveTwo recording system (BioSemi, Amsterdam, Netherlands). Two additional reference electrodes were placed on either earlobe. Vertical and horizontal ocular movements were monitored by two electrodes placed superior and inferior to the right eye and two electrodes placed on left and right temple, respectively.

EEG processing

Data for 12 subjects could not be used because equipment failure resulted in the loss of event triggers. The 422 remaining EEGs were processed offline in MATLAB (version 7.8, Mathworks Inc.) using the EEGLAB toolbox (version 9) (38) and an automatic routine developed by the first two authors that integrates artifact-pruning elements from published data pipelines (39-41). We adopted the same general approach to Nolan *et al.* (41) in that we identified artifact at a progressively finer scale, successively pruning artifact-contaminated data by examining, in order, 1) electrode, 2) time segment, 3) temporal and spatially stereotyped ocular activity (cf. 40), and finally 4) electrode/time segments. As in existing EEG processing pipelines, noise (i.e., artifact) was identified as data segments that exceeded some threshold value (e.g., 3 *SD*s) of the empirical distribution of descriptive properties (e.g., temporal deviation, maximum difference between two time-points) derived for contiguous 1-second segments of multiple-electrode EEG. The threshold was based on a robust measure of spread, the

normalized median absolute deviations (*nMADs*; 42) to minimize the influence of outliers on the thresholds used to identify them. See **Supplement 1** for details.

A total of seven cases (1.6%) were dropped as a result of automatic processing. Five additional cases (1.2%) were dropped because visual inspection (by a trained psychophysiologist) of averaged ERPs indicated irreparable drift in the vast majority of trial-epochs. This resulted in a final sample of 410 (94.5 %) cases of analyzable EEGs with an average of 69.6 (*SD* = 10.4) artifact-free trials per subject.

ERP component extraction

Energy and inter-trial phase-locking measures for the target condition were computed with custom MATLAB scripts maintained by the third author (43). We represented the average ERP in terms of its TF energy to evaluate the ERP's intensity at specific times and frequencies. This was done with the binomial TF-transform reduced interference distribution (RID) belonging to Cohen's class (43). An advantage of the RID for the current application is its uniform TF resolution, which prevents the time-smearing at lower frequencies common in widely-used wavelet transforms. Each subject-electrode's degree of phase-locking to the stimulus across trials (independent of energy) was quantified in terms of the "phase-locking factor" (PLF, also called "inter-trial phase coherence"; 38, 44)[,] [which] ranges from zero, indicating randomness of phases across trials, to one, indicating perfect phase-locking across trials. Most previous applications of the PLF with EEG data have employed wavelet transforms (44-46), but to circumvent the time-smearing associated with wavelets, we used a recently developed complex RID based on the Cohen's class Rihaczek distribution (47) that is robust to time-smearing (48) and can quantify phase-locking.

Once evoked TF energy (hereinafter denoted with "$_{EVK}$") and inter-trial phase-locking factor ("$_{PLF}$") were computed for each subject-electrode, measures were baseline corrected within frequency-band by subtracting the pre-stimulus mean (47, 49) within a -1000 and -1 millisecond window. Component scores were extracted as mean values within delta (1 – 3.5 Hz) and theta (3.5 – 8 Hz) bands spanning the window of 300 to 600 milliseconds following stimulus-onset. This time range encapsulates the grand-averaged time-domain P3 component for the current sample and is widely used (10, 12).

Statistical analyses

Generalized estimating equations (GEEs) were computed in R (version 2.13.1) using the function *geeglm* from the "geepack" package, modeling within-family correlation as exchangeable to account for the lack of independence within twin pairs. Externalizing diagnoses (1 = disorder present, 0 = control) were first regressed onto $delta_{EVK}$, $theta_{EVK}$, $delta_{PLF}$, and $theta_{PLF}$ as separate predictors and then again with within-band (e.g., $theta_{EVK}$ and $theta_{PLF}$) components in the same model as predictors, which permitted us to assess the degree to which PLF within a frequency band might mediate any associations between reduced EVK in that band and externalizing diagnoses. For all models, a form of the Quasi-likelihood under the Independence model Criterion (QIC; 50) was computed using the R package "MESS." Designed as a goodness of fit statistic for GEE models, the QICu used here is appropriate for comparing models with the same correlation structure. When comparing QICu values from two models, it can be inferred that the model with a smaller QICu accounts more adequately for the relationship between predictor(s) and outcome. Moreover, in comparing models with different numbers of predictors, the QICu for the more complex model is penalized for

added predictors; this balances the trade-off between goodness of fit and model-complexity. For instance, when we add PLF to EVK in our models, a smaller value of QICu relative to the simpler EVK-only model would indicate that adding PLF to the model effectively offsets the penalty incurred by having added a second parameter, leading one to infer that the more complex model accounts for the data better than the simpler one.

Most research showing P3AR with externalizing-diagnoses is based on the evoked ERP, which is equivalent to the phase-locked energy associated with all the frequencies comprising P3. In a mediation framework this can be thought of as the "total effect" of EVK on externalizing, corresponding to path *c* in **Figure 1A**. Theoretically, a high-degree of PLF is necessary for large values of EVK, but large values of EVK are not necessary for a high-degree of PLF. Hence, we tested the mediation model proposed in **Figure 1B** where the "total effect" has been partitioned into "direct" (path *c'*) and "indirect" (paths *a* and *b*) effects. Path *c'* is computed as the difference between the total effect of EVK (as in **Figure 1A**) and its indirect effect through PLF, which is the product of *a* and *b*. PLF is considered to partially mediate the relationship between EVK and externalizing if a significant reduction in the magnitude of *c* to *c'* occurs; or equivalently, if the indirect effect *ab* is significantly different from zero. See **Supplement 1** for details of the bootstrapping mediation method used.

Results

Analytic ERP/diagnostic data

The final diagnostic groups for analysis resulting from EEG processing and diagnostic inclusion/exclusion criteria are as follows: ADHD (n = 27), ODD (n = 43), CD

(*n* = 119), AAB (*n* = 63), AlcD (*n* = 159), NicD (*n* = 159), DrgD (*n* = 73), and externalizing diagnosis-free controls (*n* = 93). Composite variables were also made based on the presence of any childhood disruptive disorder (Any-CDD; *n* = 138), any substance use disorder (Any-SUD; *n* = 226), or any of the seven disorders studied here (Any-EXT; *n* = 251). Sixty-six individuals with usable ERP data did not meet inclusion criteria for either diagnostic or control groups (e.g., those without history of definite externalizing disorder but with a diagnosis of substance abuse). However, a recent paper by Yoon *et al.* (20) using the same sample and assessment with identical exclusion criteria has shown that included and excluded individuals did not differ based on P3 amplitude or externalizing psychopathology at their original (age-17) assessment.

Task performance

No significant task performance measures (errors of commission, reaction time) differences existed between diagnostic and control groups (Fs < 1, p's > .3).

Evoked energy and phase-locking factor associations with P3 amplitude

Figure 2A displays group-averaged time-domain voltage waveforms at the parietal site for all externalizing-diagnosis groups and controls. The adjacent head-plot displays the topographical distribution of grand-average P3 scores (mean voltage between 300 and 600 milliseconds) for all subjects. The grand-average evoked energy (EVK) transform (i.e. average TF representation of time-domain ERPs) is presented alongside topographical distributions for corresponding delta and theta scores in **Figure 2B** windows for scores (denoted by dotted boxes). TF pixels with the largest values ("hottest" colors) are consistent with time-periods where the voltage in **Figure 2A** is greatest. Likewise, **Figure 2C** shows the grand-average phase-locking factor (PLF)

loadings and topographical distributions for delta and theta scores. Here, TF pixels that are "hottest" reflect time-points/frequencies relative to the stimulus where cross-trial ERP phase is most consistent; similar to the EVK grand-average, PLF pixels of the largest value line-up with the P3-peak. Generally, grand means for delta were larger than those for theta, as reflected by the less intense heat-maps for theta relative to delta (which were plotted using the same color scale). Scores for subsequent analyses were aggregated across five electrodes in midline-frontal (electrode sites AFZ, FZ, F1, F2, and FCZ) and midline-parietal (CPZ, PZ, P1, P2, and POZ) regions.

Predicting externalizing diagnoses with EVK and PLF

Figures 2D, 2E, and **2F** display group differences between the Any-EXT and control groups on measures shown in **Figures 2A**, **2B**, and **2C**, respectively. Here, "cooler" colors indicate topographical regions where reductions in externalizing groups' amplitude, EVK, and PLF scores relative to controls are greatest. It can be seen that for all head-plots in **Figure 2D**, **2E**, and **2F**, the Any-EXT group shows a reduction in voltage, EVK, or PLF in delta- and theta-bands in comparison to controls and these reductions are generally greatest in the parietal region. This relationship between externalizing and reduced EVK and PLF is further reflected by the negative regression coefficients and odds ratios (ORs) > 1 in each of the logistic models displayed in **Table 1**.

The left side of **Table 1** indicates that for the frontal region, with one exception (DrgD), $delta_{EVK}$ was always associated with smaller values of QICu than $delta_{PLF}$, indicating that frontal EVK better accounts for the relationship between P3-related delta and externalizing diagnoses than frontal PLF. However, the obverse was true for P3-

related frontal theta: with the exception of ADHD and ODD, PLF accounted for more variance in externalizing disorders than EVK. Several effects were statistically significant. One standard deviation *decrease* in $delta_{EVK}$ was associated with a twofold *increase in the odds* for ADHD as well as increased odds for ODD and this was also true for the Any-EXT composite. No $delta_{PLF}$ effects were significant, but $theta_{PLF}$ was significantly associated with all disorders, except ADHD and ODD.

The same analyses were carried out for scores at the midline-parietal region (**Table 1**, right). Comparing delta components, for disinhibitory disorders (ADHD, ODD, CD, AAB), EVK was always associated with smaller QICu than PLF, indicating that EVK was the better predictor of these disorders. The opposite relationship was evident for SUDs (NicD, AlcD, DrgD, Any-SUD) and Any-EXT; PLF accounted for more variance in diagnoses than did EVK. Turning to theta at the parietal site, across the board, $theta_{PLF}$ prediction models were associated with better model-fit than $theta_{EVK}$, suggesting that $theta_{PLF}$ more strongly predicts all of the externalizing disorders. Additionally, the ORs for PLF were significant for all of the individual disorders and composite diagnostic groups, while EVK ORs was significant in nearly all cases. Overall, one standard deviation decrease on $delta_{EVK}$, $delta_{PLF}$, $theta_{EVK}$, or $theta_{PLF}$ was associated with a 34% to 44% increase in odds for having an externalizing diagnosis (Any-EXT).

Does adding PLF to EVK improve goodness of fit in predicting externalizing disorders?

An important consideration is whether PLF adds sufficiently to the prediction of externalizing above and beyond EVK to justify the increase in model complexity that comes with adding another predictor. When PLF as well as EVK were entered into a dual

predictor model, for frontal delta, QICu values for the dual predictor model were smaller than those for the EVK-only model for most disorders (see **Table 2** for change in QICu values), despite frontal $delta_{PLF}$ having no significant regression coefficients in **Table 1**. For frontal theta, QICu values for the dual predictor model were always smaller than for the EVK-only model. With one exception (predicting AAB from delta), the dual predictor model at the parietal site resulted in an improvement in fit over the EVK-only model. To summarize, 40 model fitting tests were carried out to see if a model that included both PLF and EVK predicted externalizing better than one using EVK alone, and in 88% (35/40) of the comparisons, the dual predictor model fit was better, pointing to the importance of PLF over and above EVK alone to the prediction of externalizing.

Does PLF mediate the effects of EVK?

Also of interest was whether the association between reduced energy and externalizing disorders was mediated by a reduction in the phase-locking of EEG activity. To simplify this analysis, we carried out these mediation analyses using the model in **Figure 1** at the parietal site (which produced stronger effects than the frontal site) for the three composite disorders. We first examined results for delta and found that path *c'* was not significantly smaller than path *c* and no indirect effect (path *ab*, **Figure 1B**) was detected, thus failing to provide evidence that for delta, the EVK effect was mediated by PLF. As illustrated in **Figure 3**, mediation effects were observed for theta. The total effect of $theta_{EVK}$ (path *c*) became non-significant (path *c'*) while the effect of $theta_{PLF}$ (path *b*) remained significant in models predicting Any-SUD and Any-EXT. This mediating role of $theta_{EVK}$ by $theta_{PLF}$ (path *ab*) was also significant for these two models (and trend-level for Any-CDD, $p < .10$), suggesting that a reduction in P3-related theta

phase-locking at the parietal site may partially explain the total effect of reduced theta energy on externalizing diagnoses.

Discussion

The present study examined how task-induced inter-trial phase-locking relates to externalizing disorders and tested the hypothesis that reduced inter-trial phase-locking contributes to the association between reduced P3-related evoked energy and externalizing psychopathology. Energy and phase-locking scores in delta and theta frequencies corresponding to P3 peak-latency were smaller for externalizing-diagnosed individuals in comparison to controls. To the best of our knowledge, this is the first report demonstrating that reduced phase-locking was a significant predictor of externalizing disorders. Additionally, by using both evoked energy and inter-trial phase-locking in the same model to predict externalizing diagnoses (rather than evoked energy by itself), we accounted for more variance in externalizing diagnoses. In some cases (e.g., frontal delta) this improvement in fit was small whereas in others (e.g., theta), it was larger (see **Table 2**). Moreover, as we showed for the three composite disorders, where theta-band associations between evoked energy and externalizing disorders were significant, theta phase-locking mediated this association to non-significance. This was not the case for delta-band evoked energy and phase-locking.

We show that externalizing disorders are associated with high variability of delta- and theta-frequency EEG phase (trial-to-trial "arrhythmia") during the P3 time-window. Inter-trial phase-locking is thought to index an energy-efficient mechanism of signal integration and ERP generation. When inter-trial phase-locking is high, it is believed that neuromodulatory system properties are well-suited to facilitate the temporal "tuning" of

ongoing activities without requiring an increase in EEG power (i.e. recruitment of additional neurons) (52). This kind of "top-down" neuromodulatory event is typical of γ-aminobutyric-acid releasing (GABAergic) inhibitory interneurons that are capable of synchronizing the ongoing rhythms of many cortical pyramidal neurons (53). A difference in GABAergic expression has been posited to have a role in the development of alcoholism and associated EEG abnormalities (54) and some evidence links GABA activity to changes in P3 amplitude (55). Additionally, cholinergic and glutamatergic systems have been implicated in P3-related delta and theta as well as alcoholism (56-58). Perhaps by understanding the *interaction* between excitatory (e.g., cholinergic, glutamatergic) and inhibitory (e.g., GABAergic) neurochemical processes, better scaffolding of externalizing-related pathophysiology might be achieved.

These findings were limited to externalizing disorders and do not rule out the possibility that similar effects might also be observed with other clinical diagnoses where P3 amplitude is abnormally low such as major depression (59-61) or schizophrenia (62, 63). Yet, viewing P3 in terms of its evoked energy and phase-locking dynamics has the potential to uncover key similarities and distinctions across multiple disorders that could better inform researchers as to the relevant neurobiological factors at play in psychopathological etiology. By combinative use of multiple P3-related measures ("multivariate endophenotype"; cf. 6, 64), perhaps greater specificity and statistical power in prediction of certain diagnoses can be achieved.

Table 1. Externalizing diagnoses regressed onto single predictors of energy or phase-locking

	Frontal site						Parietal site					
	$Delta_{EVK}$			$Delta_{PLF}$			$Delta_{EVK}$			$Delta_{PLF}$		
Diagnosis	B (SE)	OR	QICu	B (SE)	OR	QICu	B (SE)	OR	QICu	B (SE)	OR	QICu
ADHD	-4.0 (1.4)	2.06 **	**357.8**	-6.8 (3.7)	1.50	362.8	-3.1 (1.2)	2.19 *	**358.6**	-6.0 (2.8)	1.61 *	363.1
ODD	-1.6 (0.8)	1.33 *	**437.0**	-2.0 (2.0)	1.13	440.0	-2.5 (0.8)	1.86 **	**427.8**	-7.0 (1.9)	1.74 **	429.0
CD	-1.1 (0.8)	1.21	**709.7**	-2.7 (1.8)	1.18	710.6	-1.6 (0.7)	1.51 *	**706.3**	-5.1 (1.8)	1.51 **	706.7
AAB	-0.8 (0.8)	1.16	**520.8**	-2.8 (2.3)	1.19	521.2	-2.6 (0.8)	1.85 **	**510.4**	-7.5 (2.2)	1.81 **	513.0
NicD	-0.9 (0.6)	1.16	**832.9**	-1.0 (1.9)	1.06	834.9	-1.8 (0.6)	1.56 **	821.7	-5.5 (1.7)	1.53 **	**820.4**
AlcD	-1.0 (0.7)	1.20	**832.8**	-2.0 (2.1)	1.12	834.4	-0.9 (0.5)	1.27	831.9	-4.2 (1.8)	1.38 *	**830.1**
DrgD	-1.1 (0.7)	1.22	558.7	-4.5 (2.5)	1.32	**558.3**	-0.6 (0.6)	1.17	558.7	-4.7 (1.8)	1.46 *	**554.4**
Any-CDD	-1.2 (0.7)	1.23	**768.2**	-2.8 (1.8)	1.18	768.9	-1.6 (0.6)	1.50 *	**763.8**	-5.3 (1.7)	1.54 **	763.9
Any-SUD	-1.1 (0.6)	1.22	**1018.8**	-3.1 (2.0)	1.19	1020.0	-1.3 (0.5)	1.39 **	1015.0	-4.7 (1.6)	1.44 **	**1013.9**
Any-EXT	-1.3 (0.6)	1.25 *	**1084.5**	-3.2 (2.0)	1.20	1085.9	-1.3 (0.5)	1.39 *	1081.3	-4.6 (1.6)	1.44 **	**1080.4**
	$Theta_{EVK}$			$Theta_{PLF}$			$Theta_{EVK}$			$Theta_{PLF}$		
Diagnosis	B (SE)	OR	QICu	B (SE)	OR	QICu	B (SE)	OR	QICu	B (SE)	OR	QICu
ADHD	-10.1 (4.7)	1.66 *	**362.6**	-9.7 (5.9)	1.47	363.4	-15.7 (6.4)	2.12 **	358.2	-14.1 (3.9)	2.16 **	**354.2**
ODD	-7.3 (4.8)	1.43	**435.0**	-6.3 (3.4)	1.29	438.2	-11.0 (4.3)	1.67 *	430.5	-9.9 (2.5)	1.71 **	**424.8**
CD	-2.5 (1.9)	1.16	713.4	-5.5 (2.8)	1.24 *	**710.2**	-7.4 (3.1)	1.40 *	706.8	-5.8 (2.1)	1.37 *	**705.7**
AAB	-4.9 (3.2)	1.29	520.8	-8.0 (3.7)	1.37 *	**519.8**	-10.1 (4.2)	1.60 *	516.1	-8.2 (2.9)	1.57 *	**514.7**
NicD	-5.8 (2.3)	1.31 *	827.8	-8.4 (3.0)	1.36 **	**826.6**	-9.6 (3.1)	1.49 **	822.6	-8.4 (2.3)	1.56 **	**816.5**
AlcD	-5.0 (2.7)	1.26	829.5	-9.4 (3.5)	1.43 **	**828.2**	-6.7 (2.9)	1.35 *	828.4	-7.0 (2.4)	1.46 **	**824.9**
DrgD	-4.8 (2.9)	1.26	556.6	-9.5 (3.6)	1.46 **	**552.5**	-6.3 (3.6)	1.33	556.1	-6.6 (2.8)	1.45 *	**552.7**
Any-CDD	-3.6 (2.2)	1.23	771.2	-7.2 (2.9)	1.33 *	**766.5**	-8.8 (3.2)	1.49 **	762.9	-6.9 (2.3)	1.45 **	**761.0**
Any-SUD	-4.0 (2.2)	1.21	1017.4	-8.5 (3.0)	1.38 **	**1014.4**	-6.8 (2.6)	1.34 *	1014.3	-7.0 (2.2)	1.46 **	**1009.8**
Any-EXT	-3.2 (2.1)	1.19	1086.1	-7.6 (2.9)	1.34 *	**1081.9**	-6.6 (2.6)	1.34 *	1081.8	-6.8 (2.1)	1.44 **	**1077.0**

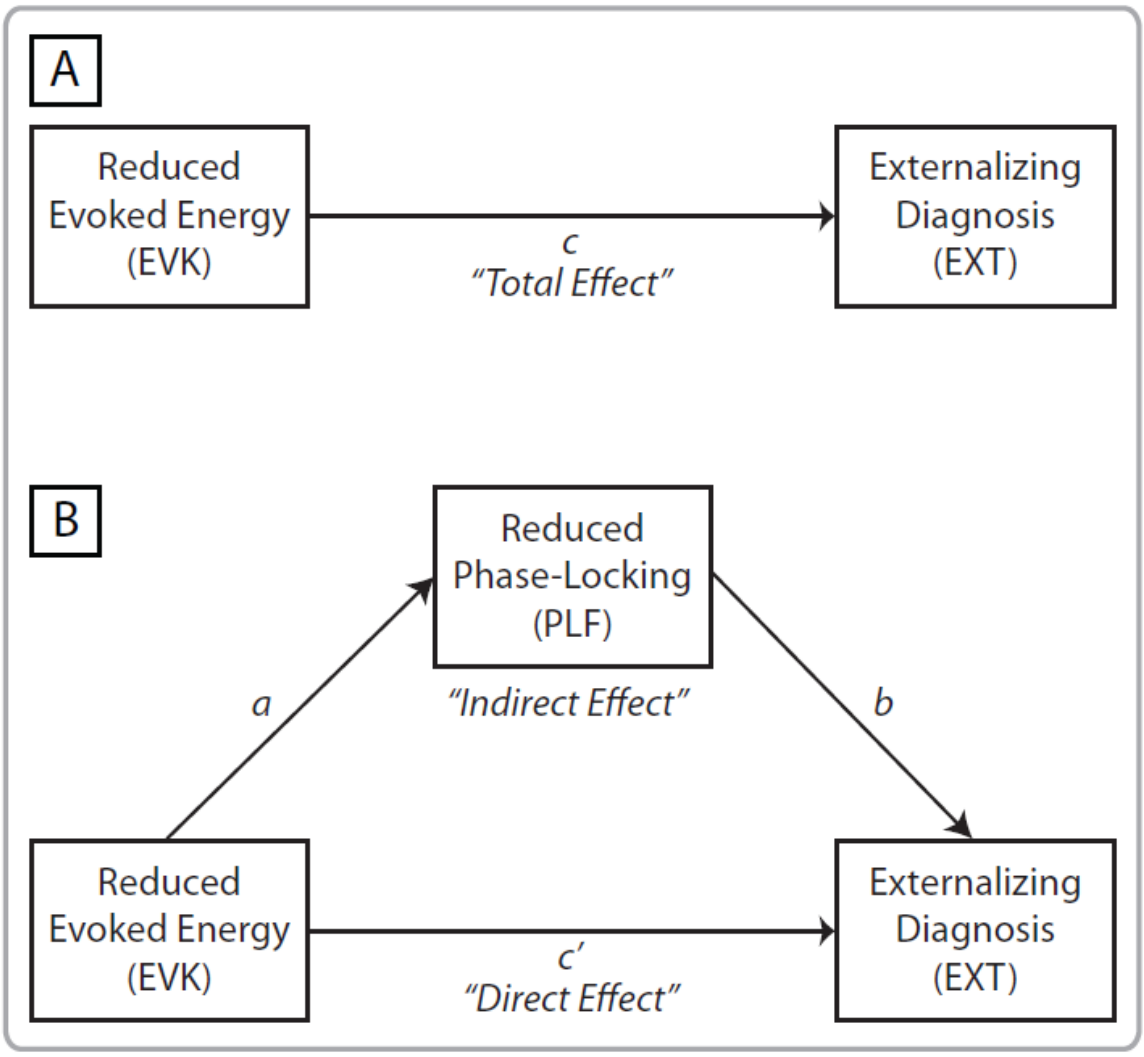
Logistic regressions for delta (1 – 3.5 Hz) and theta (3.5 – 8 Hz) evoked energy (EVK) and phase-locking factor (PLF) at frontal and parietal electrode sites in prediction of attention-deficit hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), conduct disorder (CD), adult antisocial behavior (AAB), nicotine dependence (NicD), alcohol dependence (AlcD), and illicit drug dependence (DrgD) diagnoses and composites of any childhood disruptive disorder (Any-CDD), any substance use disorder (Any-SUD), and any externalizing disorder (Any-EXT). Negative regression coefficients (Bs) reflect smaller EVK or PLF values for externalizing groups. Odds ratios (ORs) reflect *increased odds* of disorder *per one standard deviation decrease* on the predictor; this was done by inverting the exponentiated product between the standard deviation of the predictor and its corresponding B. The Quasi-likelihood under the Independence model Criterion (QICu) can be compared across rows within the same quadrant of the table (e.g., ADHD for frontal $delta_{EVK}$ versus $delta_{PLF}$); smaller values of QICu (bolded) indicate better model-fit. **p* < .05, ***p* < .01

Table 2. Change in model fit by adding phase-locking factor to evoked energy.

	Frontal site (ΔQICu)		Parietal site (ΔQICu)	
Diagnosis	Delta	Theta	Delta	Theta
ADHD	0	-1.0	-0.2	-5.6
ODD	-0.1	-0.3	-0.8	-7.1
CD	-0.3	-3.0	-0.9	-2.6
AAB	0.1	-1.3	0.1	-2.7
NicD	0.2	-3.4	-3.0	-8.2
AlcD	0	-3.0	-1.9	-4.2
DrgD	-0.3	-4.2	-5.2	-3.5
Any-CDD	-0.2	-4.4	-1.2	-3.7
Any-SUD	-0.1	-3.9	-2.0	-5.2
Any-EXT	-0.1	-4.4	-1.9	-5.2

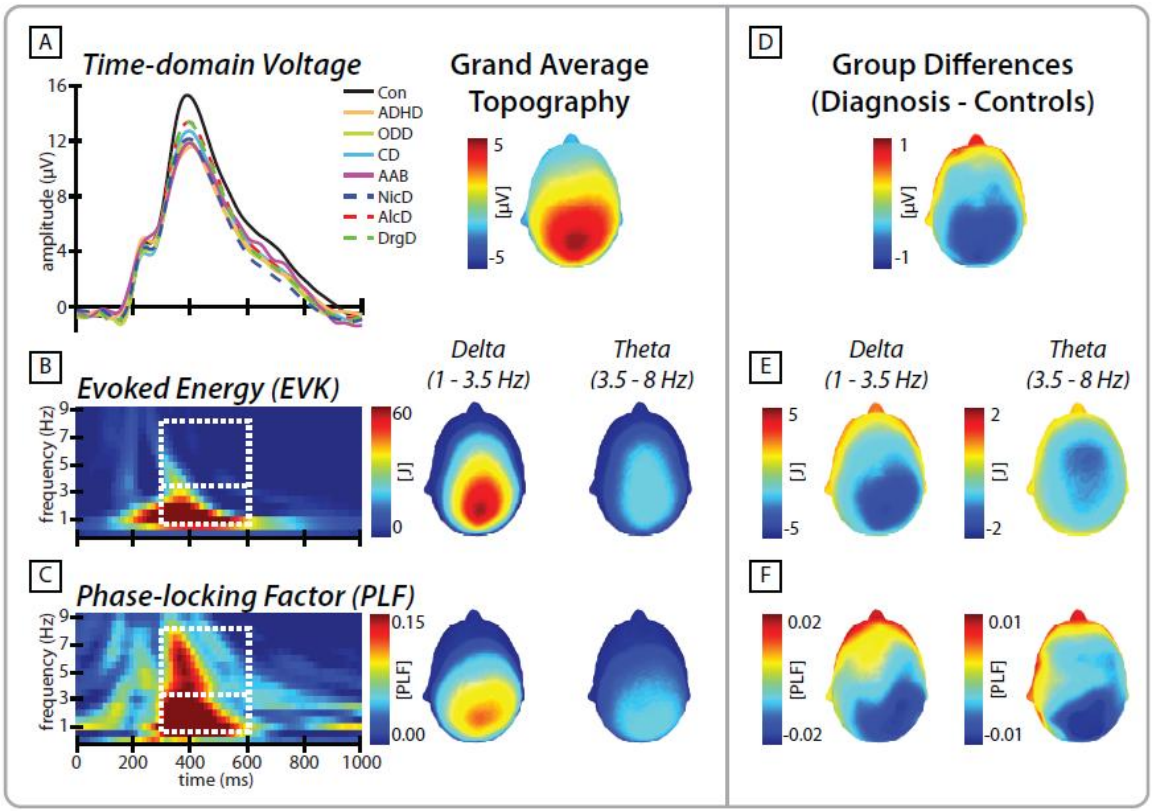
Difference in Quasi-likelihood under the Independence model Criterion (ΔQICu) between prediction of externalizing diagnoses using evoked energy (EVK) on its own and using EVK in combination with phase-locking factor (PLF) to predict externalizing diagnoses. Negative values of ΔQICu indicate that using both within-frequency EVK and PLF as dual predictors of externalizing disorders provides improved model-fit (i.e. less information loss) over solely using EVK.

Figure 1. Phase-locking mediation model.



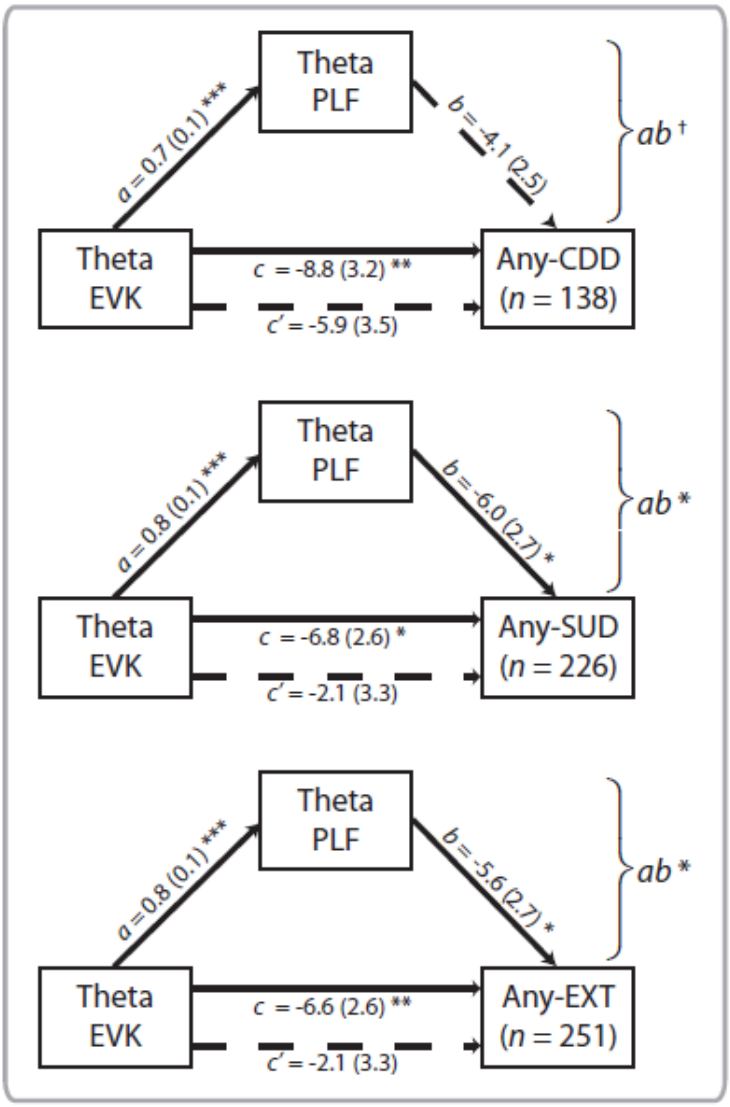
Most research showing P3-amplitude reductions with externalizing-diagnoses (EXT) uses the evoked event-related potential, which is composed of phase-locked energy (EVK). In a mediation framework, the contribution attributable to P3 amplitude can be thought of as the "total effect" of EVK on EXT, corresponding to path *c* in model **A**. Theoretically, a high-degree of phase-locking factor (PLF, phase-locking independent of energy) is necessary for large values of EVK, but large values of EVK are not necessary for a high-degree of PLF. This is reflected in the mediation model **B** where the total effect, has been partitioned into "direct" (path *c'*) and "indirect" (paths *a* and *b*, or *ab*) effects. Path *c'* is the difference between the total effect of EVK and the indirect effect through PLF, which is the product of *a* and *b*. Partial mediation is inferred when a reduction in the magnitude of *c* to *c'* has occurred; or equivalently, an indirect effect *ab* is significantly different from zero.

Figure 2. Grand averages, topographical distributions and group-differences



Grand averages, topographical distributions and group-differences. **A**) Time-domain group-averaged voltage waveforms at the parietal site for attention-deficit hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), conduct disorder (CD), adult antisocial behavior (AAB), nicotine dependence (NicD), alcohol dependence (AlcD), illicit substance dependence (DrgD), and externalizing diagnosis-free controls (Con). The adjacent head-plot displays the topographical distribution of grand-average P3 scores (mean amplitude between 300 and 600 ms) for all subjects. **B**) Grand-average time-frequency evoked energy (EVK; in Joules, or J) transform of the time-domain voltage waveforms and topographical distributions for mean P3-corresponding delta (1 – 3.5 Hertz, 300 – 600 milliseconds) and theta (3.5 – 8 Hertz, 300 – 600 milliseconds) scores (windows for time-frequency scores denoted by dotted boxes). **C**) Grand-average phase-locking factor (PLF) loadings and topographical distributions of delta and theta scores. Figures **D**, **E**, and **F** display group differences between (any disorder) Any-EXT and control groups on measures displayed in **A**, **B**, and **C**, respectively; "cooler" colors indicate topographical regions where reductions in externalizing groups relative to controls are greatest.

Figure 3. Theta phase-locking partially mediates the pathway between evoked energy and externalizing prediction.



Theta phase-locking partially mediates the pathway between evoked energy and externalizing prediction. Mediation models with parietal theta-frequency evoked energy (EVK) and phase-locking factor (PLF) as dual predictors of externalizing composites for any childhood disruptive disorder (Any-CDD), any substance use disorder (Any-SUD), and any externalizing disorder (Any-EXT). The total effect of EVK on diagnostic composites (path *c*) became non-significant (path *c'*) while the effect of PLF remained significant in models predicting Any-SUD and Any-EXT. This mediating role of EVK by PLF (i.e. the indirect path *ab*) was also significant for these two models (and trend-level for Any-CDD), suggesting that a reduction in P3-related theta phase-locking partially explains the total effect of reduced theta energy on externalizing diagnoses. ***$p < .001$, **$p < .01$, *$p < .05$, $^{\dagger}p < .1$ for path coefficients.

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Appendix A

Supplementary Methods

Rotated-heads oddball task. Stimuli consisted of four infrequently occurring target stimuli (n = 80) interspersed with one frequently occurring non-target stimulus (n = 160) presented in pseudo-random order. Target stimuli resembling "heads" were presented as an oval with one triangular "nose" appearing on either the top (oriented up) or bottom (oriented down) of the oval and one "ear" appearing on either the left or right side of the same oval. The non-target stimulus was a simple oval of the same size and shape without nose or ear. Sitting in a sound-attenuated dimly-lit room, subjects were instructed to press a button on the left or right armrest of a chair (indicating which side of the stimulus "head" the "ear" appears on) to target stimuli and ignore non-target stimuli. For half of target stimuli, the head was oriented towards the top of the screen (response-hand is congruent with ear-position). For the other half of target stimuli, the head was rotated 180° (response-hand is incongruent with ear-position). These two stimulus manipulations are regarded as "easy" and "difficult" trial-conditions, respectively.

EEG processing. For each subject, continuous EEG was downsampled to 256 Hz and highpass-filtered with a windowed sinc filter (0.1 Hz cutoff, Kaiser window, order of 1286). Gross artifacts, defined as electrode segments in which more than 75% of the samples were contaminated or time segments in which more than 15% of the electrodes were contaminated, were deleted before data were re-referenced to the averaged potential of the earlobe electrodes and lowpass-filtered to remove high-frequency noise (30 Hz cutoff, Kaiser window, order of 644). Artifact-pruned and re-referenced EEG was decomposed with Infomax independent component (ICA) analysis (1) for the purpose of correcting blinks and ocular artifacts. Each component's time course and topographic distribution (inverse-weights) were correlated with the time course of a criterion channel (bipolar vertical electrooculogram, or EOG) and a spatial template (typical IC inverse-weights of a blink IC), respectively. ICs with squared correlation-coefficients exceeding threshold derived empirically using an expectation maximization algorithm (2) were considered to represent blink activity and subtracted from the data. Horizontal eye movement artifacts were corrected in a similar manner but with a bipolar horizontal EOG as the signal. Epochs spanning -2 to 2 seconds relative to stimulus-onset were assessed for artifact using similar criteria as in our initial step. Artifact-contaminated electrodes or electrode/epochs were interpolated using a spherical-spline method (3). Trials on which >15% of electrodes required interpolation were dropped.

Mediation analysis. PLF was considered to have partially mediated the relationship between EVK and externalizing if shrinkage in the value of *c* to *c'* was significantly different than zero. The difference between c and c' can be approximated by calculating the product of paths *a* and *b*. Therefore, the size of this reduction was evaluated with a bootstrapping approach (for review, see 4) where 1,000 indirect effects (*ab*, equivalent to the change in *c* to *c'*) were simulated from the sample with replacement, keeping the proportion of matched- to unmatched-twin-pairs constant. Confidence intervals were estimated from this distribution of simulated *ab* effects to evaluate statistical significance.

Supplemental References

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