Parvalbumin Cell Ablation of NMDA-R1 Leads to Altered Phase, But Not Amplitude, of Gamma-Band Cross-Frequency Coupling

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Abstract

Altered gamma-band electrophysiological activity in individuals with autism spectrum disorder (ASD) is well documented, and analogous gamma-band alterations are recapitulated in several preclinical murine models relevant to ASD. Such gamma-band activity is hypothesized to underlie local circuit processes. Gamma-band cross-frequency coupling (CFC), a related though distinct metric, interrogates local neural circuit signal integration. Several recent studies have observed perturbed gamma-band CFC in individuals with ASD, although the direction of change remains unresolved. It also remains unclear whether murine models relevant to ASD recapitulate this altered gamma-band CFC. As such, this study examined whether mice with parvalbumin (PV) cell-specific ablation of NMDA-R1 ($PV_{cre}/NR1_{fl/fl}$) demonstrated altered gamma-band CFC as compared with their control littermates ($PV_{cre}/NR1_{+/+}$ —mice that do not have the PV cell-specific ablation of NMDA-R1). Ten mice of each genotype had 4 min of “resting” electroencephalography recorded and analyzed. First, resting electrophysiological power was parsed into the canonical frequency bands and genotype-related differences were subsequently explored so as to provide context for the subsequent CFC analyses. $PV_{cre}/NR1_{fl/fl}$ mice exhibited an increase in resting power specific to the high gamma-band, but not other frequency bands, as compared with $PV_{cre}/NR1_{+/+}$. CFC analyses then examined both the standard magnitude (strength) of CFC and the novel metric $Phase_{Max}$ —which denotes the phase of the lower frequency signal at which the peak higher frequency signal power occurred. $PV_{cre}/NR1_{fl/fl}$ mice exhibited altered $Phase_{Max}$, but not strength, of gamma-band CFC as compared with $PV_{cre}/NR1_{+/+}$ mice. As such, this study suggests a potential novel metric to explore when studying neuropsychiatric disorders.

Keywords: ASD; cross-frequency coupling; gamma; phase; phase-amplitude coupling

Introduction

CURRENT PREVALENCE RATES estimate that 1 in 59 children aged 8 years old are diagnosed with autism spectrum disorder (ASD; Baio et al., 2018), a neuropsychiatric disorder characterized by social/communication deficits and restricted/stereotyped behaviors (American Psychiatric Association, 2013). There currently exists a range of behavioral interventions (Ospina et al., 2008), as well as dietary and pharmacological treatments (Masi et al., 2017; McPheeters et al., 2011), for the symptoms of ASD. Unfortunately, none of the aforementioned interventions or treatments are

fully effective at mitigating, preventing, or resolving ASD—which likely results from their design targeting the symptoms of ASD instead of underlying neuropathophysiology. Currently, the direct targeting of such a neurobiological etiology with regard to ASD is not possible; as such principal neuropathophysiology is unresolved. Instead, the field has observed many seemingly disparate and noncontiguous neurobiological alterations in individuals with ASD, which ultimately lead to the characteristic constellation of behavioral phenotypes. In response, it has been suggested that the field should target potential zones of neurobiological convergence (Port et al., 2014). These zones of neurobiological

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convergence are biological substrates that could account for, and also provide context to, the observed neural alterations in ASD that range from preclinical subcellular protein expression to clinical whole-brain connectivity perturbations.

Local circuit activity is one such potential target zone of neurobiological convergence (Port et al., 2014). Of note, fast time-scale gamma-band (30–100 Hz) electrophysiological activity is hypothesized to underlie (Cardin et al., 2009; Gray et al., 1989; Sohal et al., 2009; Whittington et al., 2000), or at least index (Merker, 2013), local circuit activity. There is a large body of literature that reports altered gamma-band activity in ASD. Stimulus-evoked gamma-band visual (Grice et al., 2001; Keehn et al., 2015; Sun et al., 2012), as well as auditory (Gandal et al., 2010; Wilson et al., 2007), responses are decreased in individuals with ASD as compared with typically developing (TD) age-matched controls (see Rojas and Wilson, 2014 for thorough review). Moreover similar gamma-band response deficits are observed in both in first-degree relatives of individuals with ASD (Rojas et al., 2011, 2008) and in preclinical models relevant to ASD (Gandal et al., 2010, 2012a; Port et al., 2017b; Saunders et al., 2012). Resting-state (RS) gamma-band electrophysiological power is also altered in individuals with ASD as compared with TD controls, although the direction of the alterations potentially depends on both experiment design and methodological considerations (Maxwell et al., 2015). As such, gamma-band activity has been suggested as a “biomarker” (a biologically based marker) for ASD (Port et al., 2015; Rojas and Wilson, 2014). Indeed, gamma-band responses correlate with symptom severity in ASD (Edgar et al., 2015; Maxwell et al., 2015; Rojas et al., 2011), may potentially predict optimal outcome for individuals with ASD (Port et al., 2016), and recover with behaviorally efficacious interventions (Van Hecke et al., 2015), all key traits desirable in a biomarker. In addition, auditory gamma-band coherence responses positively correlate with underlying relative cortical GABA levels during typical childhood/adolescent development, but not within individuals with ASD (Port et al., 2017a), suggesting a perturbed biological coupling between physiology and neurochemistry.

A range of analogous gamma-band perturbations, and related findings, have been observed in mouse models relative to ASD. Preclinical mouse models relevant to ASD exhibit decreased auditory gamma-band responses (Gandal et al., 2010, 2012a; Nakamura et al., 2015; Port et al., 2017b; Saunders et al., 2012; Tatard-Leitman et al., 2015), as well as increased spontaneous (frequently referred to as “resting”) and prestimulus gamma-band power (Billingslea et al., 2014; Gandal et al., 2012b; Saunders et al., 2012; Sinclair et al., 2017; Tatard-Leitman et al., 2015), as compared with their wild-type (WT) littermates. Indeed, Yizhar and colleagues (2011) induced concurrent social deficits after increasing basal gamma-band activity in mice. Moreover, recovery of typical social behavior in murine models relevant to ASD is also concurrent to restoration of neurotypical gamma-band activity (Gandal et al., 2012b; Sinclair et al., 2017). Of note, although WT mice exhibited a positive correlation between auditory gamma-band coherence responses and underlying GABA levels, a mouse model with a genetic insult relevant to ASD failed to exhibit such a correlation (Port et al., 2017b)—analogous to the aforementioned clinical observations.

Although gamma-band activity alterations have been well documented in ASD, the related and more recent metric of gamma-band cross-frequency coupling (CFC) has received less attention. CFC, and more specifically, phase-amplitude coupling (PAC), is a phenomenon in the brain where the phase of a lower frequency neural signal modulates the amplitude of a concomitant higher frequency neural signal. Gamma-band PAC has been suggested to index, or even represent, the integration of fast time-scale local neural circuits with slower long-range inputs. Khan and colleagues (2013) reported decreased relative task-induced alpha to gamma PAC in individuals with ASD as compared with their TD counterparts. Separately, Berman and colleagues (2015) reported that increased RS alpha to gamma PAC was observed for children with ASD relative to TD age-matched controls at a posterior midline regional source, but not an anterior midline regional source.

Several studies have investigated murine models of relevance to ASD for alterations to the strength (amplitude) of task-related gamma-band PAC. Although these studies do report altered strength of gamma-band PAC in these mice as compared with either WT (Radwan et al., 2016) or before pharmacological insult (Michaels et al., 2018), the changes are complex and potentially inconsistent. Spontaneous RS gamma-band PAC has also been examined in a mouse model relevant to ASD, although the exact interpretation of such a “resting” period in mice is frequently disputed. After careful control and observation of the resting period for potentially confounding explanations, Port and colleagues (2017b) reported that male mice heterozygous for *PCDH10* (*Pcdh10*^{+/-}), an autism-related gene, exhibited decreased RS theta to high-gamma PAC as compared with male WT littermates. Females failed to mirror any genotypic differences in RS theta to high-gamma PAC. This observation was of particular note because sociability deficits have only been observed in the male, but not in the female, *Pcdh10*^{+/-} mice. As such, the deficit in gamma-band related PAC mirrors the presence of social dysfunction in these *Pcdh10*^{+/-} mice.

Although originally designed as a murine model of schizophrenia, mice with parvalbumin (PV) cell-specific ablation of NMDA-R1 (*PV_{cre}/NR1_{fl/fl}*) exhibit both electrophysiological and behavioral perturbations that are more analogous to individuals with ASD rather than those with schizophrenia (Billingslea et al., 2014). *PV_{cre}/NR1_{fl/fl}* mice exhibit delayed N1 component latencies compared with control mice that do not have the PV cell-specific ablation of NMDA-R1 (*PV_{cre}/NR1_{+/+}*), akin to the delayed M100 component frequently observed in magnetoencephalographic recordings of individuals with ASD (Edgar et al., 2015; Gage et al., 2003a,b; Gandal et al., 2010; Port et al., 2016; Roberts et al., 2010; see Port et al., 2015 for a detailed discussion of delayed M100 latencies in individuals with ASD). In addition, *PV_{cre}/NR1_{fl/fl}* mice exhibited markedly reduced sociability as compared with *PV_{cre}/NR1_{+/+}* controls. Therefore, this mouse model may more accurately recapitulate the phenotypes associated with ASD rather than schizophrenia. Of note, *PV_{cre}/NR1_{fl/fl}* mice also demonstrate increased prestimulus electrophysiological power in the 60 and 120 Hz frequency range. The presence of increased prestimulus electrophysiological power in other frequency bands has not been explicitly tested.

This study investigated *PV_{cre}/NR1_{fl/fl}* mice, and their *PV_{cre}/NR1_{+/+}* controls, for altered gamma-band related PAC, to determine whether a preclinical model with

relevance to ASD recapitulated the recent relevant clinical reports. We hypothesized that $PV_{cre}/NR1_{fl/fl}$ mice would demonstrate increased gamma-band PAC as compared with their $PV_{cre}/NR1_{+/+}$ counterparts.

Materials and Methods

Previous data reporting

The mice reported in this study are a subset of those reported by Billingslea et al. (2014). Billingslea and colleagues (2014) examined prestimulus gamma-band power, as opposed to the “resting” period (a duration wherein mice were not actively stimulated, while awake and coherent, but nonmobile; see Port et al., 2017b for a full delineation and discussion of this period/nomenclature) analyzed for this study. Of note, Billingslea and colleagues (2014) also reported no difference in anxiety or total distance traveled during open field testing, and so basal locomotion or anxiety differences are unlikely to account for any observed electrophysiological differences. This study additionally differs from Billingslea et al. (2014) by examining the complete canonical frequency spectrum for alterations between $PV_{cre}/NR1_{fl/fl}$ and $PV_{cre}/NR1_{+/+}$ mice rather than just the gamma-band frequency range. More importantly, this study also extends on the initial study by examining PAC differences between $PV_{cre}/NR1_{fl/fl}$ and $PV_{cre}/NR1_{+/+}$ mice.

Subjects

This study used electroencephalography (EEG) recordings from 10 $PV_{cre}/NR1_{fl/fl}$ and 10 $PV_{cre}/NR1_{+/+}$ mice. All mice were adult (3–4 months of age during EEG recordings) male mice. These sample sizes were selected in accordance with previous studies that detected significant differences when examining similar spectral metrics (Featherstone et al., 2012, 2013; Port et al., 2017b).

Mice with a cell type and regionally controllable deletion of the NMDAR1 subunit were purchased from The Jackson Laboratory (strain name: B6.129S4-Grin1^{tm2Srl}/J) to generate mice with the desired genotypes. Restriction of the knockout of NMDA receptors on corticolimbic PV containing interneurons was subsequently accomplished using a mosaic transgenic approach by breeding $NR1_{flox}$ mice with transgenic mice expressing selective expression of a Cre activator in PV-containing interneurons. As such, resulting groups will be composed of mice with 0 ($NR1_{-/-}$), 1 ($NR1_{-/+}$), or 2 ($NR1_{+/+}$) working copies of $NR1$ in PV cells. The knockout validity was confirmed using PV immunostaining in transgenic $PV_{cre}; (td)Tomato_{flox}$ mice, in which the expression of the red fluorescent protein (td)Tomato is restricted to PV_{cre} expressing cells. PV immunolabeling was observed in all (td)Tomato-positive cells, demonstrating that recombination occurred in only PV-positive interneurons (Billingslea et al., 2014). Animals were housed in temperature-controlled rooms with a 12-h light/12-h dark cycle (lights on at 07:00 h). They were given TestDiet 5001 (Purina Mills, Richmond, IN) and water *ad libitum*. Cages were changed weekly. All animal procedures were in strict accordance with the National Institutes of Health guide for the care and use of laboratory animals and were approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

In vivo (EEG) and data preprocessing

The *in vivo* EEG recording methodology was previously reported by Billingslea et al. (2014). In brief, animals were anesthetized with isoflurane and underwent stereotaxic implantation of tripolar electrode assemblies (PlasticsOne, Roanoke, VA). Low-impedance stainless steel macroelectrodes (<5 k Ω , 1000 Hz) were aligned along the sagittal axis of the skull at 1 mm intervals (anterior to posterior: negative, ground, and positive). The electrodes were precut with a length of 3 mm (positive) and 1 mm (ground and negative). The positive electrode was placed 1.8 mm posterior, 2.65 mm right lateral, and 2.75 mm deep relative to Bregma. The electrode pedestal was secured to the skull with ethyl cyanoacrylate (Loctite; Henkel, Westlake, OH) and dental cement (Ortho Jet; Lang Dental, Wheeling, IL). This electrode configuration can replicate clinical auditory event-related potential (ERP) findings in humans that are primarily sourced to the cortex (Carlson et al., 2011; Ehrlichman et al., 2009; Gandal et al., 2008; Halene et al., 2009). EEG recordings were performed on awake mice at least 1 week after electrode implantation, in a home-cage environment, as previously described (Ehrlichman et al., 2009; Gandal et al., 2008; Halene et al., 2009). Mice were brought to the room 30 min before recording for acclimation. Cages were placed in a sound-attenuated recording chamber located inside a Faraday electrical isolation cage. Electrode pedestals were connected to a 30 cm tripolar electrode cable that exited the chamber to connect to a high impedance differential AC amplifier (A-M Systems, Carlsborg, WA). Raw EEG was filtered between 1 and 500 Hz. Four minutes of “resting” data were collected after the auditory stimulation paradigm reported in Billingslea et al. (2014). These RS data had not been analyzed and/or reported before this study. EEG signals were subsequently imported into the MatLab (Mathworks, Natick, MA) toolbox Fieldtrip (Oostenveld et al., 2011) and underwent artifact rejection using Fieldtrip’s z-score-based artifact detection routine, with experimenter input to adjust the threshold for artifact determination in accordance with Port et al. (2016, 2017a,b). There was no significant difference in the length of resting data remaining between $PV_{cre}/NR1_{fl/fl}$ and $PV_{cre}/NR1_{+/+}$ mice postartifact rejection ($p > 0.05$).

RS electrophysiological power

In accordance with Port et al. (2017b), power spectral densities (PSDs) were calculated for the artifact-rejected data using fast Fourier transformations. PSDs were subsequently divided into the canonical spectral frequency bands (1–4 Hz—delta; 4–8 Hz—theta; 8–12 Hz—alpha; 15–25 Hz—beta; 30–60 Hz—low gamma; and 60–100 Hz—high gamma) and linear mixed-effects models (LMMs) were subsequently performed in R using the package “afex.” These statistical analyses examined RS electrophysiological power for the main effects of genotype, frequency band, as well as their interactions. All pairwise comparisons utilized Holm’s multiple comparison correction.

RS PAC: Strength

RS PAC was examined in accordance with previously published studies (Berman et al., 2015; Port et al., 2017b), using the estimate of PAC first reported in Tort et al.

(2010). In particular, the broadband time-domain EEG signal was band-pass filtered into two frequency bands of interest: a low-frequency and a high-frequency signal. A Hilbert transform provided the time-varying phase of the low-frequency signal and the time varying amplitude of the high-frequency signal. A composite signal was next formed using the phase time course of the low-frequency signal and the amplitude time course of the high-frequency signal. This composite signal was then used to measure the dependence of the amplitude of the high-frequency signal on the phase of the low-frequency signal, computed as the modulation index (MI). Of note, the number of possible phase values was reduced during this step, by indexing the phase time course values of the low-frequency signal by 18 equal length contiguous bins that spanned from 0° to 360° . MI measures deviation of the amplitude-phase histogram from a uniform distribution. This study used low-frequency signals ranging from 3 to 15 Hz (in 1 Hz steps) and the high-frequency signals ranging from 20 to 100 Hz (in 1 Hz steps). MI values for each frequency pair were then collected into an MI-comodulogram that indicates the magnitude (strength) of coupling between low-frequency phase and high-frequency amplitude pairs. Within the comodulogram, the abscissa represents the low-frequency phase signal (traditionally referred to as the modulation signal) and the ordinate represents the higher frequency amplitude signal (traditionally referred to as the carrier signal) that is modulated by the low-frequency signal. As discussed in prior work, the low-frequency phase signal must be lower than the amplitude modulated frequency (Berman et al., 2012).

Population statistics were performed on the MI-comodulogram using Fieldtrip's Monte Carlo cluster-based permutation testing. Mean within-genotype MI values were compared using independent *t*-tests ($\alpha=0.05$). Nonparametric cluster thresholding ($\alpha=0.05$) based on cluster size was subsequently performed with 1000 permutations of the MI values.

Additional analyses determined the strength of PAC that could be expected due to noise floor effects. The MI calculation pipeline was repeated with the following alterations. After filtering the recorded signal into a higher frequency and lower frequency signal, each higher frequency signal was divided into 10 segments. This segmented higher frequency signal was then recombined in a random time order, and MI values were generated for the coupling between this artificial higher frequency signal and the native lower frequency signal's phase (again binned into 18 equal length contiguous bins). This artificial MI represented the PAC coupling present in a randomly coupled and scrambled signal. Although it was possible to randomly generate either, or both, frequency signal(s), this methodology allowed the artificial higher frequency to keep the spectral structure of the original signal. This entire process was then repeated 1000 times, so as to generate a distribution of noise floor values within each frequency pairing. This generation of noise floor value distributions was repeated for every frequency pair. Subsequently, these values were formed into a matrix that represented the distribution of noise floor values over the entire comodulogram, which will be referred to as the noise-comodulogram.

RS PAC: Phase

A similar bandpass filtering pipeline was used to interrogate the phase relationship of the PAC, as was used in the

analysis of the strength of PAC. After filtering and Hilbert transforming the broadband time-domain EEG signal into the time-varying phase of the low-frequency signal and the time-varying amplitude of the high-frequency signal, the amplitude of the higher frequency signal was then averaged within 18 bins indexed by the same phase bins as used in the previous analysis of CFC strength. Subsequently, the phase bin of the lower frequency signal at which the peak amplitude of the higher frequency signal occurred (denoted from here out as $\text{Phase}_{\text{Max}}$) was then determined for each frequency pairing. This process was then repeated separately for each mouse. As such, phase-based comodulogram (phase-comodulogram) was subsequently generated. The axes of the phase-comodulogram are identical to the MI-comodulograms; however, the phase-comodulogram visualizes $\text{Phase}_{\text{Max}}$ instead of MI values. A two-step process of cluster-based permutation tests then tested the phase-comodulograms for significant genotype-based alterations in $\text{Phase}_{\text{Max}}$.

First, regions of the MI-comodulogram that demonstrated PAC above the corresponding noise floor were determined separately for each genotype. As with standard cluster-based permutation testing, for each frequency pairing the median observed MI value was transformed into the corresponding *t*-score with respect to the distribution of noise floor values. A similar process then occurred for the noise-comodulogram values. Then nonparametric cluster-based analysis identified clusters of significantly increased PAC ($\alpha=0.05$) in the observed data where the cluster size was significantly ($\alpha=0.05$) greater than the corresponding clusters generated by the noise floor. As such, this analysis allowed for the determination of where PAC was actually occurring within the entire range of frequency pairs, as opposed to regions where the observed PAC was not above the level of the noise floor.

Second, a similar cluster-based permutation testing was used to test for significant $\text{Phase}_{\text{Max}}$ differences between $\text{PV}_{\text{cre}}/\text{NR1}_{\text{fl/fl}}$ and $\text{PV}_{\text{cre}}/\text{NR1}_{+/+}$ mice. Standard cluster-based permutation testing could not be used for this test, as $\text{Phase}_{\text{Max}}$ represents circular phases and not linear data. As such, the Circular Statistics Toolbox for MatLab (Berens, 2009) function "circ_cmtest" (nonparametric multisample test for equal medians) was used to generate the corresponding test statistic for the between genotype comparison for each frequency pairing. This test statistic was then used within an analogous cluster identification and selection routine as the corresponding MI-comodulogram analysis, again with 1000 permutations. As such, this cluster-based permutation testing of $\text{Phase}_{\text{Max}}$ values determined regions within the phase-comodulogram that demonstrated significant $\text{Phase}_{\text{Max}}$ differences between $\text{PV}_{\text{cre}}/\text{NR1}_{\text{fl/fl}}$ and $\text{PV}_{\text{cre}}/\text{NR1}_{+/+}$ mice.

These two cluster-based permutation tests provided statistical maps across the range of investigated frequency pairs where (1) the strength of PAC was significantly above that of the noise floor and (2) $\text{PV}_{\text{cre}}/\text{NR1}_{\text{fl/fl}}$ and $\text{PV}_{\text{cre}}/\text{NR1}_{+/+}$ mice exhibited significantly different $\text{Phase}_{\text{Max}}$. Subsequently, the within genotype phase-comodulograms were masked by both of the statistical maps, so as to allow only regions that were both significantly above noise, as well as exhibited significantly different $\text{Phase}_{\text{Max}}$ between genotypes to pass. This was deemed necessary as significant genotype-

based differences in Phase_{Max} may potentially occur even without actual PAC occurring due to random chance.

Results

RS electrophysiological power

The LMM of resting electrophysiological power demonstrated the expected significant effect of frequency band, $F(1.80, 32.41) = 19130.30$ ($p < 0.001$; Fig. 1A), with RS power decreasing with increasing frequency. In addition, the Genotype X Frequency-band interaction was significant, $F(1.80, 32.41) = 15.08$, $p < 0.001$, with greater high gamma-band RS power in PV_{cre}/NR1_{fl/fl} than PV_{cre}/NR1_{+/+} mice ($p < 0.001$; Fig. 1B). PV_{cre}/NR1_{fl/fl} additionally exhibited increased low gamma-band RS power as compared with PV_{cre}/NR1_{+/+} mice, although this observation only trended toward significance ($p < 0.10$; Fig. 1B). No other main effect or interaction reached significance ($p > 0.05$).

FIG. 1. PV_{cre}/NR1_{fl/fl} exhibit greater resting high gamma-band power than PV_{cre}/NR1_{+/+} counterparts. In confirmation of Billingslea et al. (2014), PV_{cre}/NR1_{fl/fl} exhibit greater resting high gamma-band power than their PV_{cre}/NR1_{+/+} counterparts. PV_{cre}/NR1_{fl/fl} also exhibited increased resting low gamma-band power as compared with their PV_{cre}/NR1_{+/+} counterparts, although this comparison only trended toward significance. This genotype-related observation was specific to the gamma-bands, as no other frequency band demonstrated significant genotype-dependent alteration. (A) PSDs of PV_{cre}/NR1_{+/+} (blue) and PV_{cre}/NR1_{fl/fl} (red) mice. PSDs depict average (center line) and standard error of the mean (shading). (B) Binned mean power with each canonical frequency band (error bar—standard error of the mean). # $p < 0.10$, *** $p < 0.001$. PSDs, power spectral densities; PV, parvalbumin; PV_{cre}/NR1_{fl/fl}, mice with PV cell-specific ablation of NMDA-R1; PV_{cre}/NR1_{+/+}, mice that do not have the PV cell-specific ablation of NMDA-R1.

RS PAC: Strength

Cluster-based permutation testing of MI-comodulograms revealed no significantly different clusters between PV_{cre}/NR1_{fl/fl} and PV_{cre}/NR1_{+/+} mice (Fig. 2).

RS PAC: Phase

Phase-comodulograms underwent a two-step cluster-based permutation testing and masking to ensure validity of the observed phase-related alterations between PV_{cre}/NR1_{fl/fl} and PV_{cre}/NR1_{+/+} mice (see explanation in Materials and Methods). The resulting double masked phase-comodulograms revealed two clusters of altered Phase_{Max}. The first cluster of altered Phase_{Max} occurred within theta to gamma PAC and was centered at the 8–49 Hz. Within this frequency pairing, PV_{cre}/NR1_{+/+} mice demonstrated a median Phase_{Max} of 190°, whereas PV_{cre}/NR1_{fl/fl} demonstrated a different median Phase_{Max} of 100°. The second cluster of altered Phase_{Max} occurred in alpha to high-gamma PAC and was centered on the 12–65 Hz frequency pairing. Again, PV_{cre}/NR1_{+/+} mice demonstrated a different (210°) than PV_{cre}/NR1_{fl/fl} mice (140°).

Subsequently the individual PAC frequency pairs identified in the cluster-based permutation testing of the phase-comodulograms were interrogated, so as to more fully characterize the phase relationships. To do so, the average gamma-band envelope amplitude was plotted in relation to the phase of the lower frequency signal (Fig. 3C). PV_{cre}/NR1_{fl/fl} demonstrated a single peak at ~120° for both the 8–49 Hz and the 12–65 Hz, PAC frequency pairings. However, PV_{cre}/NR1_{+/+} demonstrated a larger peak at ~200°, and a second smaller peak at ~300°, for both the 8–49 Hz and 12–65 Hz PAC frequency pairings.

Discussion

This study observed increased high gamma-band, but not other frequency band, resting electrophysiological power in PV_{cre}/NR1_{fl/fl} as compared with PV_{cre}/NR1_{+/+} mice (Fig. 1), in agreement with Billingslea et al. (2014). In addition, this study observed an alteration to the phase (Fig. 3), but not strength (Fig. 2), of gamma-band PAC in PV_{cre}/NR1_{fl/fl} as compared with PV_{cre}/NR1_{+/+} mice.

Gamma-band activity alterations are well documented in ASD as compared with TD individuals, both at rest and in response to sensory stimulation (see Rojas and Wilson, 2014; Port et al., 2015, for detailed discussion, as well as summation in the Introduction). Moreover, several murine models relevant to ASD exhibit increased spontaneous gamma-band power (Billingslea et al., 2014; Gandal et al., 2012b; Saunders et al., 2012; Sinclair et al., 2017; Tatard-Leitman et al., 2015), as well as decreased auditory gamma-band responses (Gandal et al., 2010, 2012a; Nakamura et al., 2015; Port et al., 2017b; Saunders et al., 2012; Tatard-Leitman et al., 2015) as compared with WT littermates, analogous to individuals with ASD. Such gamma-band activity may be a readout of underlying local neural circuit activity (Cardin et al., 2009; Gray et al., 1989; Sohal et al., 2009; Whittington et al., 2000), and so be a marker of local circuit functional integrative and health. It may be that this circuit construct is where the many seemingly disparate and non-contiguous neurobiological alterations previously observed in individuals with ASD coalesce into the characterized

FIG. 2. Cluster-based permutation testing of MI-comodulograms revealed no significant differences between $PV_{cre}/NR1_{fl/fl}$ and $PV_{cre}/NR1_{+/+}$ mice. Mean MI-comodulograms of $PV_{cre}/NR1_{+/+}$ (left column) as well as $PV_{cre}/NR1_{fl/fl}$ (middle column). The statistical map resulting from the cluster-based permutation testing is presented in the right column. Note, while the color scale for the left two columns represents MI value, the color scale for the right-hand column represents probability. MI, modulation index.

constellation of behavioral phenotypes (Port et al., 2014). Therefore, the interrogation of gamma-band circuit dysfunction may provide a unifying context for the heterogeneous neuropathophysiological alterations observed in ASD.

CFC is thought to reflect signal integration of long-range networks with local circuits. Currently, few studies directly examine gamma-band PAC alterations in individuals with ASD (Berman et al., 2015; Khan et al., 2013; Mamashli et al., 2017; Seymour et al., 2018). Although all studies have observed alterations to gamma-band CFC in individuals with ASD, the reported alterations are inconsistent. It is not clear though whether these inconsistent gamma-band PAC observations are due to either task versus RS differences or alternatively regional site differences. Further studies are required to fully parse apart gamma-band PAC alterations observed in individuals with ASD as compared with their TD counterparts.

A similar need for additional studies surrounds the preclinical observations of altered gamma-band PAC in mouse models relevant to ASD. Several recent preclinical studies report alterations to the strength of task-related gamma-band PAC in murine models relevant to ASD (Cao et al., 2018; Michaels et al., 2018; Radwan et al., 2016), but the changes are complex and potentially not consistent. Separately RS theta to high-gamma PAC appears decreased in mice that have a genetic insult relevant to ASD (Port et al., 2017b). As such, although initial studies suggested altered gamma-band PAC in both individuals with ASD and mouse models relevant to ASD, more detailed studies of this phenotype are required before conclusions about the role of altered gamma-band PAC in ASD can be generated.

Although this study did not observe alterations to the strength of gamma-band PAC, genotype-dependent alterations

FIG. 3. Double masked cluster-based permutation testing of phase-comodulograms revealed two significantly different clusters of $Phase_{Max}$ between $PV_{cre}/NR1_{fl/fl}$ and $PV_{cre}/NR1_{+/+}$ mice. (A) Unmasked phase-comodulogram (median $Phase_{Max}$) for $PV_{cre}/NR1_{+/+}$ mice (left) and $PV_{cre}/NR1_{fl/fl}$ (right). (B) Double masked phase-comodulograms (median $Phase_{Max}$) resulting from the cluster-based permutation testing of both noise floor values and alterations to $Phase_{Max}$ between $PV_{cre}/NR1_{+/+}$ mice (left) and $PV_{cre}/NR1_{fl/fl}$ (right). (C) Visualization of the PAC relationship for each cluster identified in (B). Graphs depict the average (center line) and standard error of the mean (shading) for the envelope amplitude of the gamma-band signal by the phase of the lower frequency signal. $PV_{cre}/NR1_{+/+}$ = blue, $PV_{cre}/NR1_{fl/fl}$ = red. PAC, phase-amplitude coupling.

to the phase of the lower frequency signal at which the maximal amplitude of the gamma-band signal occurred ($\text{Phase}_{\text{Max}}$) were observed. Both the theta to gamma and alpha to high-gamma clusters exhibited higher $\text{Phase}_{\text{Max}}$ in $\text{PV}_{\text{cre}}/\text{NR1}_{+/+}$ versus $\text{PV}_{\text{cre}}/\text{NR1}_{\text{fl/fl}}$ mice. Of note, it is not immediately clear whether these observations reflect either an earlier or later phase of gamma-band PAC in $\text{PV}_{\text{cre}}/\text{NR1}_{\text{fl/fl}}$ mice, as these data were circular in nature. In addition, the role of the concurrent increased resting high gamma-band power on the alpha to high-gamma PAC is not immediately clear.

Although speculative, examining the morphology of the gamma-band signal envelope amplitude across the phase of the lower frequency signal for the two clusters that demonstrate different $\text{Phase}_{\text{Max}}$ (Fig. 3C) presents potentially interesting avenues for further exploration. The $\text{PV}_{\text{cre}}/\text{NR1}_{\text{fl/fl}}$ mice demonstrated a sharper, and more refined, single peak distribution as compared with their $\text{PV}_{\text{cre}}/\text{NR1}_{+/+}$ littermates. In contrast, $\text{PV}_{\text{cre}}/\text{NR1}_{+/+}$ mice demonstrated a potential lower bimodal peak distribution. Again, although speculative, the observed $\text{Phase}_{\text{Max}}$ distribution differences may reflect the ability of $\text{PV}_{\text{cre}}/\text{NR1}_{+/+}$ mice to appropriately vary their $\text{Phase}_{\text{Max}}$ to on-going neural processes. In contrast, $\text{PV}_{\text{cre}}/\text{NR1}_{\text{fl/fl}}$ mice may demonstrate both (1) a pathologically strict rigidity/adherence to a (2) altered $\text{Phase}_{\text{Max}}$ relationship as compared with $\text{PV}_{\text{cre}}/\text{NR1}_{+/+}$ littermates.

This study had several limitations. First, EEG was only collected from a single site. The study's chosen electrode configuration was designed so as to record analogous ERPs as observed from Cz electrodes in clinical studies (Siegel et al., 2003). However, this limits the generalizability of the current observations to other brain regions. Second, the exact nature of the behavioral/cognitive status of the mice during the recordings remains unknown. A previous study that included this study cohort in their total study population observed no difference in anxiety or total distance traveled during open field testing (Billingslea et al., 2014). As such, basal differences in locomotion or anxiety between genotypes are unlikely to account for the current electrophysiological observations. Third, this cohort of mice did not have a complete set of behavioral characterization. As such, it is unclear how the current observations of altered $\text{Phase}_{\text{Max}}$ may relate to concomitant ASD-related behavior.

Another *potential* limitation of this study was the use of an RS paradigm as opposed to an active task paradigm. Although RS PAC alterations are inherently informative, it is unclear whether/how such RS alterations generalize to task-related PAC. Often, PAC is conceptualized in terms of spatial/temporal processing within the hippocampus (Lisman and Jensen, 2013). PAC is not a unitary construct though, and differential PAC profiles (both in terms of the strength of coupling within a single frequency pairing and the frequency pairs being coupled) are observed not only between RS and active task-engagement periods, but additionally between different active tasks (i.e., social interaction and active exploration; Cao et al., 2018), and moreover between different stages within a single learning paradigm (Radwan et al., 2016). Of note, Cao and colleagues (2018) observed a significant decrease in theta to low-gamma PAC, concurrent with an increase in theta to high-gamma PAC, in a mouse model with relevance to ASD. In addition, Cao and colleagues (2018) did not observe a significant difference in the phase of coupling for theta to low-gamma coupling.

It is unclear what factors lead to the discrepancies between Cao and colleagues' (2018) report and the current study, and although the different PAC findings may be due to the different animal models utilized, the discrepancy may *potentially* relate the differential analysis methodologies implemented between the two studies. This study examined the entirety of the comodulograms (ranging across the delta, theta, alpha; beta, low gamma, and high gamma frequency bands) for genotype-dependent alterations, and remained agnostic to which frequency-band alterations would be observed in. Such a methodology necessitated multiple comparison corrections. In contrast, Chao and colleagues (2018) examined preselected frequency ranges (theta to low-gamma and theta to high-gamma) for genotype-dependent PAC alterations, and so did not lose statistical power during multiple comparison corrections. This use of preselected frequency ranges may have also resulted in the phase of theta to low-gamma coupling failing to demonstrate genotypic differences, as Chao and colleagues' (2018) methodology was less refined in frequency space. Indeed, it appears that the phases for theta to low-gamma coupling were not significantly different between genotypes in Chao and colleagues' (2018) study due to the presence of high variance, as opposed to similar mean values.

Insight into the generalizability of the current RS PAC-related findings to active-task situations arises from a recent study that utilized a similar $\text{PV}_{\text{cre}}/\text{NR1}_{\text{fl/fl}}$ mouse model of ASD (Korotkova et al., 2010). This mouse model demonstrated on average an $\sim 90\%$ reduction in NMDA currents in fast-spiking putative PV-positive interneurons, and so may be considered a model of severe NR1 hypofunction instead of NR1 loss. These $\text{PV}_{\text{cre}}/\text{NR1}_{\text{fl/fl}}$ mice demonstrate increased gamma-band activity during spontaneous exploration akin to the current RS study (Fig. 1). In addition, these $\text{PV}_{\text{cre}}/\text{NR1}_{\text{fl/fl}}$ mice exhibited decreased theta power during spontaneous exploration, an observation that is hinted to in the current data (Fig. 1). Korotkova and colleagues (2010) also reported decreased theta to gamma coupling in $\text{PV}_{\text{cre}}/\text{NR1}_{\text{fl/fl}}$ mice, which was most prominent during periods of low theta power. As with Cao et al. (2018), this study may have failed to replicate this PAC strength alteration due to the current study's necessity for multiple comparison correction in response to examining additional frequency bands. Of note, Korotkova and colleagues (2010) additionally observed that the preferred phase of discharge of theta modulated pyramidal cells, but not interneurons, within the theta cycle was different in $\text{PV}_{\text{cre}}/\text{NR1}_{\text{fl/fl}}$ mice as compared with their respective controls. This finding is *potentially* paralleled by the current $\text{Phase}_{\text{Max}}$ metric.

This study focused on the NR1 homozygous conditions (i.e., $\text{PV}_{\text{cre}}/\text{NR1}_{+/+}$ and $\text{PV}_{\text{cre}}/\text{NR1}_{-/-}$). This murine model may not be an accurate representation of the neurobiology of ASD, as individuals are frequently heterozygous for specific deletions. Currently, it remains unknown whether NR1 deletion from PV+ cells is haplosufficient for $\text{Phase}_{\text{Max}}$ activity. NR1 gene dosage *may* affect $\text{Phase}_{\text{Max}}$ in a dose-dependent manner, or even paradoxically due to homeostatic mechanisms. A more accurate model of the neurobiology of ASD may be encapsulated in the $\text{NR1}_{\text{neo}(-/-)}$ (also known as the $\text{NR1}_{\text{neo/neo}}/\text{GRIN1}_{\text{neo}(-/-)}$) hypomorph. These $\text{NR1}_{\text{neo}(-/-)}$ mice may demonstrate behavioral phenotypes associated with ASD, including perturbed sociality (Barkus et al., 2012; Gandal et al., 2012a,b). Of note, this perturbed sociality may be selective to males (Barkus et al., 2012) and

reversible with oxytocin administration (Teng et al., 2016). Deficits in sociability are absent when the NR1^{neo-/-} knockout is induced in adult mice (Belforte et al., 2010).

NR1^{neo-/-} mice also exhibit increased RS high gamma-band activity (Gandal et al., 2012b), akin to the current PV^{cre}/NR1^{-/-} mice (Fig. 1). Moreover, NR1^{neo-/-} mice demonstrate blunted *in vitro* carbachol-induced gamma-band activity (Grannan et al., 2016). It remains unknown whether the same is true for PV^{cre}/NR1^{-/-} mice. Separately, NR1^{neo-/-} also exhibits decreased phase-locked auditory gamma-band responses (Gandal et al., 2012a,b), although this is not mirrored in PV^{cre}/NR1^{-/-} mice (Billingslea et al., 2014). As such, although NR1^{neo-/-} and PV^{cre}/NR1^{-/-} mice both demonstrate perturbed RS gamma-band activity, they differ on the presence of auditory-evoked gamma-band response deficits. Therefore, it remains unclear how the PAC alterations observed in this study would generalize to the NR1^{neo-/-} mice, although at the very least it appears that NR1 function on PV+ cells is necessary for healthy RS, but not auditory-evoked gamma-band activity.

Recently, NR1^{neo+/-} mice were also examined for electrophysiological perturbations (Featherstone et al., 2015). Although these mice demonstrated a 30% reduction in NR1 expression, no electrophysiological perturbations were observed for gamma-band measures. As such, it appears that severe reductions of NR1 functioning are required for a subsequent impact on gamma-band activity. Oscillatory activity can be characterized by their phase, frequency, and amplitude. A previous study suggested that of the three characteristics, the phase of the gamma-band activity may be the most impacted by the neuropathological alterations associated with ASD (Port et al., 2017a). Such sensitivity of the phase component of neural signals may carry across to CFC, where Phase_{Max} values may be more malleable than their strength-based counterparts. Alternatively, alterations to the Phase_{Max} of gamma-band CFC may not relate to strength-based alterations of CFC. This is because it is unclear what neurobiological circuit mechanisms account for the selective alterations to the phase, but not strength, of gamma-band PAC. Although the neural mechanisms governing the phase and strength of PAC may overlap, complex developmental homeostatic changes within PV^{cre}/NR1^{fl/fl} may have produced a novel neural circuit alteration, which is unique and nonrepresentative of ASD in general. As such, it is not immediately known whether such findings will replicate in other murine models relevant to ASD. Further studies are required to elucidate these potential concerns.

The lack of alterations to the strength of PAC between PV^{cre}/NR1^{fl/fl} mice and PV^{cre}/NR1^{+/-} is of interest in itself. PV^{cre}/NR1^{fl/fl} mice demonstrate subtle cognitive alterations, including some improvements, as compared with their control counterparts (Billingslea et al., 2014; Bygrave et al., 2016). Indeed, systematic and thorough examination of PV^{cre}/NR1^{fl/fl} demonstrated a largely intact phenotype (Bygrave et al., 2016). As such, while demonstrating impaired sociability, these PV^{cre}/NR1^{fl/fl} mice are largely unscathed. Therefore, these mice may more accurately represent higher functioning individuals with ASD, as opposed to the more severely impacted. The presence of increased RS high gamma-band activity as well as altered Phase_{Max} in this relatively less impaired phenotype may suggest the higher sensitivity of RS high gamma-band activity as well as Phase_{Max} measures, as opposed to PAC strength.

PAC strength has frequently been related to cognitive processing (Lisman and Jensen, 2013), and so it is unsurprising that PAC strength remains unchanged in a murine model that fails to demonstrate many cognitive perturbations.

The current murine model does suggest that proper PV+ interneuron function is critical for RS gamma-band activity. In addition, PV+ interneuron's activity is critical for the entrainment of the local circuit to the phase of the theta (and alpha) cycle, although it does not affect the strength of the local circuit's response during this entrainment. One possible explanation for such observations could be the following: NR1 ablation on PV+ interneurons causes PV+ interneurons to have weaker synaptic inputs, and so the depolarization resulting from the on-going theta cycle would be diminished. This decrease in theta cycle-related excitation of PV+ interneurons subsequently delays their firing. Firing of the PV+ interneurons would then entrain local pyramidal cell firing to generate a PING-model gamma-burst (Traub et al., 1996). Because a similar number of pyramidal cells contribute to the generation of PING-derived gamma-band activity in both PV^{cre}/NR1^{fl/fl} and WT mice, the strength of the gamma-band burst is similar. This mechanism leads to similar theta to gamma PAC strength but with altered phase. As already mentioned, the current analyses cannot determine whether the phase of the theta to gamma coupling is advanced or delayed in the PV^{cre}/NR1^{fl/fl}.

Conclusion

To conclude, this study presents evidence for the specificity of increased resting high gamma-band electrophysiological power in a mouse model relevant to ASD. In addition, the novel concept of phase-based alterations to PAC in neuropsychiatric disorders is presented.

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