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High-Frequency Neuronal Oscillatory Abnormalities in the Phospholipase C-β1 Knockout Mouse Model of Schizophrenia

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ABSTRACT

Background: Schizophrenia is a complex neuropsychiatric disorder characterized by psychoses, socioaffective disturbances, and cognitive deficits. The phosphodiesterase enzyme phospholipase C-β1 has been reported to be reduced in postmortem tissue of schizophrenia patients. Dysregulation of neuronal oscillations, particularly those in the higher frequency range such as beta (12–30 Hz) and gamma (30–80 Hz), are also associated with this disorder. We investigated the influence of phospholipase C-β1 gene deletion on cortical oscillatory activity and sensorimotor gating behavior.
Methods: Adult phospholipase C-β1 knockout and wild-type C57Bl/6J control mice (total n = 26) underwent surgical implantation of extradural electrodes to allow electrocorticography recordings. Electrocorticography was recorded during prepulse inhibition behavior sessions to measure ongoing and auditory-evoked electrophysiological responses. Mice were also pretreated with antipsychotic drugs haloperidol (0.25 mg/kg), clozapine (2.5 mg/kg), and olanzapine (5 mg/kg).
Results: Phospholipase C-β1 knockout mice exhibited reduced prepulse inhibition and diminished power and phase synchrony of beta and gamma oscillatory responses to auditory stimuli as well as elevated ongoing beta oscillations. Reductions in prepulse inhibition were highly correlated with the power and phase synchrony of evoked oscillations. Clozapine and olanzapine ameliorated the prepulse inhibition deficit in phospholipase C-β1 knockout mice, but not the electrophysiology abnormalities.
Conclusions: Phospholipase C-β1 reduction leads to disturbances to beta and gamma oscillatory dynamics and prepulse inhibition behavior. The strong relationships between these measures demonstrate the importance of event-related oscillatory activity to sensorimotor gating behavior. However, dissociation of these measures observed in the drug studies suggests that abnormalities in neuronal networks may not necessarily need to be corrected for behavioral improvement.

Keywords: animal model, antipsychotic, electrophysiology, neural oscillations, PLC-B1 KO mice

Introduction

Schizophrenia is a complex neuropsychiatric disorder characterized by psychosis, socioaffective disturbances, and cognitive deficits. The underlying neural pathophysiology of schizophrenia is not clear, although several lines of evidence support the theory that abnormalities in coordinated neuronal activity play a central role in the disorder (Uhlhaas and Singer, 2015). In particular,

Significance Statement

High frequency oscillatory rhythms such as beta (12–30 Hz) and gamma (30–80 Hz) oscillations are abnormal in schizophrenia. These oscillatory rhythms are thought to be important for sensory and cognitive processing and it is therefore believed that abnormalities in high frequency oscillatory activity may contribute to sensory and cognitive processing deficits in schizophrenia. Mice lacking expression of the phosphodiesterase enzyme phospholipase C-β1 (PLC-β1 $^{-/-}$ mice) exhibit an array of behavioral disturbances considered relevant to schizophrenia, including deficient prepulse inhibition (PPI) as well as deficits in selective cognitive domains. This study demonstrated profound disturbances to high frequency oscillations in PLC-β1 $^{-/-}$ mice, and correlations were revealed between these electrophysiological disturbances and behavioral measures of PPI. Specifically, deficits in auditory evoked beta and gamma band responses were observed in PLC-β1 $^{-/-}$ mice and these deficits correlated with %PPI suggesting that disturbances to high frequency oscillatory activity may underlie abnormalities in sensorimotor gating.

neuronal oscillations in the higher frequency bands—namely beta (12–30 Hz) and gamma (30–80 Hz) oscillations—have gained an increasing amount of attention due to their close association with cognitive processes known to be impaired in schizophrenia, including sensory gating, perception, working memory, and attention (Tallon-Baudry and Bertrand, 1999; Herrmann et al., 2004; Hong et al., 2004). Indeed, many studies have demonstrated that gamma oscillatory dynamics are disrupted in patients with schizophrenia, with reductions in stimulus-evoked gamma oscillations and elevated background oscillations at rest being reported (Gandal et al., 2012). Although less studied, similar abnormalities in the beta frequency band have also been reported. Despite associative evidence linking these electrophysiological abnormalities with certain symptom domains, there remains a lack of direct causal evidence demonstrating the functional relevance of beta and gamma oscillatory perturbations to symptomology. In addition, the neural mechanisms underlying oscillatory abnormalities in schizophrenia are poorly understood, but an accumulation of evidence has suggested that these may be related to dysfunction in a subclass of GABAergic interneurons containing the calcium-binding protein parvalbumin. Importantly, these interneurons are thought to be impaired in schizophrenia (Lewis et al., 2005) and are necessary for the generation of gamma oscillations in vivo (Cardin et al., 2009).

Disturbances to a wide range of neurotransmitter systems have been implicated in schizophrenia, including dopaminergic (Creese et al., 1976), serotonergic (Lopez-Figueroa et al., 2004), muscarinic (Watanabe et al., 1983; Raedler et al., 2007), and glutamatergic signaling (Ohnuma et al., 1998). Interestingly, one point of convergence for these neurotransmitter systems is the phosphoinositide-specific phospholipase C (PLC) signaling cascade, one of the major G-protein-linked signal transduction pathways operating in the central nervous system. More specifically, the PLC-β1 subtype, the major neuronal isoform, is activated by several Gq-coupled receptors, including group 1 metabotropic glutamate receptors (mGluR1 and mGluR5), muscarinic receptors (M1, M3, and M5), and serotonergic receptors (5-HT2AR and 5-HT2CR) (Salles et al., 1993; Kim et al., 1997; Hannan et al., 2001). It is therefore important that the level of neuronal PLC-β1 is altered in postmortem tissue of patients with schizophrenia, with region-specific reductions in expression (Lin et al., 1999; Shirakawa et al., 2001) and genetic deletion in the orbito-frontal cortex of some patients being demonstrated (Lo Vasco et al., 2012).

In an attempt to elucidate the contribution of the PLC-β1 signaling pathway to schizophrenia pathogenesis, mice homozygous for a null mutation in PLC-β1 (PLCβ1$^{-/-}$) were found to exhibit an array of schizophrenia-relevant behavioral deficits, including hyperlocomotor activity (indicative of psychomotor agitation), impaired prepulse inhibition (PPI) of the acoustic startle, and disruptions to various aspects of cognition including working memory and spatial memory (Koh et al., 2008;

McOmish et al., 2008). While previous studies have assessed the behavioral effects of PLC-β1 deletion, few studies have looked at the effects on electrophysiological markers of clinical relevance to schizophrenia.

The aim of the present study was to investigate the role of PLC-β1 in the regulation of beta and gamma frequency oscillatory activity, and their association with sensorimotor gating, by characterizing oscillatory dynamics in PLC-β1$^{-/-}$ mice during PPI, a validated behavioral task assessing sensorimotor gating ability. Considering previous studies have shown that PPI deficits in PLC-β1$^{-/-}$ mice are ameliorated by antipsychotic drug administration (McOmish et al., 2008), we also assessed the effects of several antipsychotics on oscillatory activity and PPI to determine whether improvements in PPI are associated with changes in oscillatory activity. We hypothesized that PLC-β1$^{-/-}$ mice would exhibit oscillatory disturbances reminiscent of those observed in schizophrenia and that these would be related to deficits in PPI.

METHODS

Animals

Mice heterozygous for the PLC-β1 mutation were bred on a C57Bl6 background strain to produce PLC-β1 knockout and wild-type littermates at the Melbourne Brain Centre, University of Melbourne (McOmish et al., 2008). Genotypes were determined using PCR (primers available upon request from NCJ). The facility was maintained on a 12-h-light/-dark cycle (7:30 AM to 7:30 PM) with food and water available ad libitum. Mice were initially group-housed in single-sex groups of 2 to 5 until experiments were started, after which they were individually housed to avoid experimental loss. All the experimental procedures were approved by the Florey Institute Animal Ethics Committee (#14–054).

Electrode Implantation Surgery

At 12 to 14 weeks of age, PLC-β1$^{-/-}$ mice (n=13; 6 male, 7 female) and wild-type littermates (n=13; 8 male, 5 female) underwent stereotaxic surgery for implantation of extradural electrodes to facilitate recording of electrocorticography (ECoG) from the left frontal cortex. We chose to record local field potential signals from the left cortical surface only. When compared, our previous studies (Hakami et al., 2009; Jones et al., 2012, 2014, 2018; Anderson et al., 2014; Long et al., 2015; Hudson et al., 2016) have not observed any hemispheric differences in neural oscillations in both genetic and pharmacological models of schizophrenia, and due to the limitations of space on the mouse head, we were restricted from recording from only one hemisphere. We arbitrarily chose the left hemisphere, as opposed to randomizing between left and right hemispheres, due to possible variations

in oscillations that others have described (Shahriari et al., 2016). Extradural electrodes were custom-made by soldering small brass-plated pins (Ginder Scientific, Canada) to 1-mm anchor screws (Mr Specs Pty Ltd, Australia) as per our previous report (Long et al., 2015). Briefly, mice were anaesthetized with isoflurane (Abbott Australasia Pty Ltd, Australia), the mouse's head was secured using ear bars, and a midline incision was made to expose the skull. Three holes were drilled at the following locations: 2 mm anterior and 2 mm lateral to bregma (overlying the left frontal cortex)—active electrode; 2 mm anterior and ±2 mm lateral to lambda—reference and ground electrodes. The electrodes were screwed into each of the 3 holes with care taken not to breach the dura. Dental cement (Vertex Dental, Netherlands) was then applied to cover the skull, and the incision was sutured. Mice were allowed a minimum of 7 days recovery prior to any behavioral testing.

Live Animal Testing Protocol

Each experiment consisted of simultaneous recording of ECoG and measurement of PPI. At the start of each session, each mouse was placed inside the PPI chamber (San Diego Instruments, San Diego, CA), and the electrode pins were connected to a lightweight custom-made 3-channel cable. The PPI sessions began with a 30-minute acclimatization period followed by a 60-minute PPI session.

To determine whether any electrophysiological or behavioral phenotype was reversible, the effects of antipsychotic drugs were assessed in a subset of PLC-$\beta1^{-/-}$ mice (n=6). In these experiments, following the 30-minute acclimatization period, mice were removed from the PPI chamber and injected i.p. with haloperidol (0.25 mg/kg), clozapine (2.5 mg/kg), olanzapine (5 mg/kg), or vehicle (saline). Following injection, they were placed back into the PPI chamber for a 60-minute PPI session. Each animal received each treatment only once, and treatments were delivered in a random order, with at least 2 days in between treatments. Whereas the half-life of antipsychotics in humans is usually 12 to 24 hours, the half-life of these drugs is 4 to 6 times faster in rodents (Kapur, VanderSpek et al. 2003). Specifically, the half-lives in rodents of the antipsychotics drugs tested in the current study are as follows: haloperidol (1.5 hours) (Cheng and Paalzow, 1992), clozapine (1.5 hours) (Baldessarini et al., 1993), and olanzapine (2.5 hours) (Aravagiri et al., 1999). As such, a 48-hour interval between dosing should be sufficient to allow for elimination of drug between testing sessions.

PPI Measurement

PPI was measured using an SR-Lab acoustic startle chamber (San Diego Instruments). The sessions consisted of pulse-alone trials (115-dB startle pulse of 40-millisecond duration) interspersed with prepulse+pulse trials (78-dB pulse of 20 milliseconds preceding the startle pulse by 100 milliseconds – background 70 dB). Within a given session, 50% of trials were pulse-alone trials and the other 50% were prepulse+pulse trials, presented in a pseudorandom order with an average intertrial interval of 15 seconds. At the beginning of each session, the animals were connected to the EEG equipment and placed in a clear plexiglass cylinder with an accelerometer attached to its base. The startle responses for each trial were recorded and percentage PPI (%PPI) calculated according to the following formula: [100 × (average amplitude of pulse-alone trials – amplitude of prepulse+pulse trials) / amplitude of pulse-alone trials]. In addition, a block of pulse-alone trials was presented at the start and end of each

session to enable calculation of startle habituation [100 × (first block average – last block average)/first block average]. We used one prepulse intensity for this study to simplify the analysis of evoked oscillations occurring as a result of the prepulse (Jones et al., 2014). A weak-moderate prepulse stimulus of 8 dB above background was chosen, which in our experience reliably elicits PPI in rodents (Hudson et al., 2016) while minimizing the potential for any startle responses itself. Previous studies in mice have shown negligible startle responses with auditory stimulus <20 dB above background (Weber et al., 2015; Li et al., 2018).

ECoG Acquisition

ECoG acquisition utilized a Powerlab A-D converter and bioamplifiers (ADinstruments, Bella Vista, NSW, Australia) using Chart V 4.5 software (ADInstruments). The sampling frequency was set at 1000 Hz. Electrical noise (50 Hz) emanating from the power mains was controlled using selective eliminators (Humbugs; Digitimer, Letchworth Garden City, UK). Throughout the session, raw ECoG data were continuously recorded from the left frontal cortex. The PPI amplifier was linked to the AD-converter to allow for precise time-locking of the auditory stimuli and any resultant electrophysiological response.

Analyzing Oscillations

To assess oscillatory activity, the raw ECoG signal was processed using a custom-written MATLAB script (v7.10.0, Natick, MA; The MathWorks, 2010). We extracted 2 primary outcomes from the data: ongoing oscillatory activity and event-related oscillatory activity elicited by the prepulse stimulus, as per our previous study (Jones et al., 2018). For the measurement of ongoing oscillations, the continuous raw ECoG data were sectioned into 2-second epochs and each epoch was subjected to Fast Fourier Transforms using the MATLAB pwelch function to calculate the Power Spectral Density estimate. Each Power Spectral Density (PSD) estimate was then averaged over the entire 60-minute PPI session. Ongoing power in the beta (12–30 Hz) and gamma (30–80 Hz) frequency bands was subsequently calculated for each session by averaging all the absolute power values within the appropriate frequency interval. Logarithmic transformation of this ongoing oscillatory data was only used for graphical representation (i.e., Figure 3a) and hence presented as dB/Hz. However, absolute power values (μV^2/frequency band) were used for statistical analyses.

For the measurement of event-related oscillatory activity, the ECoG was subjected to time-frequency analysis to assess evoked oscillations elicited by the auditory prepulse. We specifically focused on the prepulse as we have previously shown this correlates with sensorimotor gating behavior (Jones et al., 2014). The onset of the prepulse was set at t=0. Single trial epochs (–400 to +600 ms) for every prepulse+pulse trial were extracted from the continuous raw ECoG data and subjected to morlet wavelet decomposition using the EEGlab newtimef function using 180 linearly spaced frequencies from 20 to 200 Hz with wavelet cycles increasing from 3 to 10. Two outcomes were then calculated from this decomposition: evoked power and inter-trial coherence. To compute evoked power, we used the newtimef function in EEGLAB. This takes the log of post-stimulus power in each trial (defined as 0 to +100 ms relative to the prepulse) and averages these. Then it takes the log of the prestimulus power in each trial (–300 to 0 ms relative to the prepulse) and averages these. It then calculates the difference of log-poststimulus vs log-prestimulus periods, which is equivalent to the logarithm

of the ratio of power, and multiplies this by 10 to get dB. The formula for this calculation is $dB = 10*(\log[P_{post}] - \log[P_{pre}])$. Therefore, any power elevation from baseline has a ratio >1 (i.e., >0 dB), and any reduction from this has a ratio of <1 (i.e., <0 dB). The newtimef function in MATLAB also provides the inter-trial coherence values, relative to baseline (see Figure 2d).

Prepulse-evoked beta power and beta inter-trial coherence were then calculated by averaging all values between 12 and 30 Hz occurring 0 to 100 ms relative to the onset of the prepulse. Similarly, prepulse-evoked gamma power and gamma inter-trial coherence were calculated by averaging all the values between 30 and 80 Hz. Inter-trial coherence was expressed as a unitless ratio between 0 and 1 and measured the degree of phase synchronization occurring consistently between subsequent trials. A score of 0 represents an absence of synchronization between repeated ECoG responses occurring as a result of the prepulse stimulus, whereas a score of 1 represents perfect phase synchronization across evoked responses.

Drugs

Clozapine (Sigma, St. Louis, MO) and olanzapine (AbCamBiochemicals, Sapphire Biosciences, Redfern, NSW, Australia) were dissolved using pure acetic acid diluted to make 10% acetic acid solution and adjusted to pH 6.0. Haloperidol solution (Sigma) was diluted in 0.9% saline. Drug doses were informed from previous studies in mice (McOmish et al., 2008; Mutlu et al., 2011).

Statistical Analyses

All datasets were first tested for normality using the Kolmogorov-Smirnov test. With the exception of startle amplitude and all evoked datasets, all measures followed a normal distribution and so parametric analyses were used. For the evoked datasets, data was first log-transformed (see "Analyzing Oscillations" above for more detail). In this case, after undergoing logarithmic transformation, these datasets were all normally distributed; transformed datasets were then analyzed using parametric tests.

As males and females were both used, analysis of the effect of gender on all measures was first assessed using 2-way ANOVA. This analysis revealed no significant effect of gender on all measures (data not provided), and as such all subsequent analysis was performed on the combined data from both male and female mice. For assessment of effects of genotype on electrophysiological and behavioral outcomes, Student's unpaired t

test was used. Effects of antipsychotic drugs on behavioral and electrophysiological data were assessed using 1-way ANOVA with repeated measures (drug treatment) and Bonferroni's post-hoc test used where appropriate. For startle amplitude, a Mann-Whitney test was used to assess the effects of genotype, and a Friedman test with Dunn's multiple comparison test was used to assess the effects of antipsychotic drugs on startle amplitude.

Pearson's correlation coefficients were calculated to assess the inter-relationships between electrophysiological and behavioral outcomes. This analysis incorporated both genotypes. In all cases, statistical significance was defined as $P < .05$. Analyses were performed using Graphpad Prism 6.0 (La Jolla, CA); with the exception of the startle response data, all data represent mean ± SEM. For startle response, data represents median ± interquartile range.

RESULTS

PLC-β1 Gene Deletion Causes Deficits in Sensorimotor Gating

PLC-β1$^{-/-}$ mice (n = 13) showed significantly reduced levels of PPI of the acoustic startle compared with wild-type littermates (n = 13) ($t_{(24)} = 2.85$, $P = .008$; Figure 1A). In addition, we identified a significant effect of genotype on the amplitude of the acoustic startle response with significantly lower startle in the PLC-β1$^{-/-}$ mice relative to wild type (U = 28.00, $P = .0041$; Figure 1B). No significant difference was observed for habituation of the startle response over time ($t_{(24)} = 1.91$, $P = .25$; Figure 1C).

PLC-β1 Gene Deletion Disturbs Auditory-Evoked Oscillatory Power and Inter-Trial Coherence

Next, we studied electrophysiological outcomes occurring during the PPI session. Event related spectral perturbation associated with the prepulse trials was first assessed for each genotype (visualized in Figure 2A). We quantified both beta power (12–30 Hz) and gamma power (30-80 Hz) evoked by the prepulse and found that PLC-β1$^{-/-}$ mice had dramatically diminished evoked power in beta ($t_{(24)} = 8.32$, $P < .0001$; Figure 2B) and gamma ($t_{(24)} = 4.59$, $P < .0001$; Figure 2C) bands compared with wild-type littermates. To complement this analysis, we also assessed inter-trial coherence (Figure 2D), a measure independent of evoked power and which is indicative of the consistency of time-locking of an oscillatory event to a stimulus. Striking reductions in beta coherence ($t_{(24)} = 10.82$; $P < .0001$; Figure 2E) and
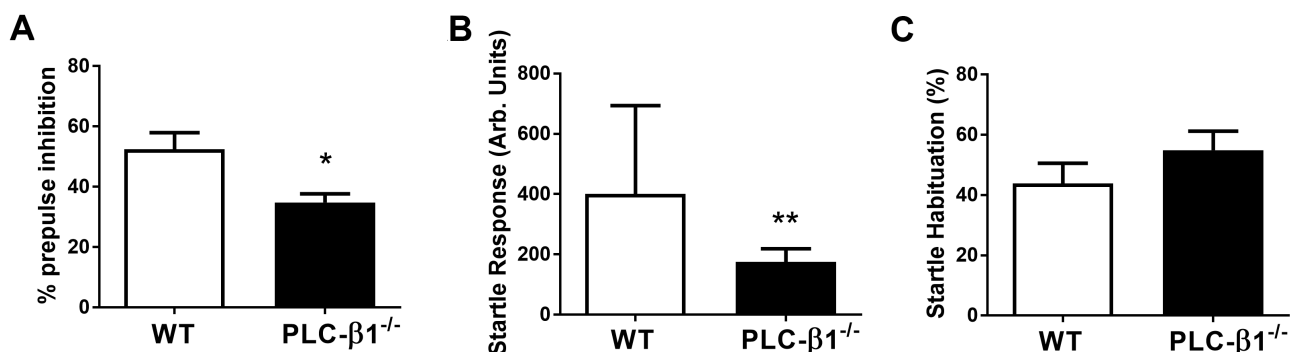

Figure 1. Effect of genotype on behavioral measures of sensorimotor gating. (A) Percentage prepulse inhibition (%PPI) was significantly reduced in phospholipase C-β1 knockout (PLC-β1$^{-/-}$) mice, relative to wild-type (WT) littermates (*$P < .05$). (B) The magnitude of the startle response was significantly reduced in PLC-β1$^{-/-}$ mice compared with WT (**$P < .01$). (C) There was no effect of genotype on habituation to startle response.
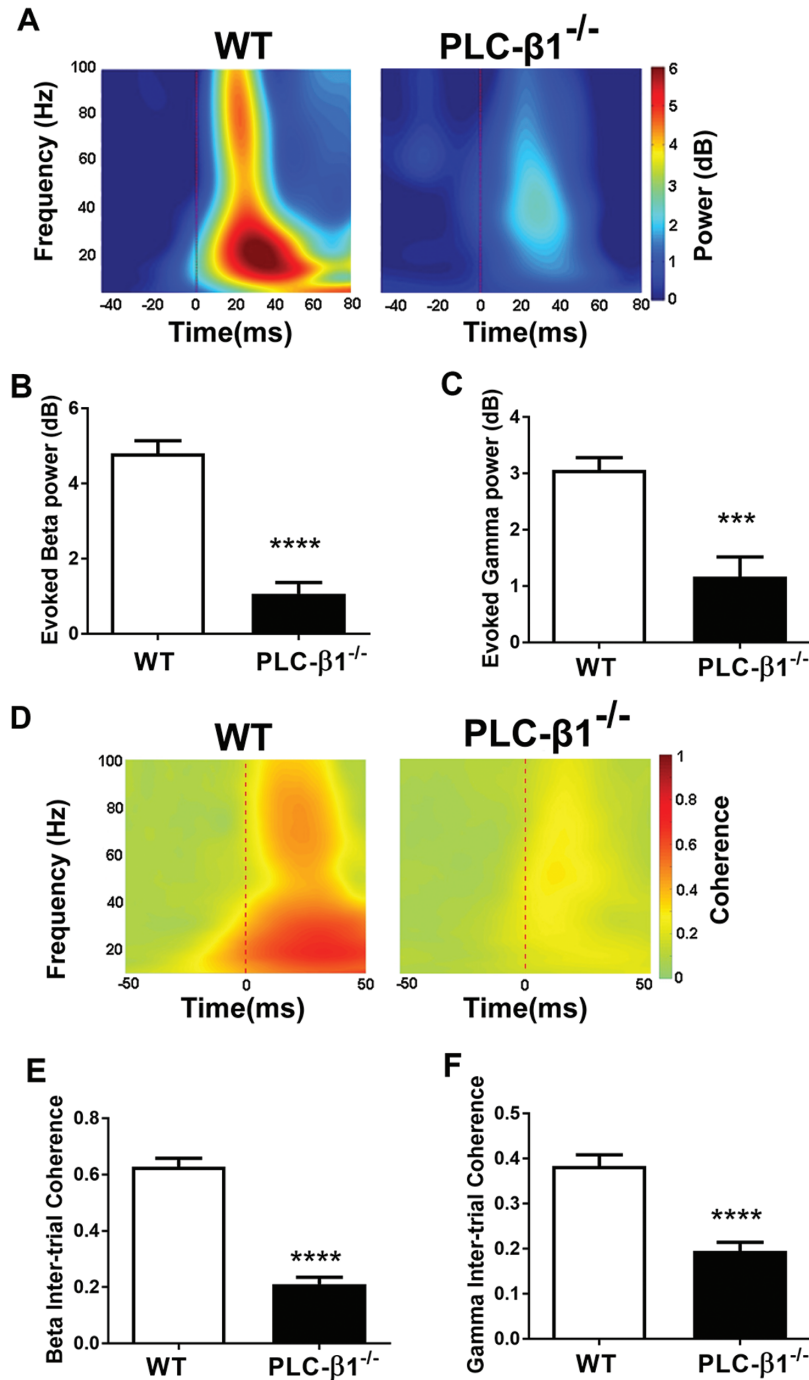
Figure 2. Effects of genotype on the power and inter-trial coherence of auditory-evoked oscillations. (A) Grand average evoked heat maps of normalized oscillatory power resulting from the auditory stimuli for wild-type (WT) mice (left) and phospholipase C-β1 knockout (PLC-β1$^{-/-}$) mice (right). Evoked beta power (B) and evoked gamma power (C) are significantly reduced in PLC-β1$^{-/-}$ mice relative to WT mice. (D) Grand average heat maps of inter-trial coherence associated with the auditory stimulus for WT mice (left) and PLC-β1$^{-/-}$ mice (right). Both beta (E) and gamma (F) inter-trial coherence are significantly reduced in PLC-β1$^{-/-}$ mice relative to WT (***$P < .05$, ****$P < .001$). The red vertical line on all images represents the onset of the auditory tone in A and D.

gamma coherence ($t_{(24)} = 6.01$, $P < .0001$; Figure 2F) were also demonstrated in the PLC-β1$^{-/-}$ mice.

PLC-β1 Deletion Alters Spontaneous ECoG Spectral Power

We then assessed the effect of genotype on ongoing oscillatory activity. The PSD estimates were averaged and used to generate PSD plots characterizing ongoing power from 1 to 100 Hz. There

were notable differences between the plots, with higher levels of ongoing power observed in the PLC-β1$^{-/-}$ mice over a broad range of frequencies (Figure 3A). Power values specifically in the beta (12–30 Hz) and gamma (30–80 Hz) bands were then isolated and compared. Compared with wild-type littermates, PLC-β1$^{-/-}$ mice had significantly higher levels of ongoing beta power ($t_{(24)} = 4.16$, $P = .0004$; Figure 3B) as well as higher levels of ongoing gamma power in the PLC-β1$^{-/-}$ mice, although this did not reach statistical significance ($t_{(24)} = 1.58$, $P = .1264$; Figure 3C). Total power was
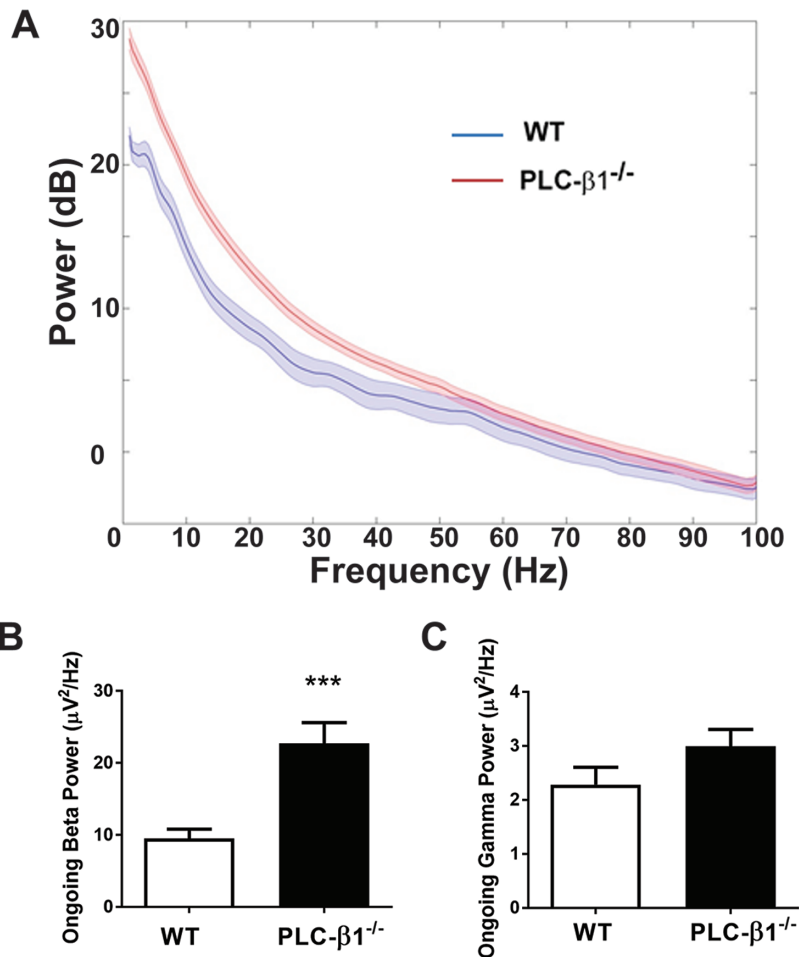
Figure 3. Effects of genotype on ongoing oscillatory activity. (A) Grand average power spectral density estimates (1–100 Hz) for phospholipase C-β1 knockout (PLC-β1$^{-/-}$) (red trace) and wild-type (WT) (blue) mice shows elevated power over a broad range of frequencies in PLC-β1$^{-/-}$ mice. Shaded areas indicate SEM. (B) Ongoing β power is significantly elevated in PLC-β1$^{-/-}$ mice (***$P < .001$). (C) Ongoing γ power was reduced, but not significantly different from PLC-β1$^{-/-}$ mice.

also significantly higher in PLC-β1$^{-/-}$ mice ($t_{(24)} = 8.46$, $P < .0001$; data not shown).

Evoked Oscillatory Power and ITC Are Highly Correlated with PPI

Correlational analysis related electrophysiological measures with corresponding PPI for each session (excluding sessions involving antipsychotic treatment). A significant positive correlation was found when relating %PPI to evoked beta power ($r = 0.57$, $P = .002$; Figure 4A), evoked gamma power ($r = 0.55$, $P = .004$; Figure 4B), beta coherence ($r = 0.52$, $P = .006$; Figure 4C), and gamma coherence ($r = 0.66$, $P < .001$; Figure 4D). For measures of ongoing power in both the beta (Figure 4E) and gamma (Figure 4F) frequency band, no significant correlations were observed when relating ongoing power measures with %PPI ($P > .05$), suggesting that these do not influence this behavior.

Antipsychotic Drugs Restore PPI, but Not Oscillatory Deficits, in PLC-β1$^{-/-}$ Mice

To determine whether antipsychotic drugs could reverse the PPI deficits, we assessed the effect of antipsychotic drug treatment

in a subset of PLC-β1$^{-/-}$ mice (n = 6). A significant effect of antipsychotic drug on PPI was found ($F_{(3, 15)} = 11.38$, $P = .0057$; Figure 5A). Posthoc comparisons demonstrated an enhancement of PPI following treatment with clozapine ($P < .001$) and olanzapine ($P < .01$) but not haloperidol ($P > .05$) compared with vehicle treatment. In addition, a nonparametric Friedman test of differences among repeated measures was conducted and rendered a chi-square value of 10.40, which was significant ($P = .0085$), indicating an overall significant effect of antipsychotic drug treatment on the startle response.

We next assessed the effects of antipsychotic drug treatment on evoked oscillatory power in PLC-β1$^{-/-}$ mice. Despite reversing PPI deficits, no significant effect of treatment was found for evoked beta power ($F_{(3, 15)} = 0.22$, $P = .88$; Figure 6A) or evoked gamma power ($F_{(3, 15)} = 1.45$, $P = .26$; Figure 6B). Similarly, antipsychotic drug treatment had no effect on beta coherence ($F_{(3, 15)} = 2.54$, $P = .09$; Figure 6C) or gamma coherence ($F_{(3, 15)} = 0.37$, $P = .77$; Figure 6D) in PLC-β1$^{-/-}$ mice. We did, however, identify significant effects of treatment on ongoing beta oscillations ($F_{(3, 15)} = 4.81$, $P = .015$; Figure 6E) and ongoing gamma oscillations ($F_{(3, 15)} = 3.98$, $P = .028$; Figure 6F). Posthoc analysis demonstrated that olanzapine significantly reduced ongoing beta power ($P < .05$) as well as ongoing gamma power ($P < .05$), normalizing these deficits observed in PLC-β1$^{-/-}$ mice.
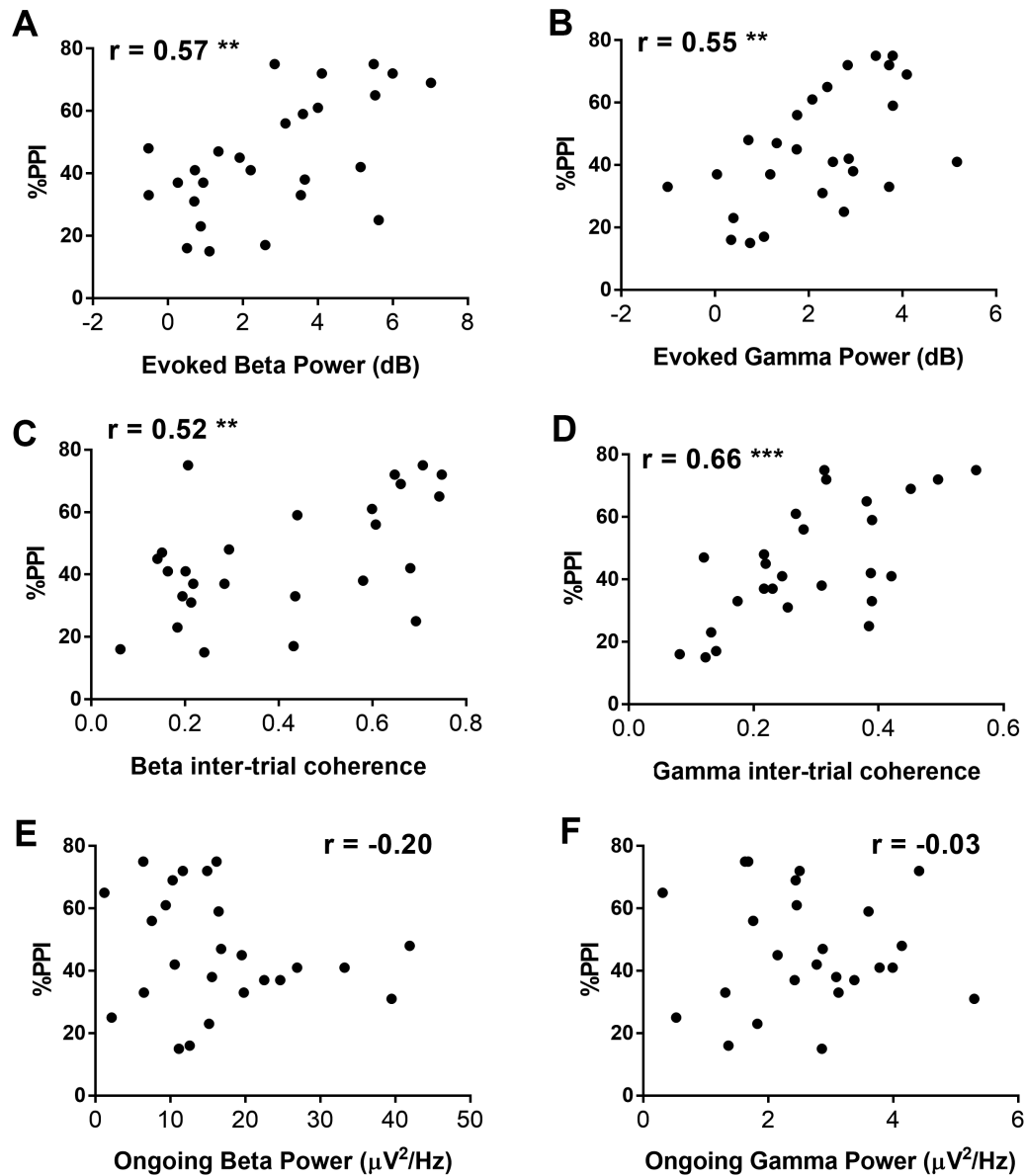
Figure 4. Correlation plots showing significant relationships between Percentage prepulse inhibition (%PPI) and electrophysiological measures, including (A) evoked beta power, (B) evoked gamma power, (C) beta inter-trial coherence, and (D) gamma inter-trial coherence, but not (E) ongoing beta power and (F) ongoing gamma power. Pearson's coefficient values (r) are given for each relationship. Open squares indicate data from phospholipase C-β1 knockout (PLC-β1⁻/⁻) mice, and closed circles are wild-type (WT) data. **P < .01, ***P < .001.
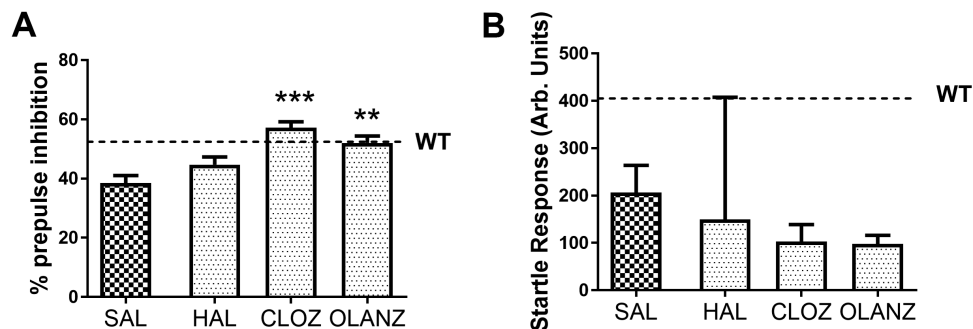

Figure 5. Effects of antipsychotic drug treatment on prepulse inhibition (PPI) in phospholipase C-β1 knockout (PLC-β1⁻/⁻) mice. (A) Treatment of PLC-β1⁻/⁻ mice with clozapine (CLOZ, 2.5 mg/kg) and olanzapine (OLANZ, 5 mg/kg) but not haloperidol (HAL, 0.25 mg/kg) significantly improved PPI relative to saline (SAL)-treated animals (**P < .001;***P < .001). (B) Treatment of PLC-β1⁻/⁻ mice with HAL, CLOZ, or OLANZ did not significantly influence startle magnitude compared with saline treatment. Dotted line represents average levels observed in wild-type (WT) littermate mice.
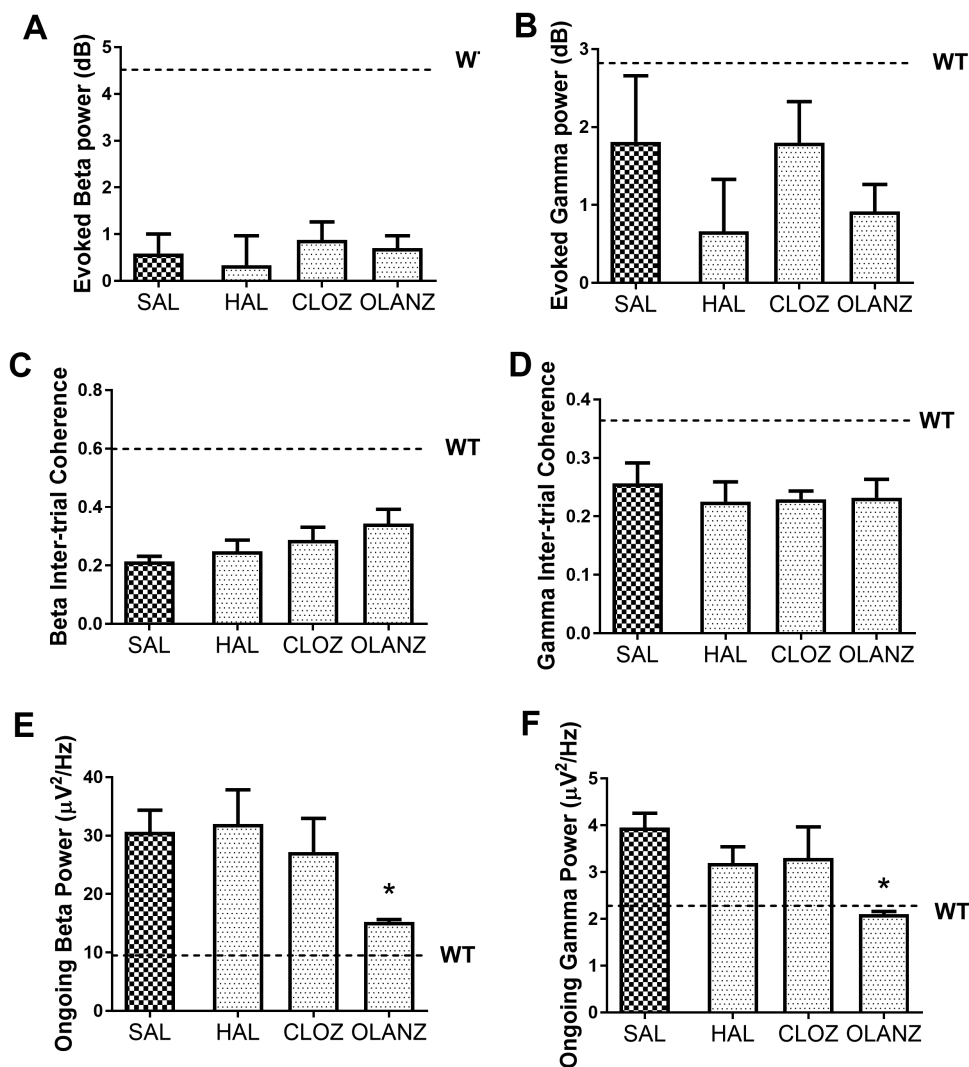
Figure 6. Effects of antipsychotic drug treatment on oscillatory responses in phospholipase C-β1 knockout (PLC-β1⁻/⁻) mice. Treatment of PLC-β1⁻/⁻ mice with haloperidol (HAL, 0.25 mg/kg), clozapine (CLOZ, 2.5 mg/kg) and olanzapine (OLANZ, 5 mg/kg) had no significant effect on (A) evoked beta power, (B) evoked gamma power, (C) beta ITC or (D) gamma ITC, when compared to treatment with saline (SAL). However, ongoing β power (E), and ongoing gamma power (F), are significantly reduced in PLC-β1⁻/⁻ mice following treatment with olanzapine (OLANZ, 5 mg/kg) (*P < .05). Dotted line represents average levels in wild-type littermates (WT).

Discussion

Here, we demonstrate that mice lacking PLC-β1 exhibit abnormalities in beta and gamma frequency oscillatory activity compared with wild-type controls. Specifically, PLC-β1⁻/⁻ mice have a deficiency in the power and phase-locking of auditory-evoked beta/gamma oscillations elicited by the prepulse stimulus during PPI as well as an elevation in the power of ongoing oscillations in the beta frequency band, and these electrophysiological abnormalities are accompanied by sensorimotor gating behavioral deficits. Importantly, evoked beta/gamma oscillatory power and phase-locking are highly correlated with PPI, suggesting that deficient beta/gamma oscillatory responses in PLC-β1⁻/⁻ mice are closely associated with deficient PPI. This agrees with our previous studies using pharmacological models of disease (Jones et al., 2014; Hudson et al., 2016). Given post-mortem findings of reduced PLC-β1 expression in schizophrenia (Lin et al., 1999), these findings enhance the face validity of this mouse model and promote its use for studying the relationship

between PLC-β1 expression and oscillatory abnormalities that are relevant to schizophrenia.

The most apparent electrophysiological finding from this study was that PLC-β1⁻/⁻ mice exhibited a reduction in the power and phase-locking (i.e., inter-trial coherence) of evoked beta and gamma oscillations elicited by auditory stimuli (specifically, the prepulse) during PPI. Importantly, the prepulse is of sufficiently high volume to elicit an electrophysiological response, but 8 dB above background, which is too low to elicit any behavioral (startle) responses itself (Weber et al., 2015; Li et al., 2018), so these measures of evoked oscillations are unlikely to be influenced by movement. While measures of evoked power and coherence both provide information regarding the oscillatory dynamics associated with a particular stimulus, they are independent—a reduction in evoked power indicates a reduction in the amplitude of an event-related oscillatory response, whereas a reduction in inter-trial coherence is indicative of a discrepancy in time-locking of the oscillatory event to the stimulus of interest. Both measures are thought to have functional relevance and both are perturbed in

schizophrenia: auditory-evoked gamma oscillatory deficits are one of the most consistent oscillatory abnormalities reported in schizophrenia, with a large number of studies demonstrating a reduction in the power and phase-locking of auditory-evoked gamma oscillations in patients (e.g., Leicht et al., 2010, 2015). Although receiving less attention, deficits in auditory-evoked beta oscillatory power and phase-locking have also been reported (Hong et al., 2004). Our findings are also somewhat consistent with one previous study that assessed oscillatory activity in PLC-β1⁻/⁻ mice (Shahriari et al., 2016). This previous study demonstrated a reduction in power and phase-locking of beta and gamma frequency oscillations resulting from an auditory entrainment paradigm. Intriguingly, these authors also found that hemispheric coherence was reduced in the left but not right frontal regions. In our study, since we recorded from only a single brain region, we could not investigate effects of antipsychotics on regional or hemispheric differences, such as those identified previously in these mice.

We also observed a significant increase in the power of ongoing beta oscillations and a trend towards higher levels of ongoing gamma power in PLC-β1⁻/⁻ mice. Importantly, elevations in ongoing gamma activity (Winterer et al., 2004; Venables et al., 2009; Spencer, 2011) as well as ongoing beta activity (Karson et al., 1988) have also been reported in schizophrenia, and this may be indicative of an increase in background neural noise that may overwhelm any transient elevations in oscillatory activity involved in the processing of sensory or cognitive stimuli (Hakami et al., 2009). As such, in summary, PLC-β1 deletion is sufficient to produce both a reduction in auditory-evoked beta/gamma band responses and an elevation in ongoing cortical beta synchrony, thereby replicating oscillatory disturbances reported in schizophrenia.

While abnormalities in event-related oscillatory dynamics were highly correlated with PPI, the ability for antipsychotic drugs to normalize PPI was not accompanied with normalization of oscillatory activity. This is demonstrated most distinctly by the ability of clozapine to normalize PPI, yet this drug did not alter any of the oscillatory measures. This suggests that, while PPI deficits may be closely tied to the oscillatory disturbances observed in PLC-β1⁻/⁻ mice, the ability for antipsychotic drugs to normalize PPI is mediated by mechanisms distinct from those that cause oscillatory activity deficits. The ability for clozapine but not haloperidol to normalize PPI in PLC-β1⁻/⁻ mice is consistent with one previous study (McOmish et al., 2008), but not another (Koh et al., 2008). It is difficult to reconcile these differences considering the similar doses of drug being used; however, the mice used in our study were obtained from the same parent colony as McOmish et al., and so it is possible that genetic drift of the mouse lines between our colony and that of Koh et al. may have influenced the responsivity to haloperidol. Of note, muscarinic transmission at M1 and M4 receptors is known to be deficient in PLC-β1⁻/⁻ mice (McOmish et al., 2008) and M1/M4 deletion has been shown to impair PPI (Thomsen et al., 2010). It is therefore of interest that clozapine and olanzapine have appreciable affinity for M1/M4, where they are thought to act as partial agonists (Miyamoto, 2012). As such, it is possible that the ability for these drugs, but not haloperidol, to normalize PPI in PLC-β1⁻/⁻ mice may be attributed to enhanced muscarinic neurotransmission, thereby alleviating the muscarinic deficiency in these mice, although further studies would be required to demonstrate this.

As mentioned previously, PLC-β1 is activated by numerous neurotransmitter systems, including glutamatergic, cholinergic, and serotonergic systems. This represents a strength of this model, as schizophrenia is associated with disturbances to a wide range of neurotransmitter systems. Moreover, PLC-β1

activation results in a complex Gq-coupled intracellular signaling cascade with a vast number of consequences to cellular processes. This lack of neuronal specificity and diversity of function of PLC-β1 makes speculation difficult as to the neuronal basis underlying phenotypic effects of PLC-β1 deletion observed here. One candidate mechanism responsible for the disturbances to gamma oscillatory activity observed in the present study is NMDAr hypofunction. First, NMDAr antagonists, such as ketamine and MK-801, produce phenotypically similar disturbances to gamma oscillatory activity to those observed in the present study—that is, an elevation in ongoing gamma activity as well as a deficit in sensory-evoked gamma oscillations (Jones et al., 2014; Anderson et al., 2017), and evidence supports a role for NMDA receptor hypofunction in PLC-β1⁻/⁻ mice (Gray et al., 2009). Upon activation of Gq-coupled receptors, PLC-β1 initiates a signal cascade, which, among other things, leads to activation of protein kinase C, which is known to potentiate NMDA receptor activity by increasing the opening time of NMDA receptor channels and upregulating synaptic NMDA receptor expression (Lu et al., 1999; Lan et al., 2001). It is therefore conceivable that mice lacking the PLC-β1 enzyme may exhibit reductions in the expression and activity of NMDA receptors. Indeed, PLC-β1⁻/⁻ mice are sensitive to the behavioral effects of the NMDAr antagonist MK-801, and radioligand binding has shown that these mice have reduced NMDAr expression in the hippocampus, suggesting that NMDAr hypofunction may be central to the schizophrenia-relevant phenotype observed in these mice (Gray et al., 2009).

One methodological aspect worth considering is whether repeated dosing of various antipsychotics alters neural circuitry and induces adaptive changes that may influence electrophysiological outcomes, since persistent antipsychotic treatment has been shown to alter glutamatergic and dopaminergic systems. For instance, changes in NMDAr-mediated activity have been observed following 21 days dosing of haloperidol and olanzapine (Duncan et al., 2003) or 28 days dosing of clozapine (Tarazi et al., 1996). However, to our knowledge, there is no evidence to indicate that acute dosing regimens of antipsychotics induce adaptive changes in neural circuits. Moreover, we have previously assessed the effects of repeated exposure of rats administered acute doses of antipsychotic drugs including haloperidol, clozapine, and olanzapine and found no effect of repeated sessions on PPI, ongoing gamma power, or evoked gamma power (Hudson et al., 2016). Based on this, it is unlikely that this markedly influenced the outcomes of our research.

To summarize, our results expand upon our understanding of the consequences of reduced PLC-β1 expression relevant to schizophrenia. High-frequency oscillatory abnormalities that closely resemble those reported in patients with schizophrenia were observed in this genetic model. These electrophysiological findings closely parallel findings from clinical studies in schizophrenia and thus enhance the face validity of PLC-β1⁻/⁻ mice representing an animal model with relevance to schizophrenia. In line with previous studies, PLC-β1⁻/⁻ mice displayed sensorimotor gating impairment, a deficit highly correlated with event-related oscillatory dynamics within the beta and gamma frequency range. Future studies examining the potential relevance of oscillatory impairments to measures of higher order cognition in PLC-β1⁻/⁻ mice may provide greater insight into the functional relevance of these oscillatory abnormalities

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Statement of Interest

None.

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