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Acute phencyclidine alters neural oscillations evoked by tones in the auditory cortex of rats

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Abstract

Background/Aims—The onset response to a single tone as measured by electroencephalography (EEG) is diminished in power and synchrony in schizophrenia. Because neural synchrony, particularly at gamma frequencies (30–80 Hz), is hypothesized to be supported by the *N*-methyl-d-aspartate receptor (NMDAr) system, we tested whether phencyclidine (PCP), an NMDAr antagonist, produced similar deficits to tone stimuli in rats.

Methods—Experiment 1 tested the effect of a PCP dose (1.0, 2.5, and 4.5 mg/kg) on response to single tones on intracranial EEG recorded over the auditory cortex in rats. Experiment 2 evaluated the effect of PCP after acute administration of saline or PCP (5 mg/kg), after continuous subchronic administration of saline or PCP (5 mg/kg/day), and after a week of drug cessation. In both experiments, a time-frequency analysis quantified mean power (MP) and phase locking factor (PLF) between 1 and 80 Hz. Event related potentials were also measured to tones, and EEG spectral power in the absence of auditory stimuli.

Results—Acute PCP increased PLF and MP between 10 to 30 Hz, while decreasing MP and PLF between ~50–70Hz. Acute PCP produced a dose-dependent broadband increase in EEG power that extended into gamma range frequencies. There were no consistent effects of subchronic administration on gamma range activity. Acute PCP increased ERP amplitudes for the P16 and N70 components.

Conclusions—Findings suggest that acute PCP induced NMDAr hypofunction has differential effects on neural power and synchrony which vary with dose, time-course of administration and

EEG frequency. EEG synchrony and power appear to be sensitive translational biomarkers for disrupted NMDAr function, which may contribute to the pathophysiology of schizophrenia and other neuropsychiatric disorders.

Keywords

NMDA receptor antagonism; phencyclidine; auditory ERP; neural synchrony; schizophrenia; rat model

Introduction

Schizophrenia is characterized by both auditory hallucinations and distortions in auditory perception [1]. Consistent with these subjective and behavioral disturbances of auditory processing, one of the most common neurobiological findings in schizophrenia is reduction in the amplitude of N100 components of the event-related potential (ERP) elicited by tones or clicks [2]. While the majority of studies of ERPs in schizophrenia have measured these responses in the time domain, there has been increasing interest in utilizing time-frequency analytic techniques to differentiate the early auditory response between frequency bands. The gamma frequency band (30–80 Hz) has been of specific interest because it has been associated with the neural processing of sensory stimuli as well as perceptual and cognitive integration [3,4].

In individuals with schizophrenia, a decrease in gamma range power [3,5–8] or phase-locking factor [9] in the EEG after the onset of single tones has been observed by most [9] but not all studies [10,11]. Hall et al. (2011) used a twin design and found that auditory gamma-band power and phase locking to standard tones in an auditory oddball paradigm were heritable responses associated with schizophrenia: both patients with schizophrenia and their non-psychotic co-twins showed reduced gamma power [3]. The pathophysiological mechanisms responsible for deficits in auditory gamma-band activity in schizophrenia have focused on the role of the parvalbumin-positive gamma-amino butyric acid (GABA) receptor and to *N*-methyl-d-aspartate receptor (NMDAr) [4,12–14]. Both in vitro and in vivo evidence suggest that excitatory principal neurons, parvalbumin inhibitory interneurons, subtype A of the gamma-amino butyric acid receptor family (GABA_A), and NMDAr modulate neural synchrony in the gamma frequency range [15–18]. NMDAr antagonists are believed to block NMDA receptors on GABAergic neurons largely in the cortic limbic, thalamocortical and intracortical neural circuits, causing disinhibition of pyramidal neurons and an increase in dopamine in the prefrontal cortex (PFC) [19,20]. Since NMDAr abnormalities have been implicated in the pathophysiology of schizophrenia [21], it has been argued that NMDAr hypofunction may contribute to the observed deficit in neural synchrony in the gamma frequency range (40–50 Hz) in responses evoked by auditory stimuli [4,9,13]. The effects of NMDAr antagonists on gamma oscillations, however, vary with experimental manipulations, paradigms, and recording procedures. Ex vivo recordings from mice [22] and rats [23] indicate that chronic ketamine exposure suppresses spontaneous gamma oscillations. However, acute in vivo administration of NMDAr antagonists generally increases spontaneous gamma activity in rodent electroencephalography (EEG) and local field potentials (LFP) [17,24].

Few studies have examined the effect of NMDAr antagonism or hypofunction on gamma activity evoked by single tones or clicks, and results have varied across paradigms. Gandal and colleagues (2012) tested mice that were genetically engineered to have an 85% downregulation of the NMDAr system [25]. Compared to wild-type mice, the NMDAr knock-down mice demonstrated selective reduction of gamma range power to click stimuli, suggesting NMDAr hypofunction as a possible mechanism for impaired gamma neural oscillatory behavior. In contrast, Ehrlichman et al. (2009) found that acute pharmacologic administration of ketamine in mice did not have a significant effect on evoked gamma power to click stimuli [26]. In humans, Hong et al. (2010) found that subanesthetic ketamine increased gamma-band oscillations elicited by click stimuli in a sensory gating paradigm [27]. One factor that may account for differences among studies is the dose of the NMDAr antagonist or degree of genetically manipulated receptor hypofunction. With auditory steady state responses (ASSRs), for example, high levels of NMDAr blockade may be required to reduce gamma synchrony [14,28]. Secondly, subchronic or chronic administration using either multiple single doses or continuous delivery of NMDAr antagonists may have quite different physiological effects [29]. Studies using MK-801 [30] and PCP found that acute, but not subchronic administration of a NMDAr antagonist, reduced ASSR gamma phase synchrony in rats [30][28].

The aim of the present study was designed to address these issues, examining intracranial EEG responses measured in both the time and frequency domains. In Experiment 1, the effects of three different subcutaneous doses of PCP (1, 2.5, and 4 mg/kg) were evaluated. In Experiment 2, the effects of acute and subchronic administration of PCP were evaluated. In both experiments, three electrophysiological measures were characterized including time domain evoked responses to tones, time-frequency measures of evoked power and phase synchronization to tones, and the frequency power spectrum in the absence of tone stimuli. It was hypothesized that 1) acute PCP would increase the amplitude of evoked responses in the time domain and decrease responses after subchronic administration, 2) both acute and chronic administration of PCP would suppress gamma activity in the frequency domain and 3) acute PCP would produce a broadband increase in EEG spectral power in the absence of tone stimuli, consistent with prior studies. This study makes unique contributions in that it evaluates evoked responses to tones 1) at different levels of NMDAr pharmacological antagonism, 2) in both the time and frequency domains, 3) for both acute and subchronic PCP administration and 4) in comparison with non-evoked (resting) EEG activity.

Materials and Methods

Ethics

The research facilities were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Indiana University (Reference number: 0000003253) in compliance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The minimal numbers of animals required were used, and efforts were made to minimize all pain and suffering. An isoflurane/air mixture was used for general anesthesia during surgical procedures, and methods of

euthanasia were based on recommendations of the Panel of Euthanasia of the American Veterinary Medical Association.

Animals

Adult male (300 g) Sprague-Dawley rats were obtained from Harlan Laboratories (Indianapolis, IN) and were acclimated to the facilities for seven days before being individually housed. Food and water were available ad libitum.

Electrode implantation

The procedure for electrode implantation was completed as described in Leishman et al. (2015) [28]. In short, at 12 weeks old (369 ± 6 g), rats were anesthetized and stainless steel screw electrodes were epidurally implanted over the temporal cortex, cerebellum (ground), and frontal sinus (reference). Rats were given two weeks of recovery before beginning EEG recordings.

Stimulation, EEG recording and PCP administration

Rats were awake and allowed to move freely during the recordings, which occurred at the same time of day for each animal. Rats were allowed 30 minutes to acclimate to the recording environment. EEG recordings began 20 minutes after injection to allow for drug absorption in treated animals with a baseline EEG recording of 78 s without auditory stimuli followed by a tone paradigm. The tone paradigm included the presentation of 100 single tones via a speaker above the animal enclosure. Tones (1000 Hz, 85 dB, and with a 50 ms rise fall time) were 1000 ms in duration and were presented at a 1000 ms inter-trial interval. Continuous EEG (bandpass 1–200 Hz) was recorded with a digitization rate of 1000 Hz (Contact Precision Instruments, Cambridge, MA) and saved for off-line processing. EEG was analyzed using Brain Vision Analyzer (Brain Products GmbH, Munich, Germany) and MATLAB (The MathWorks, Natick, MA).

Experiment 1: Dose-response effects of PCP

On day 1, all rats ($N=10$) were subcutaneously injected with saline (pH ~7.5) and baseline recordings were collected at rest. One week following baseline measures, rats received a dosing regimen of 1.0, 2.5, and 4.0 mg/kg PCP (pH~7.8) in randomized order. PCP doses were given one week apart to avoid carry-over effects. One week after the last dose of PCP, rats received an injection of saline and washout responses were recorded. All resting state (freely moving) EEG recordings were collected 20 minutes after drug injection.

Experiment 2: Acute versus subchronic effects of PCP

Rats ($N=21$), different from those included in Experiment 1, were randomly assigned to either a PCP ($n=10$) or saline control group ($n=11$). On day 1, all rats were subcutaneously injected with saline and baseline EEG recordings were obtained. Ninety minutes later, rats were injected with saline or PCP (5 mg/kg), depending on group randomization, and acute responses were obtained. This dose of PCP increased locomotor activity and stereotyped behaviors, consistent with previously reported observations after acute subanesthetic PCP administration [31–33]. On day 4, each rat was implanted between the scapulae with an

osmotic mini pump (model 2ML2, Durect Corp., Cupertino, CA) that subcutaneously delivered either saline or PCP (5 mg/kg/day) for 14 days. Continuous subchronic administration of PCP produces a lower peak serum level than a single acute injection [34–36], with two weeks of continuous PCP administration at 5 mg/kg/day producing serum levels of about 16 ng/ml [35]. Similar subchronic dosages of continuous or repeated PCP administration have produced impairments in extradimensional shift learning [37], novel object recognition [38], and potentiation of amphetamine-induced locomotion [35]. On day 18, EEG recordings were obtained to evaluate the effect of subchronic PCP exposure. After 25 days (7 days after cessation of drug delivery), EEG recordings were again collected. Three rats included in the analysis did not have a saline injection prior to their baseline recordings.

EEG data processing and outcome measures

For both Experiment 1 and 2, three types of analyses were used: 1) event-related potentials (ERP) to tone onset in the time domain, 2) time frequency analysis of the tone response, and 3) power spectra analysis of baseline EEG without auditory stimulation. For the ERP responses in the time domain, the onset of the tone elicited a large positive deflection with a mean latency of 16 ms (P16), and a negative deflection with a mean latency of 70 ms (N70) in the baseline saline condition (Figure 1A). For each rat and condition, EEG was segmented into 600 ms epochs with a 100 ms prestimulus baseline period and a 500 ms post-trial period. Automatic artifact rejection removed trials with any data point outside the range of $\pm 450 \mu\text{V}$ across conditions. Epochs were averaged and baseline and corrected by subtracting the average baseline value from all of the sample points in the epoch. P16 was measured as the most positive peak in the 14 to 24 ms range, and N70 as the most negative peak in the 55 to 100 ms range.

For the time frequency analysis of tone responses, raw data were segmented into 1500 ms epochs with a 250 ms baseline and 1250 ms post-trial period. Automatic artifact rejection removed trials containing data points outside the range of $\pm 450 \mu\text{V}$. FFT spectrograms were calculated with a moving window of 128 ms, a time step of 10 ms, and a pad ratio of 2 for each trial and channel. The frequency data was separated into eight 10 Hz step frequency bins as follows: Bin 1 = 1 to 9.8 Hz, Bin 2 = 9.9 to 19.6 Hz, Bin 3 = 19.7 to 29.4 Hz, Bin 4 = 29.5 to 39.2 Hz, Bin 5 = 39.3 to 49.0 Hz, Bin 6 = 49.1 to 58.8 Hz, Bin 7 = 58.9 to 68.6 Hz, and Bin 8 = 68.7 to 78.4. Phase locking factor (PLF) and mean power (MP) were calculated for the onset response, the period between 0 ms (stimulus onset) to 50 ms after stimulus onset, for each condition (Figure 2). PLF, also known as intertrial phase coherence, is an index of phase synchronization across trials at particular temporal intervals and frequencies. PLF is the average of normalized phase across trials for every time point and frequency, and it is measured between 0 (no synchrony), to 1 (perfect synchrony) [39]. MP measures the power in a specific frequency band relative to baseline and includes both synchronous and unsynchronized activity [40].

For baseline EEG without stimulation (“resting”), raw data recorded from the temporal electrode was segmented into 5 s intervals, and automatic artifact rejection was used to remove segments outside of $\pm 450 \mu\text{V}$ in amplitude. Next, a Fast Fourier Transform (FFT)

was calculated with a Hanning window of 20%, and segments were averaged. Mean values were exported for eight 10 Hz step frequency bins consistent with the time-frequency analysis.

Statistical analysis

For Experiment 1, an analysis of variance (ANOVA) was conducted with the repeated measure factor of *Dose* (5: baseline, 1.0 mg/kg, 2.5mg/kg, 4.0mg/kg, washout) to assess each outcome measure. When the Mauchley test for sphericity was significant ($p < .05$), the Geisser-Greenhouse correction was used for significance testing. When an ANOVA indicated significant differences between doses, paired sample t-tests were conducted between the baseline and the four other conditions (1.0 mg/kg, 2.5mg/kg, 4.0mg/kg, and washout). Bonferroni corrections were applied for multiple comparisons, and a p value .0125 (.05/4) was considered statistically significant. For outcome measures in Experiment 2, a repeated measures ANOVA was conducted with the repeated measure factor of *Time* (4: baseline, acute, subchronic, washout) for each condition (saline or PCP). When an ANOVA indicated significant effects of time, a paired samples t-test was conducted between the baseline and three other conditions (acute, subchronic and washout). Bonferroni corrections were applied for multiple comparisons, and a p value .017 (.05/3) was considered significant.

Results

Experiment 1: Dose-response effects of PCP

ERP Time Domain Analyses—There was no effect of Dose for P16 latency, P16 amplitude or N70 latency. There was a main effect of Dose for N70 amplitude ($p = .029$). Paired t-tests with showed a trend for an increased N70 amplitude to the 4.0 mg/kg dose ($t(9)=3.03$, $p = 0.014$) and an increase at the washout recording ($t(9) = 3.72$, $p = .005$). Figure 1A–B depicts the ERP waveforms and amplitude for the N70 ERP component.

Time-Frequency Analysis: Phase Locking Factor—PCP increased PLF at lower frequencies (~20 to 30 Hz) and decreased PLF at higher frequencies (~50–70 Hz) at select doses of PCP (Figure 2A–B). There was a significant effect of dose in Bin 3 between 19.7 to 29.4 Hz ($F(4,6) = 5.10$, $p = .002$), Bin 6 between 49.1 to 58.8 Hz ($F(4,6) = 4.07$, $p = .008$), Bin 7 between 58.9 to 68.6 Hz ($F(4,6) = 7.48$, $p < .001$), and Bin 8 between 68.7 to 78.4 Hz ($F(4,6) = 9.13$, $p < .001$). Post-hoc analyses determined that there were decreases in PLF between baseline and 1.0 mg/kg in Bin 7 (58.9 to 68.6 Hz) ($t(9)=3.23$, $p = .010$) as well as between baseline and 2.5 mg/kg in Bins 6 (49.1 to 58.8 Hz) and 7 (58.9 to 68.6 Hz) ($t(9)=3.12$, $p = .012$ and $t(9)=3.44$, $p = .007$, respectively). Additionally, there was an increase in PLF between baseline and 4 mg/kg in Bin 3 (19.7 to 29.4 Hz) ($t(9) = -3.73$, $p = .005$). There were no significant differences between baseline and washout conditions.

Time-Frequency Analysis: Mean Power—PCP administration decreased MP at high frequencies (~50 to 70 Hz) across all doses (Figure 2C–D). There was an effect of dose in Bin 6 between 49.1 to 58.8 Hz ($F(4,6)=4.44$, $p=.005$), Bin 7 between 58.9 to 68.6 Hz ($F(4,6)=3.36$, $p = .020$), and in Bin 8 between 68.7 to 78.4 Hz ($F(4,6) = 3.73$, $p = .012$).

Post-hoc analyses with paired sample t-tests determined that there were decreases in power between baseline and 1.0 mg/kg in Bin 7 (58.9 to 68.6 Hz) ($t(9)=3.14$, $p = .012$), between baseline and 2.5 mg/kg conditions in Bins 6 and 7 (49.1 to 68.6 Hz) ($(t(9) = 4.72$, $p = .001$, and $t(9) = 3.12$, $p = .009$, respectively), as well as between baseline and 4.0 mg/kg in Bins 6 and 7 (49.1 to 68.6 Hz) ($(t(9) = 5.66$, $p < .0001$, and $t(9) = 4.23$, $p = .002$, respectively). There were no significant differences between baseline and washout conditions.

Resting EEG Power Spectrum—PCP administration increased spectral power across frequencies (Figure 3A). There was an effect of dose in Bin 2 between 9.9 to 19.6 Hz ($F(4,6)= 5.10$, $p = .039$), Bin 3 between 19.7 to 29.4 Hz ($F(4,6) = 4.33$, $p = .005$), Bin 4 between 29.5 to 39.2 Hz ($F(4,6) = 8.56$, $p = .012$), Bin 5 between 39.3 to 49.0 Hz ($F(4,6) = 15.64$, $p = .003$), Bin 6 between 49.1 to 58.8 Hz ($F(4,6)= 18.63$, $p=.002$), Bin 7 between 58.9 to 68.6 Hz ($F(4,6)= 28.20$, $p < .001$), and in Bin 8 between 68.7 to 78.4 Hz ($F(4,6) = 17.80$, $p = .002$). Post-hoc analyses with paired sample t-tests determined that there were increases in power between baseline and 1.0 mg/kg in Bins 4 through 8 (29.5 to 78.4 Hz) ($(t(9) > -3.53$, $p < .007$ for all tests). Significant increases in spectral power were observed between baseline and 2.5 mg/kg in Bins 4 through 8 (29.5 to 78.4 Hz) ($(t(9) > -5.94$, $p < .001$ for all tests). Increases in spectral power were also observed between baseline and 4 mg/kg in Bins 2 through 8 (9.9 to 78.4 Hz) ($(t(9) > 3.72$, $p < .006$ for all tests). There were no significant differences in power between baseline and washout conditions.

Experiment 2: Acute versus subchronic effects of PCP

ERP Time Domain Analyses—P16 amplitude increased after acute PCP administration ($t(9) = 3.815$, $p = .004$) and returned to baseline values after 14 days of subchronic PCP treatment ($F(1,67) = 5.19$, $p = .006$). Similarly, acute administration of PCP resulted in a two fold increase in N70 amplitude ($t(9) = 3.93$, $p < .001$), which then returned to baseline range values ($F(1.31, 27) = 14.01$, $p = .002$). In contrast, changes over time occurred in the saline group which likely reflect developmental changes over the course of the study. In the saline condition, N70 latency decreased across recording sessions ($F(3,30) = 10.54$, $p < .001$) at the trend level at 14 days ($t(10) = 2.31$, $p = .04$) and significantly at 21 days ($t = 5.94$, $p < .001$). Similarly, N70 amplitude increased over sessions ($F(1.63, 30) = 27.25$, $p < .001$) at 14 days ($t(10) = 5.69$, $p < .001$) and 21 days ($t(10) = 6.28$, $p < .001$). Overall, these results suggest that the PCP administration resulted in a transient increase in N40 amplitude, but then interfered with developmental changes in the N40 latency and amplitude over the course of the study period. See Figure 1C–E for the ERP waveform (1C) and changes in amplitude of the N70 (1D) and P16 (1E) ERP components.

Time-Frequency Analysis: Phase Locking Factor—Compared to saline, acute PCP administration increased PLF at lower frequencies (~10 to 30 Hz) and decreased PLF at higher frequencies (~40 to 80 Hz) (Figure 2E–F). There was a significant effect of time in the PCP group for Bin 2 between 9.9 to 19.6 Hz ($F(3,7) = 5.89$, $p = .003$) and Bin 3 between 19.7 to 29.4 Hz ($F(3,7) = 7.05$, $p = .001$) (10 to 30 Hz). Post-hoc analyses demonstrated a statistically significant increase in PLF between baseline and acute conditions in Bin 2 ($t(9) = -4.39$, $p=.002$) and Bin 3 ($t(9) = -4.39$, $p = .002$) (9.9 to 29.4 Hz). There was also a significant effect of time in the PCP group for Bin 5 to 8 (39.3 to 78.4 Hz ($F(3,7) > 4.58$, $p <$

0.020). Post-hoc analyses demonstrated statistically significant decreases in PLF between baseline and acute conditions within Bins 5, 6 and 8 ($t(9) = 4.06$, $p = .003$; $t(9) = 4.55$, $p = .001$; $t(9) = 4.47$, $p = .002$, respectively). Additionally, PLF increased between baseline and washout in Bin 2 only ($t(9) = -3.18$, $p = .011$). There were no significant effects of time for the saline group.

Time-Frequency Analysis: Mean Power—Compared to the saline, acute PCP administration increased MP at lower frequencies (~10 to 30 Hz) (Figure 2G–H). In the PCP group, there was a significant effect of time in Bin 2 between 9.9 to 19.6 Hz ($F(3, 7) = 7.30$, $p = .001$) and Bin 3 between 19.7 to 29.4 Hz ($F(3, 7) = 8.18$, $p < .001$). Post-hoc analyses revealed that there was an increase in MP in both Bins 2 and 3 (9.9 to 29.4 Hz) between baseline and acute conditions ($t(9) = -4.20$, $p = .002$; $t(9) = -4.05$, $p = .003$, respectively). There was also a significant effect of time in Bin 8 between 68.7 to 78.4 Hz ($F(3, 7) = 4.62$, $p = .01$), however no post-hoc comparisons were significant. There was, however, a trend for decreased MP in the acute condition ($t(9) = 2.789$, $p = .021$). In the saline group, EEGs showed a change from baseline ($p < .05$) for Bin 3 only ($F(3, 8) = 3.41$, $p = .030$) (19.7 to 29.4 Hz), with post-hoc comparisons showing an increase in MP between baseline and the subchronic condition ($t(10) = -3.85$, $p = .003$). There were no statistically significant differences between baseline and washout conditions for either group.

Resting EEG Power Spectrum—Acute PCP administration increased spectral power (Figure 3C), compared to saline (Figure 3B), for most frequencies. In the PCP group, there was a nearly significant effect of time in Bin 2 (9.9 to 19.6 Hz) ($F(3, 7) = 4.19$, $p = .054$) and significant effects for Bins 3 through 8 (19.7 to 78.4 Hz, $F(3, 7) > 5.59$, $p < .029$ for all ANOVAs). Post-hoc analyses demonstrated significant increases in spectral power between baseline and acute conditions between Bins 2 through 8 (9.9 to 78.4 Hz) ($t(9) > 3.19$, $p < .012$ for all tests). Significant increases in power were also observed between baseline and subchronic administration for Bins 6 and 7 (49.1 to 68.6 Hz) ($t(9) = 3.19$, $p = .011$) and ($t(9) = 3.02$, $p = .014$), respectively). There were no significant differences in power observed in the PCP administration group between baseline and washout. There was a significant effect of time for the saline group observed in Bins 5 through 8 (39.3 to 78.4 Hz) ($F(3, 7) = 4.54$, $p = 0.39$), ($F(3, 7) = 7.97$, $p = .009$), ($F(3, 7) = 6.23$, $p = .017$), and ($F(3, 7) = 6.55$, $p = .015$), respectively). Post-hoc analyses demonstrated, however, only a significant difference in Bin 5 (39.3 to 49.0) between baseline and washout conditions ($t(9) = -3.70$, $p = .004$).

Discussion

Acute *in vivo* administration of PCP produced pervasive dose-related alterations in the neural response to a single tone in rats. As hypothesized, NMDAr antagonism suppressed gamma-band neural activity (~50–70 Hz) in response to auditory stimuli, while simultaneously increasing neural synchrony and power at lower frequencies (i.e., 10–30 Hz). These results support a possible role for NMDAr hypofunction in gamma frequency band deficits observed in schizophrenia. The magnitude of these effects were dose-dependent, with higher doses of acute PCP producing greater gamma suppression. In contrast, acute PCP produced a broad band, dose-dependent increase in resting spectral power including gamma range activity. Continuous, low level administration of PCP (5 mg/kg/day) had little

effect on neural power or synchrony in response to a single tone or to resting EEG spectral power.

The suppression of high frequency gamma activity by PCP in the present study is similar to several other studies of auditory ERPs in rodents with NMDAr function impaired through genetic manipulations [25] and other NMDAr antagonists, such as MK-801 [30], ketamine [14], and PCP [28]. In contrast, relatively low dose ketamine administration in humans [27] and mice [26] failed to suppress auditory evoked gamma activity. These divergent results may be related to the level of NMDAr suppression by each manipulation. For instance, high levels of NMDAr suppression (> 80% receptor occupancy) by PCP, ketamine, or genetic inactivation of NMDAr channels may reduce auditory gamma synchrony, whereas low levels of NMDAr suppression may increase gamma synchrony.

These findings suggest that PCP induced NMDAr hypofunction increases both power and neural synchrony at low frequency oscillations. Beta neural synchrony (15–30 Hz) has been suggested to be responsible for multi-modal binding across long-range neural networks [41]. The elevated low frequency neural synchrony and power in response to PCP administration is similar to our previous findings with ASSRs [28] and the broadband increase in spontaneous EEG activity commonly elicited by NMDAr antagonists. The cellular mechanisms for a PCP-induced increase in alpha and low gamma range activity generated by auditory stimulation are not well understood. Computational modeling by Spencer (2009) suggested that reducing NMDAr input to fast spiking interneurons increases network excitability, including gamma power [42].

Experiment 2 suggested that acute administration of 5 mg/kg has a larger physiological impact than the same dose over an extended period of time. Similarly, acute NMDAr antagonist administration, but not subchronic administration, reduced gamma range activity in the auditory steady state response in rats [28,30]. Steady-state serum levels associated with continuous administration are much lower than the maximum concentration in response to the same amount of drug produced by injection. In addition, changes in neurotransmission occur with continued exposure, as acute PCP administration increases glutamate in the prefrontal cortex, while chronic administration decreases the production of dopamine and glutamate [29,43,44]. It has also been shown that acute PCP administration impairs temporal cortex function, although it may be unaffected by chronic exposure [29].

The early auditory ERP components to tone onset increased in amplitude to acute PCP, different from the usual reduction in early auditory ERP amplitude in schizophrenia. However, in Experiment 2 there was an increase in N70 amplitude in the saline arm over time which failed to occur in the PCP arm, which suggests that NMDAr hypofunction could result in smaller auditory ERPs over development. The relationship between auditory evoked potentials elicited by a single tone and the course of schizophrenia or in high-risk groups has not been well characterized, although gamma-band neural synchrony deficits in auditory evoked potentials have been found in the relatives of patients with schizophrenia, suggesting an effect of genetic risk in the absence of psychotic symptoms [27,45].

There are several important limitations regarding the mechanistic implications of the present data. First, in vivo administration affected NMDAR in the entire brain, and therefore direct effects on local auditory circuits cannot be differentiated from modulation of auditory circuits by projections from other brain regions. Second, the continuous dosing using an osmotic mini-pump may not have maintained levels of PCP in the CNS sufficient to impact EEG measures. Finally, though the auditory cortex recording site in the present study was proximal to the primary generator of the neural response to the single tone, it is unclear which intracranial recording sites in rats generate activity most similar to that recorded from scalp electrodes in humans.

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Figure 1. ERP Time Domain Analyses

Analyses for Experiment 1 and 2 are displayed in the left and right columns, respectively. The figure depicts the ERP waveforms for the P16 and N70 ERP components across all doses in Experiment 1 (baseline, 1.0 mg/kg, 2.5 mg/kg, 4 mg/kg and washout) (Fig. 1A) and time in Experiment 2 for the PCP arm (baseline, acute PCP, subchronic PCP and washout conditions) (Fig. 1C). Figure 1 also displays the change in N70 (Figs. 1B and 1D) and P16 (Fig. 1E) amplitudes. All comparisons are in relation to the baseline condition, and asterisks and p-values refer to Bonferroni adjusted significance levels.

Figure 2. Time-Frequency Analyses

Analyses for Experiment 1 and 2 are displayed in the left and right columns, respectively. The figure shows phase locking factor (PLF) (Figs. 2A and 2E) and mean power (MP) (Figs. 2C and 2G) across all doses in Experiment 1 (baseline, 1.0 mg/kg, 2.5 mg/kg, 4 mg/kg and washout) and time in Experiment 2 for the PCP arm (baseline, acute PCP, subchronic PCP and washout) with exemplary spectrograms of PCP effects (Figs. 2B and 2F (PLF) and 2D and 2H (MP)). MP is shown in microvolts² (μV^2) and PLF is depicted from 0 to .9, indicating minimum to maximum synchrony. Statistically significant differences ($p < .05$) from baseline are indicated with colored asterisks (*); the color of the asterisks corresponds

with the condition in which a significant difference from baseline was observed. Statistically significant differences ($p < .05$) from baseline are indicated with an asterisk (*), and represent Bonferroni adjusted significance levels.

Experiment 1

Experiment 2

Figure 3. Resting State Power Spectrum

Analyses for Experiment 1 and 2 are displayed in Figures 3A–C. Figure 3A depicts spectral power across all doses in Experiment 1 (baseline, 1.0 mg/kg, 2.5 mg/kg, 4 mg/kg and washout). Figures 3B and 3C depict the changes in spectral power in both the saline (3B) and PCP (3C) arms of Experiment 2 (conditions: baseline, acute PCP or saline, subchronic PCP or saline, and washout). Power spectra was measured microvolts² and plotted in logarithmic mean values. Statistically significant differences ($p < .05$) from baseline are indicated with colored asterisks (*); the color of the asterisks corresponds with the condition in which a significant difference from baseline was observed. Asterisks and p-values refer to Bonferroni adjusted significance levels.