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Alterations in High-Frequency Neuronal Oscillations in a Cynomolgus Macaque Test of Sustained Attention Following NMDA Receptor Antagonism

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A growing body of evidence indicates that neuronal oscillations in the gamma frequency range (30–80 Hz) are disturbed in schizophrenic patients during cognitive processes and may represent an endophenotype of the disease. *N*-methyl-D-aspartate (NMDA) receptor antagonists have been used experimentally to induce schizophrenia-like symptoms including cognitive deficits in animals and humans. Here we characterized neuronal oscillations and event-related potentials (ERPs) in Cynomolgus macaques fully trained to perform a continuous performance test (CPT) in the presence and absence of the NMDA antagonist phencyclidine (PCP). Macaques ($n = 8$) were trained to touch 'target' stimuli and ignore 'distractor' stimuli presented randomly on a touchscreen. Subsequently, all subjects were implanted with epidural EEG electrodes over frontal (FC) and parietal cortices (PC) and later tested under vehicle (saline, i.m.) or acute PCP (0.1–0.3 mg/kg, i.m.) conditions. Compared with vehicle treatment, PCP produced a significant dose-dependent decrease in CPT performance accuracy and increased reaction times. Furthermore, PCP elevated the amplitudes of 'low' (30–50 Hz) and 'high' (51–80 Hz) gamma oscillations in FC and PC around target presentations for all correct responses. The CPT accuracy was inversely correlated with the gamma band amplitude in the presence of PCP. Additionally, PCP delayed the N100 peak latency in FC, and prolonged and suppressed the cognitively relevant P300 component of mean ERPs in FC and PC, respectively. The NMDA receptor antagonist-induced alteration in neuronal oscillations and ERPs may contribute to the observed cognitive deficits in macaques, and enhance our understanding of EEG recordings as a translatable biomarker.
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INTRODUCTION

Cognitive impairments associated with schizophrenia (CIAS) include deficits in attention, memory and executive functions, and significantly impact long-term functionality (Sun *et al*, 2011; Gandal *et al*, 2012; Green, 1996). Emerging evidence suggests disturbances in the dynamic coordination between cortico-cortical regions (Phillips and Silverstein, 2003; Friston, 1999) may contribute to ineffective neuronal communication that underlies CIAS (Uhlhaas and Singer, 2010). Neuronal oscillations enable coordinated activity during normal brain function (Buzsaki and Draguhn, 2004; Fries, 2009; Singer, 1999). Oscillations in the lower frequencies (eg, alpha, theta) preferentially establish synchronization over longer distances, whereas high-frequency (ie, gamma) oscillations establish synchronization with great precision in local cortical networks (von Stein and Sarnthein, 2000; Womelsdorf and Fries, 2007). These temporal correlations are functionally relevant as there is abundant evidence for a close relationship between the occurrence of oscillations and cognitive/behavioral responses, such as attention-dependent stimulus selection, working memory, executive function, and sensory processing (Uhlhaas and Singer, 2010). In particular, oscillations within the gamma frequency band (30–80 Hz) integrate neural networks involved in higher order cognitive processes. Alterations in the gamma band in schizophrenia have been reported in a variety of contexts (Carlen *et al*, 2012; Gandal *et al*, 2012; Uhlhaas and Singer, 2010), including enhanced gamma band synchronization in response to visual stimuli in monkeys (Fries *et al*, 2001, 2008) and visual/auditory stimuli in humans (Gruber *et al*, 1999; Tiitinen *et al*, 1993) in selective attention processes.

Mechanistically, gamma band oscillations (GBOs) are mediated by fast-spiking GABAergic cortical interneurons (Tamas *et al*, 2000). Within the prefrontal cortex (PFC), local inhibitory parvalbumin (PV)-containing GABAergic interneurons modulate the temporal-spatial excitatory activity of

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glutamatergic neurons and the generation of GBOs (Lett *et al*, 2014; Sohal *et al*, 2009; Lewis *et al*, 2005). Importantly, overall reductions in mRNA expression of PV and the GABA synthesizing enzyme, GAD67, in PV-containing GABAergic interneurons are two of the most robust postmortem results in the schizophrenic brain (Glausier *et al*, 2014; Lewis *et al*, 2012) and may contribute to abnormal GBOs and/or cognitive deficits.

Experimentally, the administration of *N*-methyl-D-aspartate receptor (NMDAR) antagonists (eg, ketamine) to humans or animals produces symptoms related to schizophrenia and can exacerbate psychotic symptoms in patients (Lahti *et al*, 2001). For example, repeated exposure of NMDAR antagonists to rodents impairs cognition, and suppresses GAD67 and PV expression in GABAergic interneurons (Cochran *et al*, 2003; Behrens *et al*, 2007), and acute administration produces cognitive deficits, evidence of increased positive and negative symptoms and augments GBOs that have been likened to prodromal/first episode stages of the disease (Hong *et al*, 2010; Krystal *et al*, 1994).

Abnormalities in electroencephalography (EEG) measures such as event-related potentials (ERPs) (eg, mismatch negativity, P300) have been well-characterized in schizophrenia and other psychiatric diseases, and the approach itself has been successfully translated across species (Gil-da-Costa *et al*, 2013; Gandal *et al*, 2010; Friedman and Squires-Wheeler, 1994). Similarly, assessing EEG power across frequency bands provides insight into cortical network function and dysregulation in disease state. The intent of the present study was to simultaneously measure neuronal oscillations and cognitive function in non-human primates (NHPs), whose brains are structurally and functionally similar to humans. We placed epidural EEG electrodes in NHPs trained on a sustained attention task and evaluated their performance in the presence/absence of the NMDAR antagonist phencyclidine (PCP) in attempt to evaluate the translational potential of our approach to humans.

MATERIALS AND METHODS

Animals

Eight male Cynomolgus macaques (6–9 kg; 8–9 years old) were housed individually with a 12 h light/dark cycle. Animals received a full daily regimen of food (Purina Animal Nutrition, Gray Summit, MO) and enrichment (eg, fresh fruit, vegetables) after training/testing and had access to water *ad libitum* in their home cage. All protocols were approved by the SRI International IACUC and were in compliance with NIH guidelines for care and use of laboratory animals. SRI is an AAALAC accredited institution.

Continuous Performance Test (CPT)

For the CPT, animals were seated in standard restraint chairs and placed into sound-attenuated chambers (30"W, 30"D, 59"H; Med Associates, St Albans, VT, USA) equipped with a monitor delivering visual stimuli (Cambridge Neuropsychological Test Automated Battery (CANTAB) version 9.4; Lafayette Instruments, Lafayette, IN, USA). NHPs were required to attend to a stream of 200 rectangular stimuli (50% targets (100 yellow) and 50% distractors (50 blue and 50 white)) presented individually for 2 s with a 15 s interstimulus interval (ISI). Each correct touch to a target (ie, hit) was rewarded with a food pellet (190 mg banana-flavored pellet; TestDiet, Newco Distributors, Hayward, CA, USA) and a 'correct' auditory tone, and incorrect touches to distractors were punished with an 'incorrect' tone and a 15 s time-out period. The percentage of 'hits' (correct responses to targets), 'misses' (withheld responses to targets) and 'reaction time to targets' was calculated.

Drug Administration

Drug doses and pre-treatment times were selected from available literature and internal data. NHPs were administered PCP (0.1, 0.18, and 0.3 mg/kg; Sigma-Aldrich, St Louis, MO, USA) or vehicle (saline) intra-muscularly (i.m.) 10 min before testing.

Surgery

EEG/EMG electrodes and transmitters (Model: 4ET-S1; DSI, St. Paul, MN, USA) were implanted in fully anesthetized (telazole (4 mg/kg; i.m.) and 1% isoflurane) animals by trained surgeons. Following a dorsal midline skin incision ($\leqslant$5 cm), the transmitter module was implanted subcutaneously below the scapular region. Biopotential leads were guided subcutaneously from the back to the head via a midline incision from the brow ridge to the occipital notch. Using a stereotaxic approach, stainless steel screws were implanted into the skull over left/right frontal cortex (FC; AP: +23.0 mm, ML $\pm$15.0 mm) and parietal cortex (PC; AP: − 18.4 mm; ML $\pm$16.0 mm) (from Bregma; Paxinos *et al*, 1999) until the tips were on the surface of the dura mater. The biopotential leads were wrapped around the screws and referenced to C3/C4. The EMG leads were sutured into the temporalis muscle. Animals received postoperative analgesia for $\geqslant$48 h and antibiotics for 10–14 days, and recovered for a minimum of 21-days before testing.

EEG Recording and Signal Processing

Receiver boards (Model: RPC-2; DSI) were mounted to the chairs directly behind the animals to facilitate real-time EEG/EMG recordings during testing. Stimuli presented on the monitors during the task were time-stamped and relayed into data exchange matrices (DEMs) via analog-to-digital converters and the two systems were synchronized to 0.5 ms using a customized MATLAB script. All EEG/EMG recordings and behavioral events were acquired at 2 kHz in Dataquest A.R.T. Data were further processed with a customized MATLAB script as follows: (i) stimulus presentation time was obtained from the rising edge of the stimulus signal within 0.5 ms of onset; (ii) 6 s epochs centered on the stimulus time were obtained from the EEG traces and plotted. Epochs with artifacts that were empirically identified as abnormally high amplitude ($\geqslant$170 μV) in a 3 s window around stimulus time were discarded from the analysis; (iii) EEG traces that were not rejected ($>$80%

correct trials) were filtered with a two-pass fourth-order Butterworth band-pass filter with zero lag in the bands of interest: alpha (8–12 Hz), beta (16–24 Hz), 'low' gamma (30–50 Hz), and 'high' gamma (51–80 Hz); (iv) Root mean square amplitudes (RMS-Amp) of the filtered signal were calculated and the central 3 s around targets were binned in 100 ms averages to obtain the equivalent intensity of the signal for each frequency band; and (v) time-frequency analysis was done applying fast Fourier transform (FFT) on 0.5 s periods filtered by a Hamming window that advanced in 10 ms steps. Power in each 2 Hz band was normalized by the averaged power during 1 s before target. Reliable recordings were obtained from FC in $n=6$, whereas data from $n=8$ were obtained from PC.

Statistical Analysis

For the behavioral studies, 'accuracy' (% hits: (number of hits/number of target trials) × 100%)) and 'reaction time to target' measures were analyzed using a one-way repeated measure (RM) ANOVA, followed by Fisher's *post hoc* tests. Paired *t*-tests were used for comparisons between the impairing dose of PCP and vehicle, and % hits versus '% misses' ((number of misses/number of target trials) × 100%)). For the EEG analyses, neuronal oscillations for all frequency bands (RMS-Amp) were analyzed using a two-way RM-ANOVA followed by Fisher's *post hoc* tests. Each component of the mean ERP was assessed using paired *t*-tests. Linear correlation between % accuracy and pre-target and post-target RMS-Amp (gamma) was assessed using a Pearson's product moment correlation coefficient analysis. Significance was defined as $P \leqslant 0.05$.

RESULTS

Effects of PCP on the NHP CPT

Following the administration of PCP (0, 0.1, 0.18, and 0.3 mg/kg), main effects of treatment for both overall accuracy (% hits) ($F_{(3,15)}=11.02$, $P<0.001$; Figure 1a) and 'reaction time to targets' ($F_{(3,15)}=8.07$, $P=0.002$; Figure 1c) were identified. *Post hoc* analyses revealed that PCP produced a dose-related decrease in accuracy and an increase in reaction time at all doses ($P<0.05$) in comparison with vehicle. Figure 1b and d depict significant impairments in CPT accuracy ($t_{(7)}=4.934$, $P=0.002$; vehicle: 94.6 ± 1.9%; PCP: 55.5 ± 7.0%) and reaction time ($t_{(7)}=-3.731$, $P=0.007$; vehicle: 765 ± 50 ms; PCP: 1050 ± 58 ms), respectively, after identifying the cognitive-impairing dose of PCP (range 0.1–0.18 mg/kg) for each animal that did not cause sedation. Because of individual animal sensitivity to PCP, $n=8$, $n=7$, and $n=3$ animals completed the CPT at 0.1, 0.18, and 0.3 mg/kg doses, respectively.

Effects of PCP on High-Frequency Gamma Oscillations in FC and PC During CPT Performance

The acute effects of PCP on both 'high' gamma (51–80 Hz) and 'low' gamma (30–50 Hz) oscillatory activity were assessed during correct choices of the CPT. A main effect of treatment (PCP vs vehicle) ($F_{(1,5)}=42.51$, $P=0.001$) was identified for 'high' gamma in the FC, with a significant increase in RMS-Amp (13.4%) in the PCP condition as compared with control (Figure 2a). In addition, there was a main effect of stimulus (pre- vs post-target) ($F_{(1,5)}=13.20$, $P=0.015$) that revealed a significant increase in 'high'

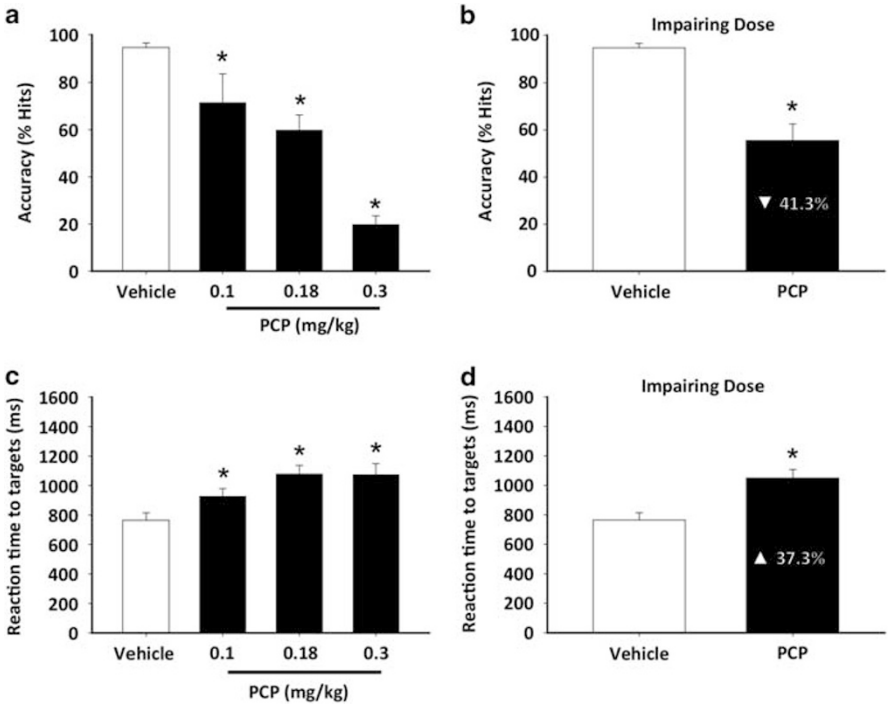


Figure 1 Phencyclidine (PCP) produced dose-related decreases in accuracy (% hits) (a) and increases in reaction time to targets (c) in non-human primates performing the continuous performance test. (b) and (d) depict the dose that impairs continuous performance test accuracy and reaction time, respectively, without impairing the ability to perform the task. Data represent the mean ± SEM. *$P \leqslant 0.05$.

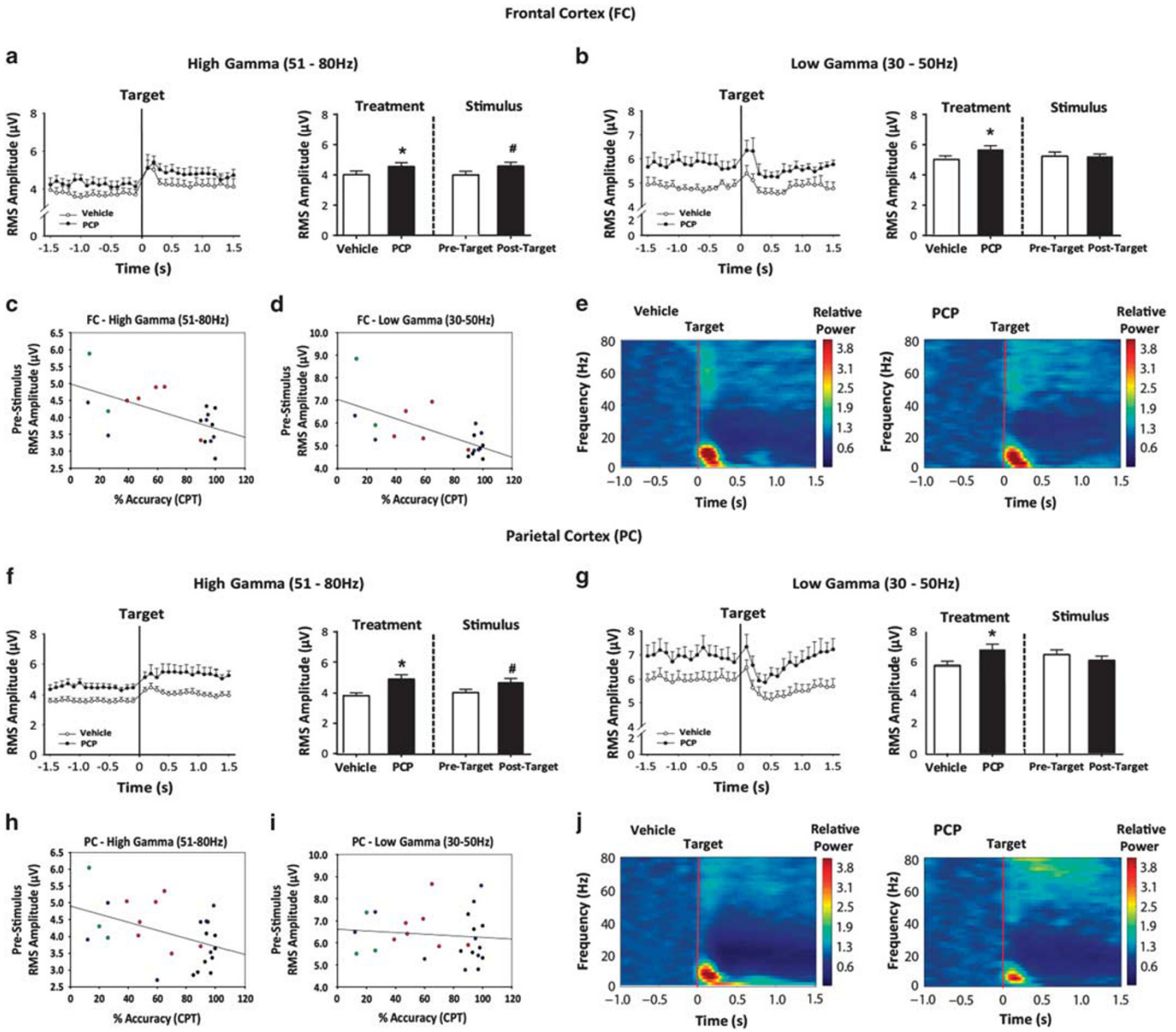


Figure 2 Phencyclidine (PCP) elevates 'high' gamma (51–80 Hz) and 'low' gamma (30–50 Hz) oscillations in frontal cortex (a, b) and parietal cortex (f, g) around 'target' presentations for all 'correct' responses (hits) during continuous performance test performance. Pre-stimulus gamma RMS-Amp was inversely correlated with continuous performance test performance accuracy in frontal cortex (high (c) and low (d) gamma), and in parietal cortex (high (h), but not low (i) gamma) across doses (black = vehicle; blue = 0.1 mg/kg phencyclidine; red = 0.18 mg/kg phencyclidine; green = 0.3 mg/kg phencyclidine). Post-target changes in mean relative power (normalized to pre-stimulus baseline) are equivalent across all frequency bands independent of treatment (vehicle and phencyclidine) in both frontal cortex (e) and parietal cortex (j). Data represent mean ± SEM. $p \leqslant 0.05$ (*vehicle vs phencyclidine; #pre-target vs post-target).

gamma RMS-Amp in the post-target period independent of treatment. This post-stimulus elevation in 'high' gamma activity may be indicative of binding task-relevant information together in order to perform the CPT task successfully. There was no main treatment–stimulus interaction ($F_{(1,5)} < 1.0$, $P = 0.994$) identified for 'high' gamma in the FC. Similarly, there was a main effect of treatment on 'low' gamma in the FC ($F_{(1,5)} = 18.55$, $P = 0.008$) with an overall increase in RMS-Amp (17.9%) in the PCP condition, but no stimulus effect ($F_{(1,5)} = 0.184$, $P = 0.686$) or treatment–stimulus interaction ($F_{(1,5)} = 0.656$, $P = 0.455$) (Figure 2b).

Furthermore, we assessed the relationship between accuracy and RMS-amplitude within high and low gamma bands in FC across all doses. We observed a significant inverse correlation between pre-target RMS-Amp and CPT performance accuracy in the frontal cortex for high gamma ($r = -0.59$; $P = 0.008$; Figure 2c) and for low gamma ($r = -0.66$; $P = 0.002$; Figure 2d). Similar, but less robust effects were observed with post-stimulus gamma and accuracy for high gamma ($r = -0.46$; $P = 0.05$) and for low gamma ($r = -0.53$; $P = 0.02$).

FFT of the mean relative power (post-target relative to pre-target baseline) was equivalent between treatment conditions in 'high' and 'low' gamma bands, as well as in the lower frequencies within FC (Figure 2e). Taken together with the RMS-Amp data, PCP elevates the overall magnitude of

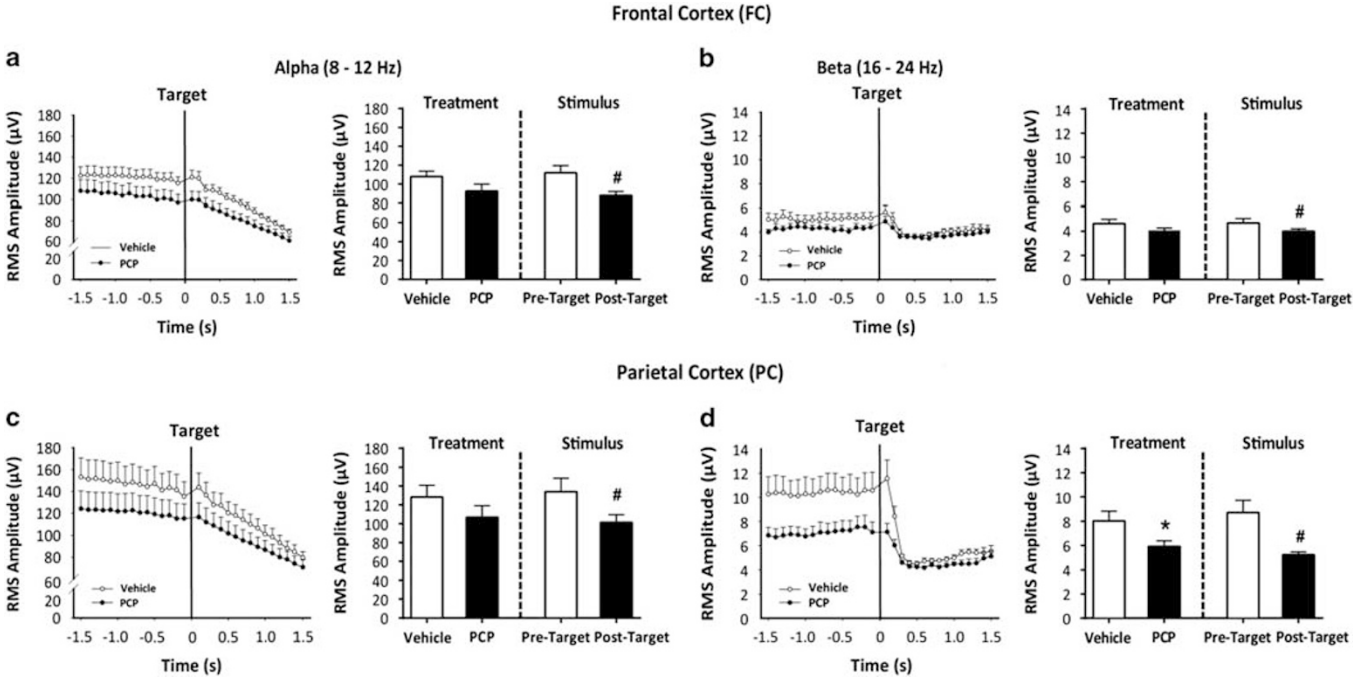


Figure 3 Alpha (8–12 Hz) and beta (16–24 Hz) oscillations showed reductions in RMS-Amp in frontal cortex (a, b) and parietal cortex (c, d) following 'target' presentations for all 'correct' responses (hits) during continuous performance test performance. Phencyclidine (PCP) produced a reduction in beta activity in parietal cortex. Data represent mean ± SEM. $P \leqslant 0.05$ (*vehicle vs phencyclidine; #pre-target vs post-target).

gamma oscillatory activity independent of stimulus condition.

Similar effects were observed in PC as were reported in FC. Namely, there was a main effect of treatment ($F_{(1,7)} = 22.20$, $P = 0.002$) and stimulus ($F_{(1,7)} = 22.00$, $P = 0.001$), but no treatment–stimulus interaction ($F_{(1,7)} = 0.628$, $P = 0.458$) on the RMS-Amp in the 'high' gamma band during correct responding (Figure 2f). Similarly, 'low' gamma oscillations in PC showed a main effect of treatment ($F_{(1,7)} = 12.60$, $P = 0.009$), but no stimulus ($F_{(1,7)} = 5.02$, $P = 0.06$) or treatment–stimulus interaction ($F_{(1,7)} = 1.01$, $P = 0.348$; Figure 2g). PCP produced overall increases of 20.7 and 17.9% in RMS-Amp of 'high' and 'low' gamma oscillations respectively in PC.

In the PC, there was a similar, but more moderate negative correlation between pre-stimulus RMS-Amp and CPT accuracy for high gamma ($r = -0.44$; $P = 0.02$; Figure 2h), but not low gamma ($r = -0.11$; $P = 0.60$; Figure 2i); and for post-stimulus RMS-Amp and accuracy for high gamma ($r = -0.43$; $P = 0.03$), but not low gamma ($r = -0.15$; $P = 0.46$).

Post-stimulus changes in mean relative power across all frequency bands were similar for vehicle and PCP conditions in the PC, similar to FC (Figure 2j).

Effects of PCP on Alpha and Beta Oscillations in FC and PC During CPT Performance

Although high-frequency gamma oscillations have been linked with cognition, frequencies in the lower bands (eg, alpha and beta) are also associated with attention and long-range synchronization of information between distant brain regions. Analysis of the RMS-Amp of the alpha (8–12 Hz) and beta (16–24 Hz) oscillations revealed a main effect of stimulus in the FC (alpha: $F_{(1,5)} = 42.54$, $P = 0.001$ (Figure 3a); beta: $F_{(1,5)} = 7.81$, $P = 0.038$ (Figure 3b)) with a significant reduction in RMS-Amp in the post-target period. There were no significant main treatment effects (alpha: $F_{(1,5)} = 5.623$, $P = 0.064$; beta: $F_{(1,5)} = 5.66$, $P = 0.063$) or treatment–stimulus interactions (alpha: $F_{(1,5)} = 2.23$, $P = 0.196$; beta: $F_{(1,5)} = 2.78$, $P = 0.156$) during correct responding in the FC.

Similar to FC, the amplitude for alpha oscillations in PC, revealed a main effect of stimulus ($F_{(1,7)} = 24.59$, $P = 0.002$) but, no treatment ($F_{(1,7)} = 4.22$, $P = 0.079$) or treatment–stimulus interaction ($F_{(1,7)} = 2.36$, $P = 0.169$) during correct responding (Figure 3c).

In contrast to FC, the amplitude of beta oscillations showed a more robust decrease in PC following target presentations for all correct responses irrespective of treatment (Figure 3d). A two-way repeated measures ANOVA performed on RMS amplitude for beta oscillations, revealed a main effect of treatment ($F_{(1,7)} = 19.50$, $P = 0.003$), stimulus ($F_{(1,7)} = 17.73$, $P = 0.004$) and treatment–stimulus interaction ($F_{(1,7)} = 11.45$, $P = 0.012$) in the PC. Overall, PCP reduced the RMS-Amp of beta oscillations by 26.4% compared with vehicle controls. Following *post hoc* analyses, it was evident that PCP significantly suppressed beta amplitude before ($P < 0.001$), but not after ($P = 0.144$) target presentations for all correct responses.

Overall, these results suggest that PCP elevated the amplitudes of both 'high' and 'low' gamma oscillations above baseline levels in FC and PC while suppressing the amplitude of beta oscillations particularly in PC. Alpha showed a more general reduction of the post-target amplitude regardless of treatment in both brain regions.

Effects of PCP Across All Frequency Bands During 'Correct' vs 'Incorrect' Responses During CPT Performance in FC and PC

Next, we evaluated the acute effects of PCP on cognitively relevant frequency bands (gamma, beta and alpha) during 'correct' (hit) or 'incorrect' (miss) responses during CPT performance. The choice between a 'hit' (correct response to a target stimulus) or a 'miss' (incorrect withholding of a response to a target stimulus) determines whether or not an animal successfully responds within a given trial. Given that each of these events is cognitively relevant and unique, concurrent EEG recordings may provide insight into how different frequency oscillations behave when making 'correct' or 'incorrect' responses during the CPT. Only 'hits' and 'misses' in the presence of PCP were analyzed because control animals had an accuracy $\geqslant 95\%$ with too few 'misses' to be analyzed.

Paired *t*-tests revealed a significant decrease in post-target RMS-Amp of 'high' gamma oscillations during 'miss' trials compared with 'hits' ($t_{(5)} = 2.69$; $P = 0.043$) in the FC (Figure 4a) but not in PC ($t_{(7)} = 1.93$; $P = 0.095$) (Figure 4b). No differences in pre-target baselines of RMS-Amp between 'hits' and 'misses' were noted for 'high' gamma in either FC or PC regions ($P > 0.05$).

In contrast, similar pairwise comparisons revealed a significant elevation in post-target RMS-Amp of beta oscillations during 'miss' trials in comparison to 'hits' ($t_{(7)} = -2.61$; $P = 0.035$) in PC (Figure 4d) but not in FC ($t_{(5)} = -1.33$; $P = 0.241$; Figure 4c). No difference in pre-target baseline amplitudes between 'hits' and 'misses' was evident for beta oscillations in either brain region. Similar analysis failed to show significant differences in post-target RMS-Amplitudes of 'low' gamma and alpha oscillations during 'hits' vs 'misses' in both FC and PC (Supplementary Figure S1a–d).

These results suggest that 'high' gamma activity is elevated in FC, while beta activity is suppressed in PC when animals make 'correct' as opposed to 'incorrect' responses. Hence, it is evident that 'high' gamma and beta oscillations are inversely modulated in different brain regions depending on the type of choice animals make during this particular sustained attention task.

Effects of PCP on Event-Related Potentials (ERPs) in FC and PC During CPT Performance

Given that ERPs have been used extensively as neurophysiological indices of sensory and cognitive function in patients with schizophrenia, we also examined the acute effects of PCP on ERPs in FC and PC during the sustained attention task. Statistical analyses were performed on two main ERP components, visual N100 and P300 which provide information about 'sensory' and 'cognitive' processing in the brain respectively. Paired *t*-tests revealed that PCP significantly prolongs the mean 'peak latency' of both visual N100 ($t_{(5)} = -2.64$, $P = 0.046$) and P300 ($t_{(5)} = -2.67$, $P = 0.044$) components in the post-target period for all correct responses in FC (Figure 5a and b), but not in PC ($P \geqslant 0.05$) (Figure 5c and d). Interestingly, whereas there was no significant difference in mean 'peak amplitude' observed for the N100 or P300 in the FC (p's > 0.05), PCP induced a significant suppression in mean 'peak amplitude' of the P300 component compared to vehicle in PC ($t_{(7)} = 2.37$, $P = 0.049$; Figure 5c and d). It is also interesting to note that the peak range of the P300 component, especially in the FC,

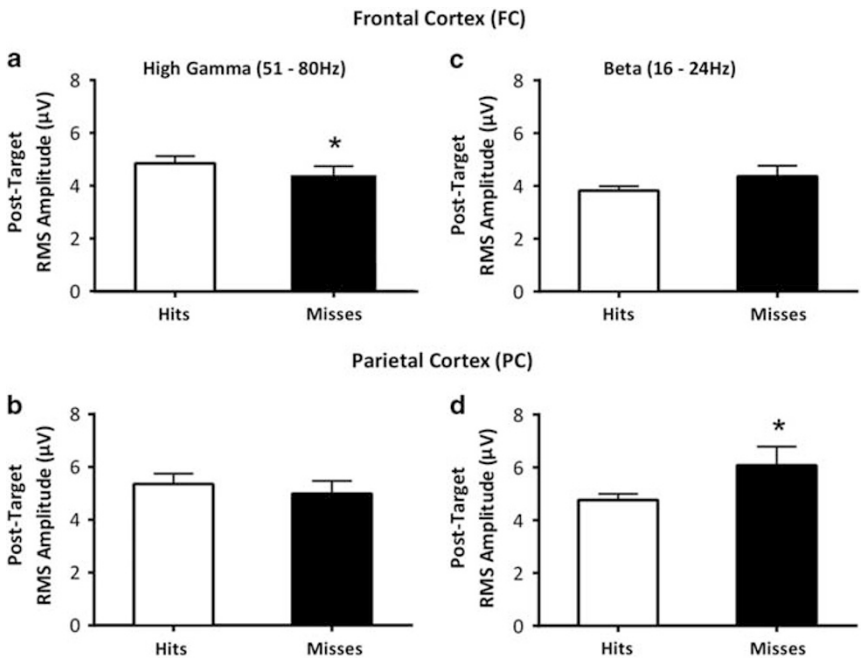
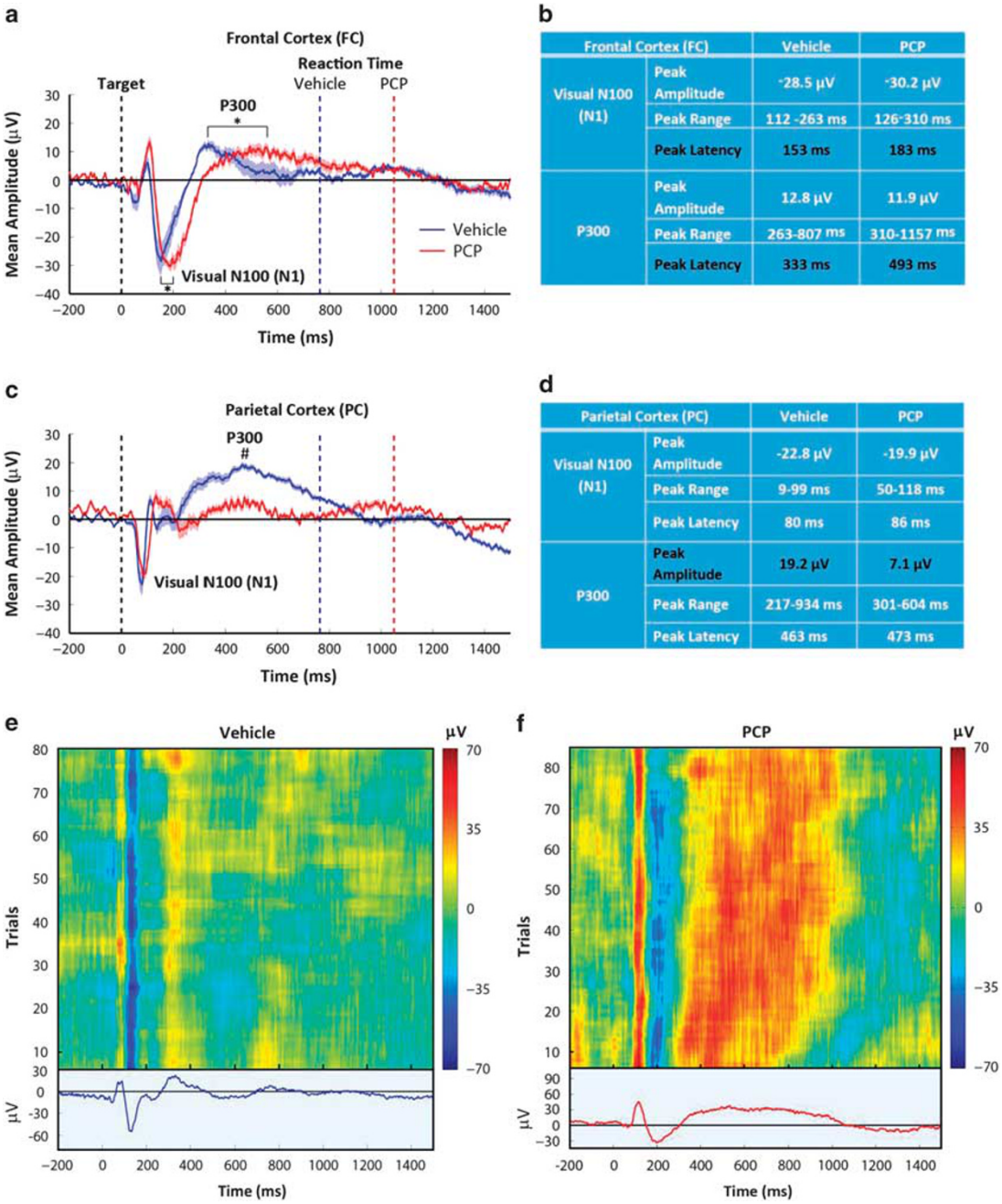


Figure 4 Post-target RMS-Amp of 'high' gamma oscillations was suppressed in frontal cortex (a) while beta was enhanced in parietal cortex (d) when subjects made 'incorrect' (misses) compared with 'correct' (hits) responses in the presence of phencyclidine. No significant changes in post-target RMS-Amp were observed in 'high' gamma and beta oscillations in parietal cortex (b) and frontal cortex (c) respectively. Data represent mean ± SEM. $^*P \leqslant 0.05$.



Frontal Cortex (FC)		Vehicle	PCP
Visual N100 (N1)	Peak Amplitude	-28.5 μV	-30.2 μV
	Peak Range	112 -263 ms	126-310 ms
	Peak Latency	153 ms	183 ms
P300	Peak Amplitude	12.8 μV	11.9 μV
	Peak Range	263-807 ms	310-1157 ms
	Peak Latency	333 ms	493 ms

Parietal Cortex (PC)		Vehicle	PCP
Visual N100 (N1)	Peak Amplitude	-22.8 μV	-19.9 μV
	Peak Range	9-99 ms	50-118 ms
	Peak Latency	80 ms	86 ms
P300	Peak Amplitude	19.2 μV	7.1 μV
	Peak Range	217-934 ms	301-604 ms
	Peak Latency	463 ms	473 ms

Figure 5 Phencyclidine (PCP) significantly prolonged the 'peak latency' of N100 and P300 components of the event-related potential in frontal cortex (a, b) and suppressed the 'peak amplitude' of P300 in parietal cortex (c, d). Data represent mean event-related potentials for all 'correct' responses (hits) following vehicle and phencyclidine treatments across all non-human primates. $P<0.05$ (*vehicle vs phencyclidine: peak latency; #vehicle vs phencyclidine: peak amplitude). The heat map shows the trial-by-trial voltage amplitudes of the individual event-related potentials (top) and the mean event-related potentials (bottom) for all 'correct' responses in the same animal ($n=1$) treated with either vehicle (e) or an impairing dose of phencyclidine (f).

corresponded closely with the reaction time to respond to the target following vehicle and PCP administration respectively (Figure 5a). Representative heat maps of the individual ERPs across 'correct' trials illustrate the differences in N100 and P300 voltages following vehicle (Figure 5e) and PCP (Figure 5f) treatments ($n=1$), and also shows low trial-by-trial variability across trials within a treatment. These results suggest that PCP has differential effects on both sensory and cognitively relevant ERP components in FC and PC during CPT performance.

DISCUSSION

In this study, we investigated the acute effects of the NMDA receptor antagonist, PCP, on different neuronal oscillations and ERPs during performance of a sustained attention task in macaques. In our study, PCP dose-dependently impaired accuracy and increased reaction time in NHPs performing the CPT. Interestingly, the PCP-induced behavioral effects were accompanied by significant changes in high-frequency oscillations (gamma and beta) and sensory and cognitively

relevant ERPs as measured by epidural electrodes placed over the FC and PC. These data suggest the functional consequences of impaired sustained attention under conditions of NMDA receptor antagonism may be associated with altered neuronal communication.

Overall, PCP significantly elevated 'high' and 'low' gamma amplitude (FC and PC) around target trials compared to vehicle treated animals independent of stimulus condition (ie, pre and post). In our study, the effect on 'high' gamma following PCP administration is consistent with EEG and magnetoencephalography results reported in healthy volunteers receiving acute ketamine (Hong *et al*, 2010; Rivolta *et al*, 2015), as well as with *in vivo* local field potentials and EEG recordings following the administration of NMDA receptor antagonists to rodents (Ehrlichman *et al*, 2009; Pinault, 2008; Lazarewicz *et al*, 2010; Sullivan *et al*, 2015). The enhancement in gamma oscillations following acute NMDA receptor inhibition could result from at least two separate mechanisms in which the NMDA receptor antagonist: (i) acts directly on PV-containing GABAergic interneurons and thereby disinhibits pyramidal cells (eg, layer 5 of dlPFC); or (ii) acts directly on pyramidal cells causing a reduction in glutamate release which in turn reduces the stimulation of AMPA receptors on GABAergic interneurons which may also transiently disinhibit pyramidal cells for example in layer 3 of dlPFC (Rotaru *et al*, 2011; Wang *et al*, 2013). Both mechanisms could increase extracellular glutamate release and enhance gamma oscillations, leading to schizophrenia-like symptoms in humans and animals (Krystal *et al*, 1994; Moghaddam *et al*, 1997).

PCP-treated animals showed an overall reduction in performance accuracy (~56%) as compared to vehicle treated animals (~95%). However, in trials in which PCP-treated animals responded correctly they exhibited an evoked response following the presentation of the visual stimulus, and similar to the vehicle treated animals, showed a sustained, although increased elevation in 'high' gamma amplitude, which was not observed in incorrect responses. These data suggest that animals treated with an impairing dose of PCP still maintain some ability to modulate gamma band activity depending on stimulus contingencies. However, the elevation in pre-stimulus baseline gamma amplitude was inversely associated with accuracy in the CPT, and we observed a similar inverse correlation with the post-stimulus gamma response and accuracy. These data may suggest an inappropriate increase in non-task related activity that negatively impacts overall performance, and are similar to findings of elevated gamma following the acute administration of NMDAR antagonists that have been reported in rodents (Ehrlichman *et al*, 2009; Saunders *et al*, 2012b) and humans (Hong *et al*, 2010) in auditory-evoked stimulus paradigms.

In contrast to enhancements in 'high' gamma activity in the correct trials, we observed a significant suppression of alpha (8–12 Hz) and beta (16–24 Hz) amplitude in the FC and PC following the presentation of the visual stimuli. Moreover, the suppression in beta amplitude was PCP-dependent showing attenuated response differences between pre- and post-stimulus conditions in PC. The suppression of event-related alpha and beta activity may reflect local disinhibition of cortical regions, permitting them to engage in detailed stimulus processing. Both alpha and beta are involved in long-range synchronization of information between cortico-cortical regions (Uhlhaas and Singer, 2010). In particular, alterations in alpha are related to sensory encoding and evaluation of the cue while changes in beta are associated with response preparation (Bickel *et al*, 2012; Dias *et al*, 2013). Moreover, stimulus-related reductions in alpha and beta oscillatory activity observed under control conditions during successful CPT performance have also been reported in parieto-occipital region of healthy humans performing a similar sustained attention task, AX-CPT (Bickel *et al*, 2012; Dias *et al*, 2013). Furthermore, these results are consistent with other local field potential studies in monkeys that show suppression in alpha activity during performance of a visual selective attentional task in macaques (Fries *et al*, 2001, 2008).

Although PCP showed a trend towards reducing alpha activity globally in this study, the more robust effect of PCP was seen in PC, where overall beta oscillatory activity was suppressed further. This effect is consistent with event-related desynchronizations of beta activity observed in parieto-occipital areas of patients with schizophrenia and healthy human volunteers performing the AX-CPT task (Dias *et al*, 2013).

Evidence of impaired sensory and cognitively relevant ERP components suggests a contribution to cognitive deficits observed in schizophrenia. In our study, we showed PCP significantly prolonged the peak latency, but not amplitude, of both the N100 and P300 following stimulus presentation in the FC, and suppressed the peak amplitude of the P300 in PC compared to vehicle treatment. These PCP-induced deficits in early (sensory) and late (cognitive) ERP components in response to visual stimuli during CPT performance, may contribute to impaired cue encoding that could lead to the observed behavioral deficits. The PCP-induced suppression in P300 in the PC was also observed in healthy macaques and humans performing an auditory 'oddball' task following an acute subanesthetic dose of ketamine (Gil-da-Costa *et al*, 2013). Similarly in mice, prolongation of the N100 latency has been observed following acute administration of MK-801 (Saunders *et al*, 2012a) and in some (Ehrlichman *et al*, 2008), but not all (Maxwell *et al*, 2006), studies with ketamine using auditory-evoked paradigms.

Overall, PCP-induced alterations in high-frequency neuronal oscillations and ERPs may result in the observed cognitive deficits. It is likely that the disinhibition of principal cells resulting from the NMDA receptor blockade of interneurons and/or the principal cells themselves facilitates an uncoordinated generation and transient increase of gamma oscillations. Potentially, this dysregulation may lead to a decoupling of local gamma oscillators from the controlling influence of extended networks that are fundamental for processing information during cognition. This global, hyperconnectivity following acute NMDA receptor antagonist treatment has been reported previously in healthy humans (Driesen *et al*, 2013) and rodents (Kocsis *et al*, 2013), and supports the hypothesis that pathological increases in resting brain functional connectivity contribute to the emergence of symptoms associated with schizophrenia. Further investigation is warranted for our studies, perhaps including sub-chronic treatment of NMDA receptor antagonists to allow cortical changes and neurophysiological

deficits correlated with CIAS to emerge and more closely mimic observations in schizophrenic patients.

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The authors declare no conflict of interest.

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REFERENCES

Behrens MM, Ali SS, Dao DN, Lucero J, Shekhtman G, Quick KL *et al* (2007). Ketamine-induced loss of phenotype of fast-spiking interneurons is mediated by NADPH-oxidase. *Science* **318**: 1645–1647.

Bickel S, Dias EC, Epstein ML, Javitt DC (2012). Expectancy-related modulations of neural oscillations in continuous performance tasks. *Neuroimage* **62**: 1867–1876.

Buzsaki G, Draguhn A (2004). Neuronal oscillations in cortical networks. *Science* **304**: 1926–1929.

Carlen MK, Meletis JH, Siegle JA, Cardin K, Futai D, Vierling-Claassen C *et al* (2012). A critical role for NMDA receptors in parvalbumin interneurons for gamma rhythm induction and behavior. *Mol Psychiatry* **17**: 537–548.

Cochran SM, Kennedy M, McKerchar CE, Steward LJ, Pratt JA, Morris BJ (2003). Induction of metabolic hypofunction and neurochemical deficits after chronic intermittent exposure to phencyclidine: differential modulation by antipsychotic drugs. *Neuropsychopharmacology* **28**: 265–275.

Dias EC, Bickel S, Epstein ML, Sehatpour P, Javitt DC (2013). Abnormal task modulation of oscillatory neural activity in schizophrenia. *Front Psychol* **4**: 540.

Driesen NR, McCarthy G, Bhagwagar Z, Bloch M, Calhoun V, D'Souza DC *et al* (2013). Relationship of resting brain hyperconnectivity and schizophrenia-like symptoms produced by the NMDA receptor antagonist ketamine in humans. *Mol Psychiatry* **18**: 1199–1204.

Ehrlichman RS, Gandal MJ, Maxwell CR, Lazarewicz MT, Finkel LH, Contreras D *et al* (2009). N-methyl-d-aspartic acid receptor antagonist-induced frequency oscillations in mice recreate pattern of electrophysiological deficits in schizophrenia. *Neuroscience* **158**: 705–712.

Ehrlichman RS, Maxwell CR, Majumdar S, Siegel SJ (2008). Deviance-elicited changes in event-related potentials are attenuated by ketamine in mice. *J Cogn Neurosci* **20**: 1403–1414.

Friedman D, Squires-Wheeler E (1994). Event-related potentials (ERPs) as indicators of risk for schizophrenia. *Schizophr Bull* **20**: 63–74.

Fries P (2009). Neuronal gamma-band synchronization as a fundamental process in cortical computation. *Annu Rev Neurosci* **32**: 209–224.

Fries P, Reynolds JH, Rorie AE, Desimone R (2001). Modulation of oscillatory neuronal synchronization by selective visual attention. *Science* **291**: 1560–1563.

Fries P, Womelsdorf T, Oostenveld R, Desimone R (2008). The effects of visual stimulation and selective visual attention on rhythmic neuronal synchronization in macaque area V4. *J Neurosci* **28**: 4823–4835.

Friston KJ (1999). Schizophrenia and the disconnection hypothesis. *Acta Psychiatr Scand Suppl* **395**: 68–79.

Gandal MJ, Edgar JC, Ehrlichman RS, Mehta M, Roberts TP, Siegel SJ (2010). Validating gamma oscillations and delayed auditory responses as translational biomarkers of autism. *Biol Psychiatry* **68**: 1100–1106.

Gandal MJ, Edgar JC, Klook K, Siegel SJ (2012). Gamma synchrony: towards a translational biomarker for the treatment-resistant symptoms of schizophrenia. *Neuropharmacology* **62**: 1504–1518.

Gil-da-Costa R, Stoner GR, Fung R, Albright TD (2013). Nonhuman primate model of schizophrenia using a noninvasive EEG method. *Proc Natl Acad Sci USA* **110**: 15425–15430.

Glausier JR, Fish KN, Lewis DA (2014). Altered parvalbumin basket cell inputs in the dorsolateral prefrontal cortex of schizophrenia subjects. *Mol Psychiatry* **19**: 30–36.

Green MF (1996). What are the functional consequences of neurocognitive deficits in schizophrenia? *Am J Psychiatry* **153**: 321–330.

Gruber T, Muller MM, Keil A, Elbert T (1999). Selective visual-spatial attention alters induced gamma band responses in the human EEG. *Clin Neurophysiol* **110**: 2074–2085.

Hong LE, Summerfelt A, Buchanan RW, O'Donnell P, Thaker GK, Weiler MA *et al* (2010). Gamma and delta neural oscillations and association with clinical symptoms under subanesthetic ketamine. *Neuropsychopharmacology* **35**: 632–640.

Kocsis B, Brown RE, McCarley RW, Hajos M (2013). Impact of ketamine on neuronal network dynamics: translational modeling of schizophrenia-relevant deficits. *CNS Neurosci Ther* **19**: 437–447.

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD *et al* (1994). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry* **51**: 199–214.

Lahti AC, Weiler MA, Tamara Michaelidis BA, Parwani A, Tamminga CA (2001). Effects of ketamine in normal and schizophrenic volunteers. *Neuropsychopharmacology* **25**: 455–467.

Lazarewicz MT, Ehrlichman RS, Maxwell CR, Gandal MJ, Finkel LH, Siegel SJ (2010). Ketamine modulates theta and gamma oscillations. *J Cogn Neurosci* **22**: 1452–1464.

Lett TA, Voineskos AN, Kennedy JL, Levine B, Daskalakis ZJ (2014). Treating working memory deficits in schizophrenia: a review of the neurobiology. *Biol Psychiatry* **75**: 361–370.

Lewis DA, Curley AA, Glausier JR, Volk DW (2012). Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. *Trends Neurosci* **35**: 57–67.

Lewis DA, Hashimoto T, Volk DW (2005). Cortical inhibitory neurons and schizophrenia. *Nat Rev Neurosci* **6**: 312–324.

Maxwell CR, Ehrlichman RS, Liang Y, Trief D, Kanes SJ, Karp J *et al* (2006). Ketamine produces lasting disruptions in encoding of sensory stimuli. *J Pharmacol Exp Therapeut* **316**: 315–324.

Moghaddam B, Adams B, Verma A, Daly D (1997). Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci* **17**: 2921–2927.

Paxinos G, Huang X, Toga A (1999). *The Rhesus Monkey Brain in Stereotaxic Coordinates*, 1st edn. Academic Press: San Diego, CA.

Phillips WA, Silverstein SM (2003). Convergence of biological and psychological perspectives on cognitive coordination in schizophrenia. *Behav Brain Sci* **26**: 65–137.

Pinault D (2008). N-methyl d-aspartate receptor antagonists ketamine and MK-801 induce wake-related aberrant gamma oscillations in the rat neocortex. *Biol Psychiatry* **63**: 730–735.

Rivolta D, Heidegger T, Scheller B, Sauer A, Schaum M, Birkner K *et al* (2015). Ketamine dysregulates the amplitude and connectivity of high-frequency oscillations in cortical-subcortical networks in humans: evidence from resting-state magnetoencephalography-recordings. *Schizophr Bull* **41**: 1105–1114.

Rotaru DC, Yoshino H, Lewis DA, Ermentrout GB, Gonzalez-Burgos G (2011). Glutamate receptor subtypes mediating synaptic activation of prefrontal cortex neurons: relevance for schizophrenia. *J Neurosci* **31**: 142–156.

Saunders JA, Gandal MJ, Roberts TP, Siegel SJ (2012a). NMDA antagonist MK801 recreates auditory electrophysiology disruption present in autism and other neurodevelopmental disorders. *Behav Brain Res* **234**: 233–237.

Saunders JA, Gandal MJ, Siegel SJ (2012b). NMDA antagonists recreate signal-to-noise ratio and timing perturbations present in schizophrenia. *Neurobiol Dis* **46**: 93–100.

Singer W (1999). Neuronal synchrony: a versatile code for the definition of relations? *Neuron* **24**: 49–65, 111–125.

Sohal VS, Zhang F, Yizhar O, Deisseroth K (2009). Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature* **459**: 698–702.

Sullivan EM, Timi P, Hong LE, O'Donnell P (2015). Reverse translation of clinical electrophysiological biomarkers in behaving rodents under acute and chronic NMDA receptor antagonism. *Neuropsychopharmacology* **40**: 719–727.

Sun Y, Farzan F, Barr MS, Kirihara K, Fitzgerald PB, Light GA *et al* (2011). Gamma oscillations in schizophrenia: mechanisms and clinical significance. *Brain Res* **1413**: 98–114.

Tamas G, Buhl EH, Lorincz A, Somogyi P (2000). Proximally targeted GABAergic synapses and gap junctions synchronize cortical interneurons. *Nat Neurosci* **3**: 366–371.

Tiitinen H, Sinkkonen J, Reinikainen K, Alho K, Lavikainen J, Naatanen R (1993). Selective attention enhances the auditory 40-Hz transient response in humans. *Nature* **364**: 59–60.

Uhlhaas PJ, Singer W (2010). Abnormal neural oscillations and synchrony in schizophrenia. *Nat Rev Neurosci* **11**: 100–113.

von Stein A, Sarnthein J (2000). Different frequencies for different scales of cortical integration: from local gamma to long range alpha/theta synchronization. *Int J Psychophysiol* **38**: 301–313.

Wang M, Yang Y, Wang CJ, Gamo NJ, Jin LE, Mazer JA *et al* (2013). NMDA receptors subserve persistent neuronal firing during working memory in dorsolateral prefrontal cortex. *Neuron* **77**: 736–749.

Womelsdorf T, Fries P (2007). The role of neuronal synchronization in selective attention. *Curr Opin Neurobiol* **17**: 154–160.

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