International Journal of Neuropsychopharmacology (2014), **17**, 1671–1681. © CINP 2014
doi:10.1017/S1461145714000492

ARTICLE

Effects of aberrant gamma frequency oscillations on prepulse inhibition

Nigel C. Jones[1], Paul Anderson[1], Gil Rind[1], Caley Sullivan[1], Maarten van den Buuse[2,3] and Terence J. O'Brien[1]

[1] *Department of Medicine (RMH), University of Melbourne, Melbourne Brain Centre, Parkville, Victoria, Australia*
[2] *Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne Brain Centre, Parkville, Victoria, Australia*
[3] *School of Psychological Science, La Trobe University, Bundoora, Melbourne, Victoria, Australia*

Abstract

Emerging literature implicates abnormalities in gamma frequency oscillations in the pathophysiology of schizophrenia, with hypofunction of N-methyl-D-aspartate (NMDA) receptors implicated as a key factor. Prepulse inhibition (PPI) is a behavioural measure of sensorimotor gating, which is disrupted in schizophrenia. We studied relationships between ongoing and sensory-evoked gamma oscillations and PPI using pharmacological interventions designed to increase gamma oscillations (ketamine, MK-801); reduce gamma oscillations (LY379268); or disrupt PPI (amphetamine). We predicted that elevating ongoing gamma power would lead to increased 'neural noise' in cortical circuits, dampened sensory-evoked gamma responses and disrupted behaviour.

Wistar rats were implanted with EEG recording electrodes. They received ketamine (5 mg/kg), MK-801 (0.16 mg/kg), amphetamine (0.5 mg/kg), LY379268 (3 mg/kg) or vehicle and underwent PPI sessions with concurrent EEG recording.

Ketamine and MK-801 increased the power of ongoing gamma oscillations and caused time-matched disruptions of PPI, while amphetamine marginally affected ongoing gamma power. In contrast, LY379268 reduced ongoing gamma power, but had no effect on PPI. The sensory gamma response evoked by the prepulse was reduced following treatment with all psychotomimetics, associating with disruptions in PPI. This was most noticeable following treatment with NMDA receptor antagonists.

We found that ketamine and MK-801 increase ongoing gamma power and reduce evoked gamma power, both of which are related to disruptions in sensorimotor gating. This appears to be due to antagonism of NMDA receptors, since amphetamine and LY379268 differentially impacted these outcomes and possess different neuropharmacological substrates. Aberrant gamma frequency oscillations caused by NMDA receptor hypofunction may mediate the sensory processing deficits observed in schizophrenia.

Received 19 November 2013; Reviewed 17 December 2013; Revised 10 March 2014; Accepted 13 March 2014; First published online 15 May 2014

Key words: Animal behaviour, electrophysiology, gamma frequency oscillations, NMDA receptor antagonist, prepulse inhibition, schizophrenia.

Introduction

Schizophrenia is a debilitating psychiatric condition characterized by deficits in many aspects of higher order behaviour, including information processing, sensory perception, and cognition (Elvevag and Goldberg, 2000). The broad nature of the cognitive deficits has led to the conceptualization of schizophrenia as a disorder of neural networks (Spencer et al., 2003; Lewis et al., 2005; Uhlhaas and Singer, 2010). In particular, high frequency (30–80 Hz; gamma) neuronal oscillations have received significant attention (Lee et al., 2003a; Uhlhaas and Singer, 2010; Gandal et al., 2012) due to a growing understanding of their important role in cognitive processes, which are disrupted in schizophrenia (Fell et al., 2001; Fries et al., 2001; Jensen et al., 2007). Many reports document abnormal gamma activity in schizophrenia patients, with the findings largely falling into two distinct categories depending on the nature of the oscillations studied: (1) several reports describe *reduced* stimulus-evoked gamma responses, suggesting an inappropriate sensory response to a given stimulus (e.g. (Kwon et al., 1999; Leicht et al., 2010); and (2) others report *increases* in ongoing, or baseline gamma frequency activity in schizophrenia patients (Baldeweg et al., 1998; Flynn et al., 2008; Spencer, 2011), which might represent aberrant cortical network noise disrupting normal cognitive processing.

One prominent theory describing the underlying neurobiological mechanisms of schizophrenia revolves

Address for correspondence: N. C. Jones, Department of Medicine (RMH), University of Melbourne, Melbourne Brain Centre, Parkville 3052, Australia.
Tel.: +61 3 9035 6402 *Fax*: +61 3 9347 1863
Email: ncjones@unimelb.edu.au

around N-methyl-D-aspartate receptor (NMDAr) hypofunction (Goff and Coyle, 2001; Coyle, 2012). This theory was proposed based on observations that NMDAr antagonists, such as ketamine, induce symptoms in healthy volunteers reminiscent of those seen in schizophrenia, and exacerbate these symptoms in schizophrenia patients (Adler et al., 1998; Newcomer et al., 1999). The molecular substrates mediating the neurophysiological deficits in schizophrenia are not fully understood, but NMDAr hypofunction presents a strong candidate mechanism. GABAergic parvalbumin (PV)-expressing inhibitory interneurons are believed to contribute to the generation of gamma frequency oscillations (Cobb et al., 1995), and several lines of evidence suggest that NMDAr hypofunction can influence the activity of these interneurons and alter the regulation of gamma frequency oscillations. For example, ablation of NMDAr on PV interneurons increases ongoing gamma frequency spectral power (Korotkova et al., 2010; Carlen et al., 2012). Administration of NMDAr antagonists dose-dependently increases ongoing cortical gamma oscillations in rodents (Pinault, 2008; Ehrlichman et al., 2009; Hakami et al., 2009; Jones et al., 2012; Kocsis, 2012; Kulikova et al., 2012) and reduces sensory-evoked gamma band responses (Kulikova et al., 2012; Saunders et al., 2012), patterns which are akin to those commonly observed in patients with schizophrenia. However, there exists a substantial gap in our understanding of the causal connections between alterations in gamma oscillations induced by NMDAr antagonists and the behavioural and symptomatic consequences of these drugs. Specifically, does a state of NMDAr hypofunction cause any schizophrenia-like symptoms directly because of the effects on the regulation of gamma frequency oscillations? Further, the relationship between reductions in sensory-evoked gamma activity observed in schizophrenia and the symptom profile of these patients is similarly complex (Lee et al., 2003b).

Sensorimotor gating deficits are prevalent endophenotypes of schizophrenia (Braff and Geyer, 1990). While sensorimotor gating is not considered to be a cognitive measure *per se*, abnormalities in pre-attentive information processing may be predictive of, or lead to, complex cognitive deficits. Several studies have demonstrated that sensorimotor gating deficits are induced by administration of NMDAr antagonists, such as ketamine and MK-801, and also by other psychotomimetic compounds such as amphetamine (van den Buuse, 2010), but the underlying neurophysiology regulating this behaviour has not been studied. Prepulse inhibition (PPI) is a widely used model of sensorimotor gating (Swerdlow et al., 2008). It describes the behavioural observation that when a startling stimulus, such as a loud sound, is preceded by a non-startling stimulus, the response to the startle stimulus is reduced. We designed this study to investigate the relationships between gamma frequency oscillations – both ongoing and evoked – and the regulation of sensorimotor gating in the form of PPI of the acoustic startle response. To study sensory evoked gamma oscillations, we measured the electrophysiological response occurring immediately after the prepulse in the behavioural tests. This was based on the assumption that the prepulse is a defining element of the behaviour exhibited during a PPI session (see discussion in Yee and Feldon, 2009), and allowed us to correlate electrophysiological changes with behavioural deficits. We hypothesized that drugs which elevate ongoing gamma power, such as NMDAr antagonists, would lead to a concurrent disruption of PPI, and, conversely, that drugs which reduce ongoing gamma power, such as the mGluR2/3 agonist LY379268 (Jones et al., 2012), would facilitate PPI. We also anticipated a strong relationship between the gamma frequency signal evoked by the prepulse and the regulation of PPI. Finally, we predicted that the strength of the evoked signal would be inversely related to background ongoing gamma activity.

Method

Animals

Adult male Wistar rats, aged 14–16 weeks at the time of surgery (n=12; 250–350 g), were obtained from the Australian Resource Centre and housed at the Bio-Resources Facility of the Melbourne Brain Centre, Parkville, Australia. The facility was maintained on a 12 h light/dark cycle, lights on from 06:00–18:00, and food (standard rat chow) and water were available *ad libitum*. All procedures performed in this study were approved by the University of Melbourne Animal Ethics Committee (#1011868).

Surgical electrode implantation

At least one week following delivery of animals, surgical implantation of electrocorticogram (ECoG) recording electrodes was performed, as previously described (Jones et al., 2008). Rats were anaesthetized by inhalation of isoflurane (5% induction, 1–2% maintenance) with equal parts of medical air and oxygen, and positioned in a stereotaxic frame. An incision was made along the midline of the scalp, and six holes were drilled through the skull without breaching the dura. Brass recording electrodes were gently screwed into the skull at 2 mm anterior and 2 mm lateral to bregma bilaterally (active electrodes); 2 mm posterior and 2 mm lateral to bregma bilaterally (reference electrodes); and 2 mm posterior and 2 mm lateral to lambda bilaterally (ground electrodes). The protruding ends of the electrodes were fitted to a plastic pedestal (PlasticsOne, Bioscientific Pty Ltd, Australia) and the pedestal was secured to the skull using dental cement.

Experimental design

This study consisted of simultaneous recording of ECoG and measurement of PPI. Prior to commencing the

study, animals were handled several times and habituated to the equipment. At the start of each session, animals were connected to an ECoG cable (PlasticsOne, Bioscientific Pty Ltd, Australia) and placed in a 9 cm diameter Plexiglas cylinder inside an electrostatically shielded PPI startle chamber (SR-LAB, San Diego Instruments, USA). ECoG recording was initiated and, following a baseline EEG recording, the animal was taken from the chamber and injected s.c. with ketamine, MK-801, *d*-amphetamine, LY379268, or vehicle (0.9% saline), and then replaced within the chamber. The drug doses were chosen based on our previous work, which demonstrated that, in rats, 0.5 mg/kg ketamine and 0.16 mg/kg MK-801 both increase ongoing gamma activity (Hakami et al., 2009), that 3.0 mg/kg LY379268 reduces ongoing gamma power (Jones et al., 2012), and that 0.5 mg/kg amphetamine disrupts PPI (Choy et al., 2009). PPI sessions were initiated after injection and continued for the following 90 min, during which time the ECoG was recorded. Each animal received each drug on subsequent sessions in a pseudo-randomized, repeated measures design with a minimum of two rest days between sessions to allow drug 'wash-out'.

PPI measurement

A piezoelectric accelerometer unit attached underneath the platform of the Plexiglas cylinders detected the startle responses of the animals. Acoustic stimuli were delivered, and responses recorded, using SR-LAB startle software (San Diego Instruments). Sessions were programmed to include two trial types: 115 dB startle pulse-alone trials of 40 ms duration; and prepulse-pulse trials, in which the startle pulse was preceded by 100 ms (onset to onset) by a 78 dB prepulse of 20 ms duration (i.e., 8 dB above background which does not, by itself, cause a behavioural response). These two trial types were interspersed, and herein referred to as either pulse-alone trials or prepulse trials. Background noise was set at 70 dB. Average behavioural PPI was calculated in 5-min, 12 trial blocks which included six pulse-alone and six prepulse trials delivered in a pseudo-random order. The inter-trial interval varied randomly (15, 20, 25 or 40 s). After 30 min, PPI blocks were separated with 5 min of background noise. Percentage prepulse inhibition (%PPI) was calculated for each PPI block, with the formula 100×[(startle from pulse-alone trials−startle from prepulse trials)/startle from pulse-alone trials)].

ECoG recording

The experiment used standard EEG equipment including a MacLab amplifier and A-D converter (ADInstruments, Australia), and Chart V4.5 Software (ADInstruments, Australia). ECoG was recorded continuously (sampling rate 1 kHz) on three channels for the duration of the session, one channel for each hemisphere (left and right), and the third channel was connected to the PPI amplifier to record the acoustic pulses. Noise emanating from the power mains (50 Hz) was controlled using selective eliminators (Humbugs; Digitimer, UK).

Analysing gamma oscillations

Ongoing gamma frequency data was analysed using NEUROSCAN® software (Compumedics, Australia), and evoked electrophysiological responses were analysed using MATLAB software (v7.10.0, Natick, Massachusetts: The MathWorks Inc., 2010). For measurement of ongoing gamma power, initially the recording was notch filtered (49–51 Hz) and divided into 5 min blocks consisting of 2.05 s epochs (148 epochs per block). Every epoch was inspected visually for signal distortion or movement artifact – this process also removed all EEG epochs which contained the startling pulses. Using fast Fourier transformations (FFT), average power in the gamma frequency band (30–80 Hz) was determined for each 2.05 s epoch over the entire recording, and average power was calculated for each 5 min block (Hakami et al., 2009). This process was repeated for all animals in all conditions. The data were collated using custom-designed scripts written for MATLAB software. For the purpose of this study, the left and right hemisphere measures were averaged together. The first 5 min of habituation and the ~5 min during injection were excluded from final analyses. The remaining values in the pre-injection period were averaged together to generate a baseline value for each individual recording, obtained while the animals were sitting quietly in the startle chamber. All subsequent values were expressed as a percentage of baseline [(raw power/baseline)×100].

For measurement of stimulus-evoked gamma power, individual epochs covering 600 ms centred around the prepulse were isolated, and the baseline-corrected spectral power, or event-related spectral perturbation (ERSP), calculated using the EEGLAB toolbox (Delorme and Makeig, 2004). ERSP was calculated from 20 to 120 Hz, with morlet wavelets ranging from three cycles at lower frequencies to ten at the highest. Baseline gamma power (mean power 30–80 Hz, −400 to −150 ms relative to onset of the prepulse), prepulse gamma power (0 to +60 ms relative to onset of prepulse) and pulse gamma power (+100 to +160 ms relative to prepulse, which is 0 to +60 ms relative to the pulse) was then extracted for each individual trial and averaged into 5 min blocks for correlation with PPI data.

Drugs

Isoflurane was obtained from Abbott Australasia Pty Ltd, Australia and carprofen (Rimadyl), obtained from Pfizer Animal Health Group, UK, was administered as post-surgery analgaesic. Ketamine (Troy Laboratories Pty Ltd, Australia), MK-801 and *d*-amphetamine

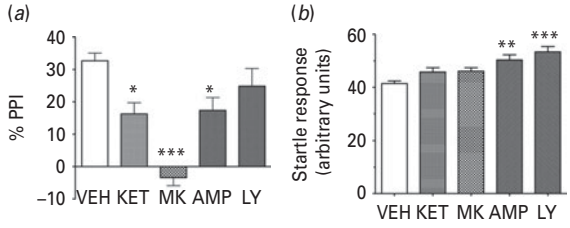

Fig. 1. (*a*) Average % prepulse inhibition (PPI) is significantly disrupted by psychotomimetic drugs ketamine (KET), MK-801 (MK) and amphetamine (AMP), but is not affected by LY379268 (LY), relative to vehicle (VEH) treatment. (*b*) The average startle response was enhanced by amphetamine (AMP) and LY379268 (LY), but not the other drugs. Data represent mean+S.E.M. *$p<0.05$, ***$p<0.001$ compared to vehicle control.

(Sigma-Aldrich, Australia) and LY379268 (Abcam Biochemicals, Sapphire Biosciences Pty Ltd, Australia) were used as the test drugs in the study. Drugs were dissolved in 0.9% saline prior to each session. To dissolve MK-801 and LY379268, solutions were sonicated until clear.

Statistical analysis

The behavioural data and evoked gamma responses were analysed using one-way analysis of variance (ANOVA) with repeated measures (drug), comparing all drug conditions to control (saline). Ongoing gamma power was normalized to the pre-injection period for each trial, and compared between drug treatments over time using two-way ANOVA with repeated measures. Correlational analyses between PPI and either ongoing or stimulus-triggered gamma power incorporated values at each 5 min time-point in each drug condition and were done using Pearson's correlation. All statistical comparisons were performed using GraphPad Prism®, and statistical significance was set at $p<0.05$ for all tests. Data are presented as mean±standard error of the mean (S.E.M.). A number of animals were removed over the course of the study due to headpieces dislodging. Our final analysis includes animals ($n=6$) that completed all treatments, allowing for repeated measures analyses to be conducted.

Results

Psychotomimetic drugs disrupt prepulse inhibition of the acoustic startle response

As anticipated, we observed significant overall differences in the regulation of PPI between the different drugs measured over the entire session ($F_{(1,5)}=12.9$, $p<0.001$; Fig. 1*a*). *Post-hoc* analysis revealed that ketamine (50% reduction, $p<0.05$), MK-801 (>100% reduction, $p<0.001$) and amphetamine (47% reduction, $p<0.05$) all significantly disrupted PPI compared to vehicle

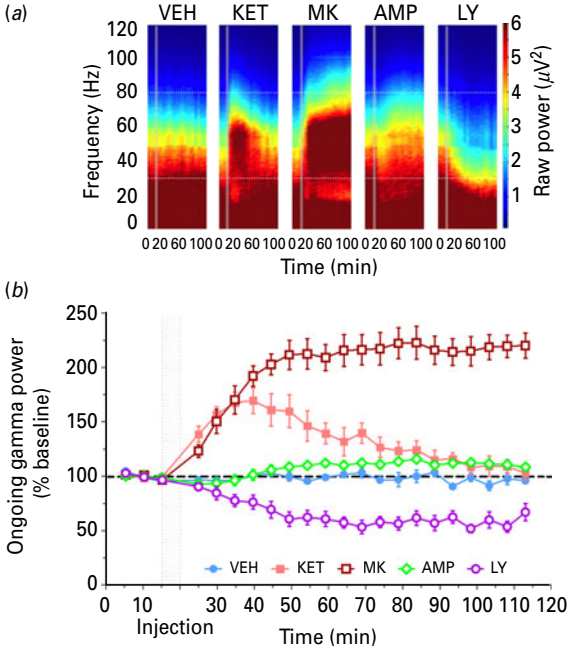

Fig. 2. Electrophysiological responses caused by the different drugs over time. (*a*) Heat maps of spectral power changes over the experimental session. (*b*) Quantification of the changes in gamma power over time measured in response to different drugs, relative to baseline (100%). The shaded vertical bar at $t=15$–20 min represents the injection period, and the dashed horizontal line represents 100% of baseline. Ketamine (KET; red solid squares), MK-801 (MK; brown open squares) and amphetamine (AMP; green open diamonds) all significantly increased ongoing gamma power compared to vehicle (VEH; closed blue circles). In contrast, LY379268 (LY; open purple circles) significantly reduced ongoing gamma power. Data represent mean±S.E.M. $p<0.05$ for all treatment conditions compared to vehicle.

treatment, but that LY379268 was without effect. We also observed differences in the mean behavioural startle response to the pulse-alone trials between treatments ($F_{(1,5)}=7.5$, $p<0.001$; Fig. 1*b*). Amphetamine (22% increase, $p<0.01$) and LY379268 (29% increase, $p<0.001$) both increased the startle response compared to vehicle, but treatment with either of the NMDAr antagonists did not influence this measure.

Differential drug effects on EEG spectra and ongoing gamma power

All test drugs substantially impacted frequency spectra over the duration of the experiments (see Fig. 2*a*). Overall analysis of ongoing gamma frequency oscillations revealed a significant effect of drug treatment ($F_{(1,5)}=60.9$, $p<0.001$; Fig. 2*b*). As previously reported, both ketamine ($p<0.001$) and MK-801 ($p<0.001$) increased ongoing gamma power when compared to vehicle treatment, but with different temporal profiles: the effect of ketamine was immediate, peaking 20 min after injection, and then

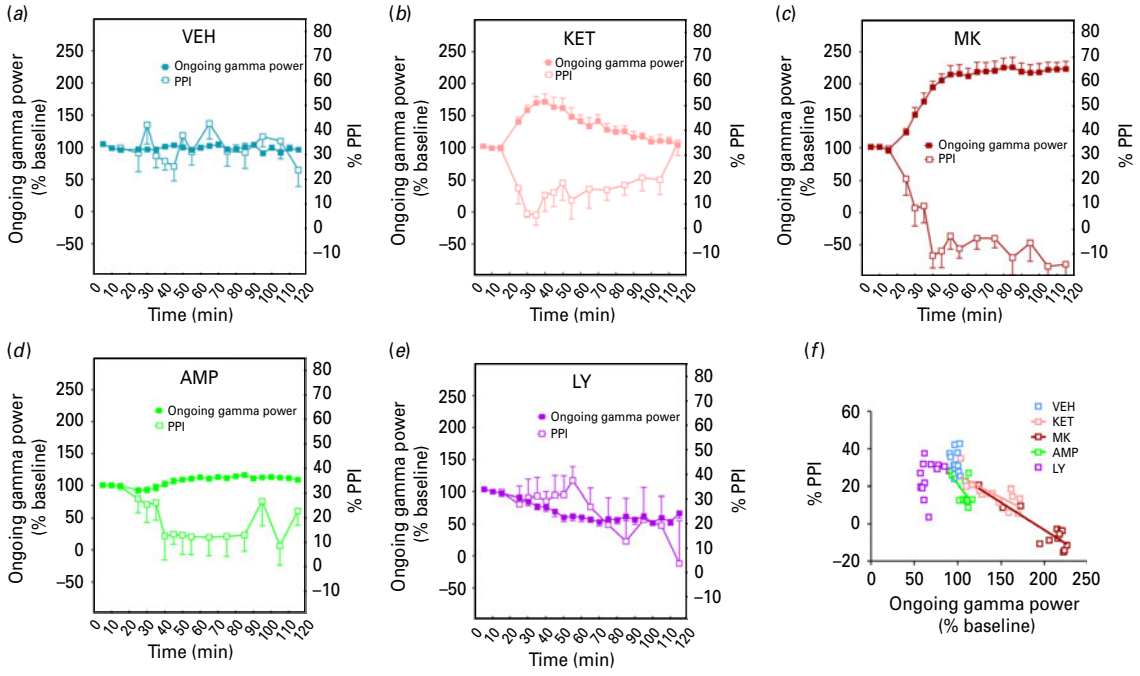

Fig. 3. (*a–e*) Temporal comparisons of the changes in ongoing gamma power (closed squares, left abscissa) and %PPI (open squares, right abscissa) in the different drug conditions. (*f*) Significant correlations were observed between %PPI and ongoing gamma power after treatment with ketamine (r^2=0.56), MK-801 (r^2=0.80) and amphetamine (r^2=0.39); regression lines represent these significant correlations.

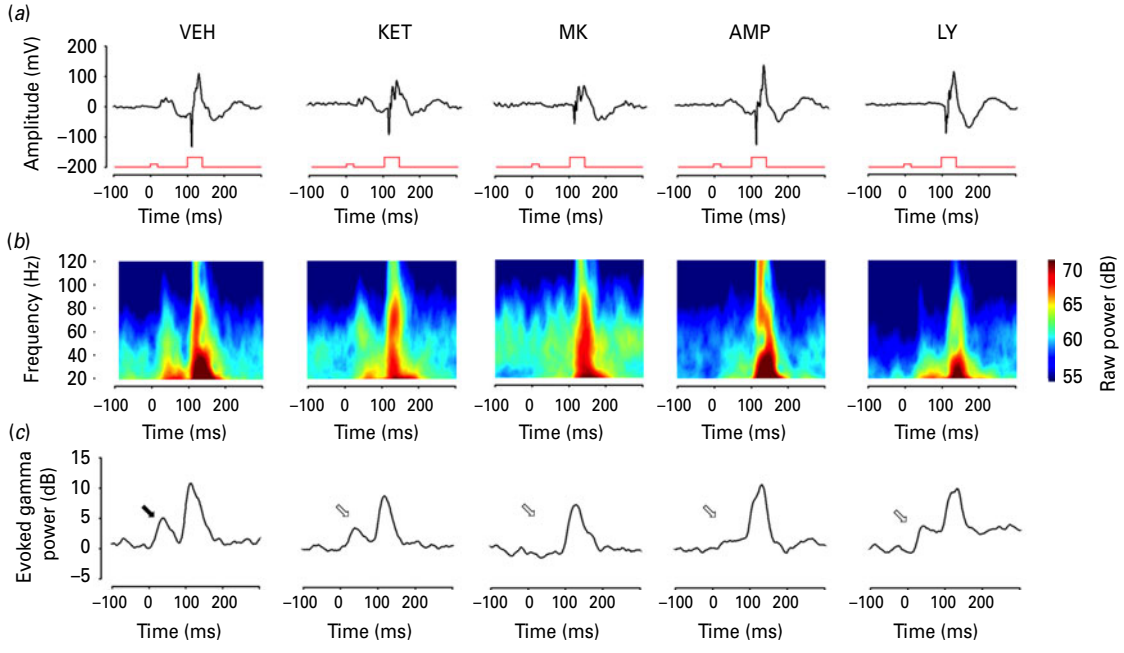

Fig. 4. Effect of psychotomimetic drugs (represented in the different columns) on ECoG responses measured during the behavioural task. (*a*) Averaged EEG waveform evoked by the prepulse trials. The acoustic stimuli are represented under the waveforms (time-point of 0 refers to initiation of the prepulse). (*b*) Time-frequency heat maps showing mean spectral power generated in response to all prepulse trials from 30–60 min post injection. Note the ability of NMDA receptor antagonists ketamine and MK-801 to increase the pre-stimulus gamma power. (*c*) Averaged event-related spectral perturbation (ERSP) gamma power measured during the behavioural trials. Note the substantially smaller gamma power responses in the drug-treated conditions (open arrowheads) compared to vehicle (solid arrowhead) recorded immediately following presentation of the prepulse (i.e. between 0–100 ms). These data are generated from one animal under all five drug conditions (60 epochs): ketamine (KET), MK-801 (MK), amphetamine (AMP), LY379268 (LY) and vehicle (VEH).

returning to baseline over the time of recording, whereas the effect of MK-801, which peaked around 30 min after injection, was sustained for the duration of the study. Amphetamine also slightly, but significantly, increased gamma power ($p=0.021$), an effect that became clear about 40 min after injection. LY379268 was the only compound to significantly reduce gamma power ($p<0.001$), achieving a steady reduction to about 55% of baseline levels.

Psychotomimetics disrupt PPI and increase ongoing gamma power in a temporally related fashion

To investigate whether changes in ongoing gamma power were directly related to impaired PPI, we compared the time courses of effects of these two variables for the different drugs (Fig. 3). Both NMDAr antagonists induced temporally matched disruptions in PPI and elevations in ongoing gamma power (Fig. 3*b*, *c*). Amphetamine also caused a concurrent increase in gamma power and disruption of PPI (Fig. 3*d*), but the magnitude of its effect on PPI appeared much greater than its effect on cortical gamma oscillations. In contrast to the other drugs, there was no relationship between the effects of LY379268 on gamma power and sensorimotor gating (Fig. 3*e*), perhaps because of the inconsistent effect on PPI of this drug. Linear regression analysis (Fig. 3*f*) showed that the effects of ketamine ($p<0.001$), MK-801 ($p<0.001$), and amphetamine ($p=0.02$) on behaviour and ongoing gamma power were significantly anti-correlated.

Psychotomimetic drugs reduce sensory-evoked responses and this temporally coincides with impaired PPI

Figure 4*a* illustrates the average event-related spectral perturbation (ERSP) triggered in the ECoG waveform during the PPI trials in the different conditions and Fig. 4*b* shows the associated ERSP without baseline correction, highlighting the frequency-specific changes caused by each drug. Following a baseline correction, when comparing each drug condition (Fig. 4*c*), we observed striking differences in the gamma response immediately following the prepulse, which appears dampened following psychotomimetic administration. When quantifying the power of the sensory-evoked gamma frequency responses occurring within 60 ms following onset of the prepulse, we found significant differences between treatments in stimulus-elicited gamma power, when averaged over the entire recording session (60 epoch averages; $F_{(1,5)}=28.6$; $p<0.0001$; Fig. 5*a*). The three psychotomimetic drugs, ketamine, MK-801 and amphetamine, all significantly reduced the gamma ERSP response to the prepulse compared to saline. LY379268 on the other hand, did not have any effect on the sensory-evoked response. These data associated well with the effects of these drugs on prepulse inhibition (see Fig. 1), suggesting a relationship between this electrophysiological measure and behaviour.

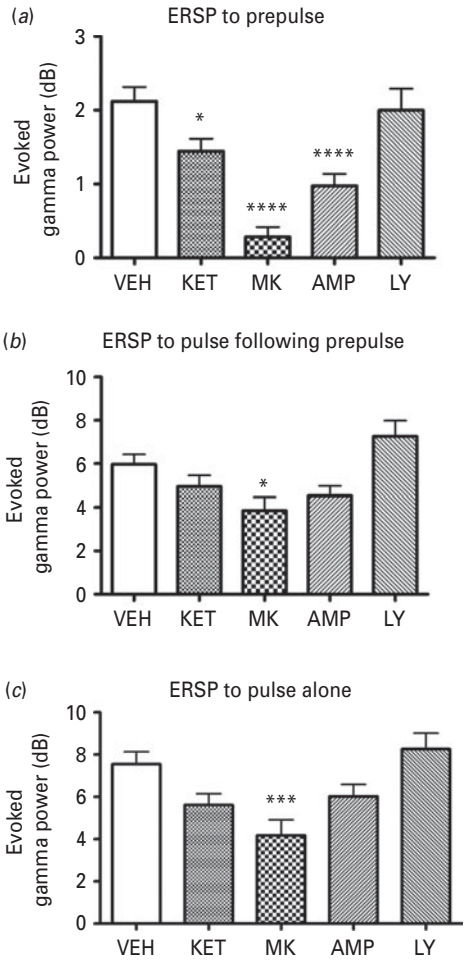

Fig. 5. Gamma power responses evoked by the prepulse are reduced by psychotomimetic drugs. (*a*) The gamma ERSP response to the prepulse is significantly reduced by ketamine (KET), MK-801 (MK) and amphetamine (AMP), compared to vehicle (VEH). LY379268 (LY) had no effect. (*b*) The gamma ERSP response to pulses which followed a prepulse was reduced by MK-801, but no other treatment significantly altered this response. (*c*) When assessing the gamma ERSP response evoked by the pulses which were not coupled to a prepulse, MK-801 was the only compound to significantly reduce the response. Data represent mean+S.E.M.; $n=6$ for all groups. *$p<0.05$, ***$p<0.001$, ****$p<0.0001$ compared to vehicle control.

We also measured the gamma response triggered by the pulse which followed the prepulse. The effects of drug were somewhat similar, although much less dramatic, to those elicited by the prepulse (overall ANOVA: $F_{(1,5)}=9.661$; $p<0.0001$; Fig. 5*b*). When examining the response to trials that were without a prepulse – i.e. pulse alone – the same pattern of EEG responses were observed, with significant reductions in the MK-801 condition (overall ANOVA: $F_{(1,5)}=11.37$; $p<0.0001$; Fig. 5*c*). Because of the potential for movement artefacts to compromise the electrophysiological recordings immediately following the

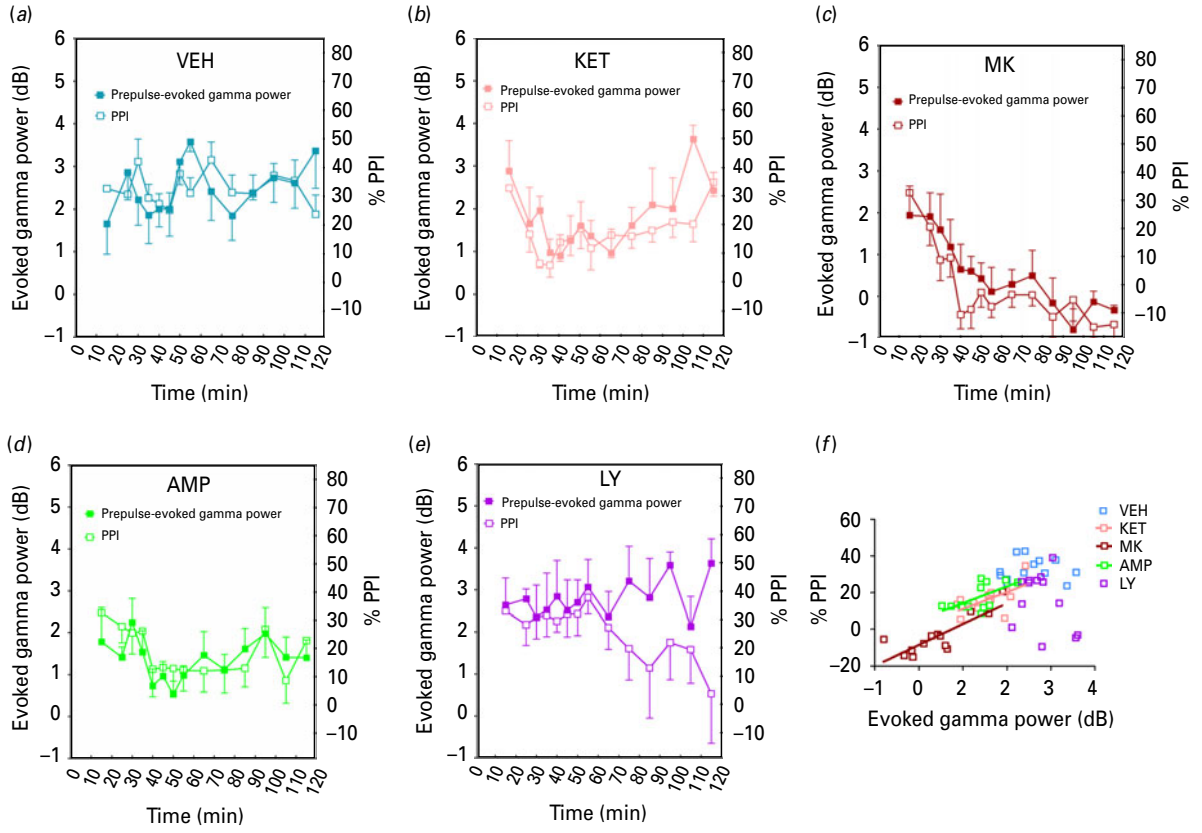

Fig. 6. (*a–e*) Time course of effects of drugs on prepulse-evoked gamma power (left abscissa) and %PPI (right abscissa) measured over the course of the experiments. Note the close temporal relationship in effects on these two measures after psychotomimetic drug treatment. (*f*) Significant correlations were observed between prepulse-evoked gamma power and PPI after ketamine (r^2=0.36), MK-801 (r^2=0.70) and amphetamine treatments (r^2=0.37), p<0.05 for these conditions; regression lines represent significant correlations.

startling pulse, all other electrophysiological data reported refer to responses evoked by the prepulse.

Analysis of the time courses of the effects of the drugs on PPI and on prepulse-evoked gamma responses (Fig. 6) showed that, for the three psychotomimetic drugs, temporal changes in these two measures matched well, with significant correlations being observed (ketamine: r^2=0.36, p=0.03; MK-801: r^2=0.70, p<0.001; amphetamine: r^2=0.37, p=0.03; Fig. 6*f*). No such relationship was observed following LY379268 or vehicle treatment.

NMDAr antagonists simultaneously increase ongoing gamma power and reduce sensory-evoked gamma power responses

Analysis of the relationship between ongoing and evoked gamma power over the course of the experiment showed that, in the presence of NMDAr antagonists, the reductions in evoked responses were temporally related to the increase in ongoing gamma power (Fig. 7). Correlational analysis demonstrated that this was highly significant for both ketamine (r^2=0.52, p=0.006) and MK-801 (r^2=0.80, p<0.001), but no relationship for the other treatment conditions existed.

Discussion

Several reports document abnormal regulation of gamma frequency oscillations in schizophrenia (reviewed in Uhlhaas and Singer, 2010), but whether these are causal to the disease, consequential to the psychotic illness, or unrelated epiphenomena has been difficult to establish. Using a series of drugs with different pharmacological substrates and electrophysiological effects, here we investigated the relationship between evoked and ongoing gamma oscillations and PPI deficits, prominent behavioural features of psychiatric conditions, in particular of schizophrenia (Braff and Geyer, 1990). We found that treatment with the psychotomimetic compounds MK-801, ketamine and amphetamine all increased ongoing gamma oscillations and reduced sensory-evoked gamma responses, and that these effects were temporally related with behavioural disruptions in prepulse inhibition, used as a measure of sensorimotor gating. LY379268, a preclinical antipsychotic (Cartmell et al., 2000), reduced ongoing gamma oscillations but did not influence PPI. This work complements previous studies examining electrophysiological activity during a PPI task in healthy humans and patients (Ford et al., 1999;

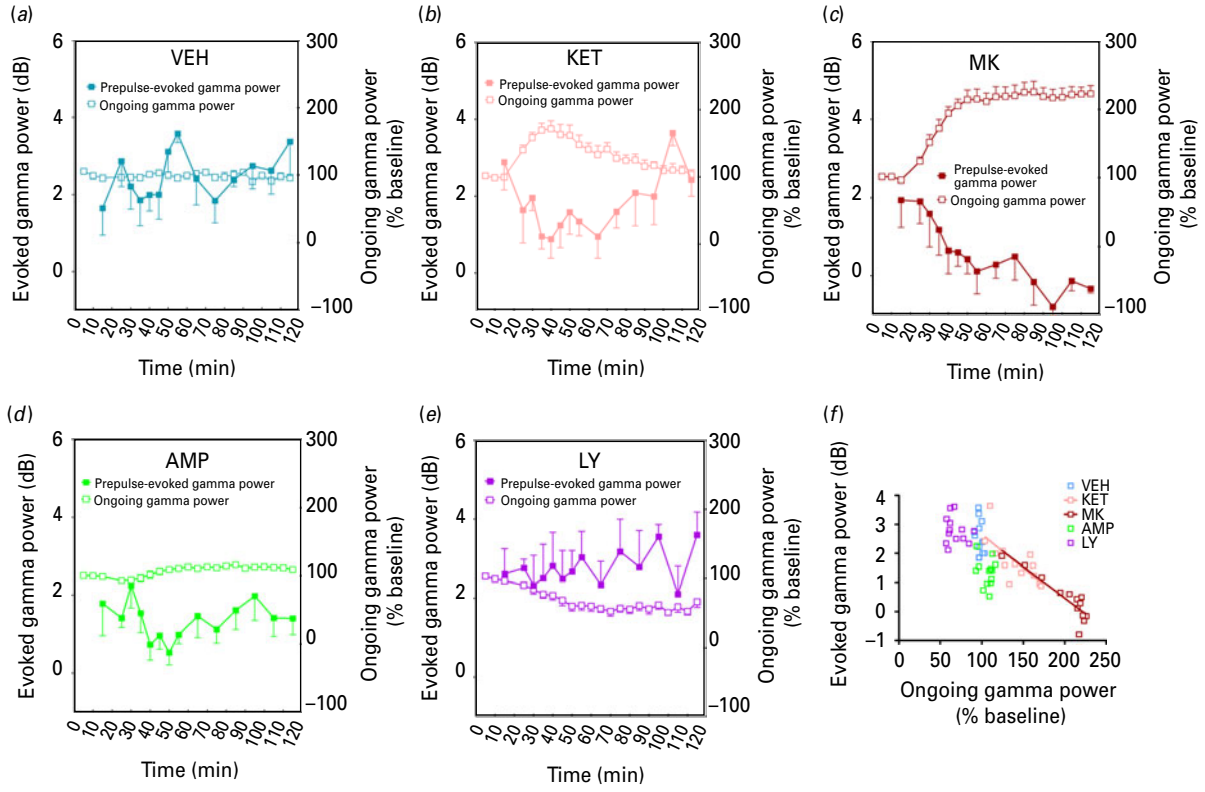

Fig. 7. (*a–e*) Time course of effects of drugs on prepulse-evoked (left abscissa) and ongoing (right abscissa) gamma power measured over the course of the experiments. Note the inverse relationship in effects after ketamine and MK-801 administration, which is not as marked following the other treatments. (*f*) Significant correlations were only observed between evoked and ongoing gamma power after ketamine (r^2=0.52) and MK-801 (r^2=0.80), p<0.001 for these conditions; regression lines represent significant correlations.

Kedzior et al., 2007), and others which compare and contrast PPI with N40 and P50 gating (Oranje et al., 2006; Swerdlow et al., 2006, 2012; Holstein et al., 2013), electrophysiological phenomena used to investigate sensory gating mechanisms. Our study demonstrates strong associations between abnormalities in gamma power and deficits in sensorimotor gating induced by psychotomimetics, and supports the notion that gamma power abnormalities may be relevant to sensory processing disturbances observed in psychiatric disorders particularly those associated with glutamatergic deficiency such as schizophrenia.

We, and others, have previously reported that NMDAr antagonists elevate the power of ongoing gamma frequency oscillations (Pinault, 2008; Ehrlichman et al., 2009; Hakami et al., 2009; Jones et al., 2012; Kocsis, 2012; Kulikova et al., 2012), and proposed that this may represent an electrophysiological correlate of a psychotic-like state. To investigate the functional consequences of alterations in ongoing gamma power, we pharmacologically manipulated this electrophysiological outcome and examined the consequences of this on sensorimotor gating. We found a strong relationship between elevations in ongoing gamma power and disruptions in PPI caused by the NMDAr antagonists, ketamine and MK-801. However, reducing ongoing gamma power with LY379268 did not achieve the heightened PPI levels, which would have been expected if a direct and independent relationship between these outcomes existed. Instead, it appears from our data that there is a relationship between ongoing gamma power and PPI regulation in the context of NMDAr hypofunction. While we also observed such a relationship following amphetamine, this drug induced marked effects on PPI but only caused a small, albeit significant, elevation in ongoing gamma power, making this harder to interpret. Only limited studies have examined ongoing gamma power in schizophrenia, and none have attempted to correlate behavioural endophentoypes to this outcome. However, our evidence suggests a relationship between ongoing gamma power and PPI regulation in pathological conditions particularly associated with NMDA hypofunction, and this should be interrogated in the clinical situation.

While the majority of studies examining gamma frequency oscillations in schizophrenia have grouped patients together regardless of the specific symptom profile or severity of the symptoms, some have attempted to strengthen the association by correlating specific behavioural features with neurophysiological abnormalities (Lee et al., 2003a). For example, phase locking to a

40-Hz auditory stimulation train is reduced in first-episode patients with schizophrenia, and this correlates with total positive symptoms in these patients (Spencer et al., 2008). Also, when performing an auditory oddball task, different schizophrenia syndromes (i.e. psychomotor poverty, reality distortion and disorganization) were differentiated by contrasting patterns of gamma synchrony (Lee et al., 2003b). Here we show that gamma responses evoked by the prepulse are impaired in rats treated with psychotomimetic compounds, and that these reductions correlate with the disruptions in PPI. Sensory-related gamma oscillations appear to be important for the early stages of perception (Haenschel et al., 2000; Kopell et al., 2000), so reducing the intensity of the signal evoked by the prepulse would be expected to impact the resultant behavioural response. This finding directly links changes in evoked electrophysiological responses with behavioural dysfunction. The relationship was most prominently observed in the NMDAr antagonist conditions, supporting the notion that NMDAr hypofunction-mediated impairment of PPI is related to its effects on gamma frequency oscillations.

We also made measurement of the evoked gamma responses, which immediately followed the startling pulse. While these responses were reduced following NMDAr antagonism, just as the response to the prepulse was, these data should be interpreted with caution. Following the presentation of the startling pulse, there is a significant amount of movement – the behavioural startle response – that could create electrophysiological artefacts and potentially interfere with any measure of evoked power made at this time. This limitation does not exist for measurement of the gamma power response evoked by the prepulse, since this does not, in itself, cause any behavioural response.

Instead of focussing on a particular brain region or specific neuronal circuit, we chose to measure cortical EEG during the PPI sessions. The regulation of PPI involves a complex neural circuitry (Swerdlow et al., 2001) and measurement of local field potentials in any number of relevant brain regions, such as the supramammillo–septal–hippocampal pathway (Ma and Leung, 2007), may provide further insight into the electrophysiological regulation of this behaviour. We chose to assess cortical activity because of the ability of this structure to generate gamma oscillations during cognitive processes (Joliot et al., 1994), and literature is suggestive of functional dysconnectivity in schizophrenia within the cortex (e.g., Friston, 2002; Lewis et al., 2005; Liu et al., 2008).

Abnormalities in dopaminergic neurotransmission have long been associated with the pathophysiology of schizophrenia (Howes and Kapur, 2009). Here we show that amphetamine treatment, which releases dopamine from presynaptic terminals, elevated ongoing gamma power and reduced sensory-evoked responses, suggesting that dopaminergic activation can influence these rhythms. This is in contrast with previous studies, which have suggested that dopaminergic stimulation does not impact either ongoing or sensory-evoked cortical responses (Ehrlichman et al., 2009), although dopamine antagonism with haloperidol can reduce ongoing gamma activity (Ehrlichman et al., 2009; Jones et al., 2012). It is not clear why such discrepancies are observed, but it should be noted that the effects of amphetamine in this study on ongoing activity were modest (see also Pinault, 2008).

In summary, we demonstrated a close temporal relationship between abnormalities in both ongoing and evoked gamma oscillatory power and disruption of PPI following psychotomimetic drug administration. The data from this study significantly strengthen the evidence of a mechanistic link between NMDAr hypofunction, gamma frequency abnormalities and behavioural disturbances, which may have relevance for the pathophysiology of schizophrenia. Future studies should investigate these relationships more closely in patients, and focus on development of therapies, which can alleviate the electrophysiological dysfunction caused by NMDAr hypofunction.

Acknowledgments

The authors appreciate the contribution of A/Prof Didier Pinault for critical review of the manuscript.

Funding and disclosure statement

The authors declare no competing interests, financial or otherwise, associated with this work.

References

Adler CM, Goldberg TE, Malhotra AK, Pickar D, Breier A (1998) Effects of ketamine on thought disorder, working memory, and semantic memory in healthy volunteers. Biol Psychiatry 43:811–816.

Baldeweg T, Spence S, Hirsch SR, Gruzelier J (1998) Gamma-band electroencephalographic oscillations in a patient with somatic hallucinations. Lancet 352:620–621.

Braff DL, Geyer MA (1990) Sensorimotor gating and schizophrenia. Human and animal model studies. Arch Gen Psychiatry 47:181–188.

Carlen M, Meletis K, Siegle JH, Cardin JA, Futai K, Vierling-Claassen D, Ruhlmann C, Jones SR, Deisseroth K, Sheng M, Moore CI, Tsai LH (2012) A critical role for NMDA receptors in parvalbumin interneurons for gamma rhythm induction and behavior. Mol Psychiatry 17:537–48.

Cartmell J, Monn JA, Schoepp DD (2000) Attenuation of specific PCP-evoked behaviors by the potent mGlu2/3 receptor agonist, LY379268 and comparison with the atypical antipsychotic, clozapine. Psychopharmacology (Berl) 148:423–429.

Choy KH, de Visser YP, van den Buuse M (2009) The effect of 'two hit' neonatal and young-adult stress on dopaminergic modulation of prepulse inhibition and dopamine receptor density. Br J Pharmacol 156:388–396.

Cobb SR, Buhl EH, Halasy K, Paulsen O, Somogyl P (1995) Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. Nature 378:75–78.

Coyle JT (2012) NMDA receptor and schizophrenia: a brief history. Schizophr Bull 38:920–926.

Delorme A, Makeig S (2004) EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods 134:9–21.

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

Elvevag B, Goldberg TE (2000) Cognitive impairment in schizophrenia is the core of the disorder. Crit Rev Neurobiol 14:1–21.

Fell J, Klaver P, Lehnertz K, Grunwald T, Schaller C, Elger CE, Fernandez G (2001) Human memory formation is accompanied by rhinal-hippocampal coupling and decoupling. Nat Neurosci 4:1259–1264.

Flynn G, Alexander D, Harris A, Whitford T, Wong W, Galletly C, Silverstein S, Gordon E, Williams LM (2008) Increased absolute magnitude of gamma synchrony in first-episode psychosis. Schizophr Res 105:262–271.

Ford JM, Roth WT, Menon V, Pfefferbaum A (1999) Failures of automatic and strategic processing in schizophrenia: comparisons of event-related brain potential and startle blink modification. Schizophr Res 37:149–163.

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

Friston KJ (2002) Dysfunctional connectivity in schizophrenia. World Psychiatry 1:66–71.

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.

Goff DC, Coyle JT (2001) The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. Am J Psychiatry 158:1367–1377.

Haenschel C, Baldeweg T, Croft RJ, Whittington M, Gruzelier J (2000) Gamma and beta frequency oscillations in response to novel auditory stimuli: a comparison of human electroencephalogram (EEG) data with in vitro models. Proc Natl Acad Sci USA 97:7645–7650.

Hakami T, Jones NC, Tolmacheva EA, Gaudias J, Chaumont J, Salzberg M, O'Brien TJ, Pinault D (2009) NMDA receptor hypofunction leads to generalized and persistent aberrant gamma oscillations independent of hyperlocomotion and the state of consciousness. PLoS One 4:e6755.

Holstein DH, Vollenweider FX, Geyer MA, Csomor PA, Belser N, Eich D (2013) Sensory and sensorimotor gating in adult attention-deficit/hyperactivity disorder (ADHD). Psychiatry Res 205:117–126.

Howes OD, Kapur S (2009) The dopamine hypothesis of schizophrenia: version III – the final common pathway. Schizophr Bull 35:549–562.

Jensen O, Kaiser J, Lachaux JP (2007) Human gamma-frequency oscillations associated with attention and memory. Trends Neurosci 30:317–324.

Joliot M, Ribary U, Llinas R (1994) Human oscillatory brain activity near 40 Hz coexists with cognitive temporal binding. Proc Natl Acad Sci USA 91:11748–11751.

Jones NC, Salzberg MR, Kumar G, Couper A, Morris MJ, O'Brien TJ (2008) Elevated anxiety and depressive-like behavior in a rat model of genetic generalized epilepsy suggesting common causation. Exp Neurol 209:254–260.

Jones NC, Reddy M, Anderson P, Salzberg MR, O'Brien TJ, Pinault D (2012) Acute administration of typical and atypical antipsychotics reduces EEG gamma power, but only the preclinical compound LY379268 reduces the ketamine-induced rise in gamma power. Int J Neuropsychopharmacol 15:657–668.

Kedzior KK, Koch M, Basar-Eroglu C (2007) Auditory-evoked EEG oscillations associated with prepulse inhibition (PPI) of auditory startle reflex in healthy humans. Brain Res 1163:111–118.

Kocsis B (2012) Differential role of NR2A and NR2B subunits in *N*-methyl-D-aspartate receptor antagonist-induced aberrant cortical gamma oscillations. Biol Psychiatry 71:987–995.

Kopell N, Ermentrout GB, Whittington MA, Traub RD (2000) Gamma rhythms and beta rhythms have different synchronization properties. Proc Natl Acad Sci USA 97:1867–1872.

Korotkova T, Fuchs EC, Ponomarenko A, von Engelhardt J, Monyer H (2010) NMDA receptor ablation on parvalbumin-positive interneurons impairs hippocampal synchrony, spatial representations, and working memory. Neuron 68:557–569.

Kulikova SP, Tolmacheva EA, Anderson P, Gaudias J, Adams BE, Zheng T, Pinault D (2012) Opposite effects of ketamine and deep brain stimulation on rat thalamocortical information processing. Eur J Neurosci 36:3407–3419.

Kwon JS, O'Donnell BF, Wallenstein GV, Greene RW, Hirayasu Y, Nestor PG, Hasselmo ME, Potts GF, Shenton ME, McCarley RW (1999) Gamma frequency-range abnormalities to auditory stimulation in schizophrenia. Arch Gen Psychiatry 56:1001–1005.

Lee KH, Williams LM, Breakspear M, Gordon E (2003a) Synchronous gamma activity: a review and contribution to an integrative neuroscience model of schizophrenia. Brain Res Brain Res Rev 41:57–78.

Lee KH, Williams LM, Haig A, Gordon E (2003b) "Gamma (40 Hz) phase synchronicity" and symptom dimensions in schizophrenia. Cogn Neuropsychiatry 8:57–71.

Leicht G, Kirsch V, Giegling I, Karch S, Hantschk I, Moller HJ, Pogarell O, Hegerl U, Rujescu D, Mulert C (2010) Reduced early auditory evoked gamma-band response in patients with schizophrenia. Biol Psychiatry 67:224–31.

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

Liu Y, Liang M, Zhou Y, He Y, Hao Y, Song M, Yu C, Liu H, Liu Z, Jiang T (2008) Disrupted small-world networks in schizophrenia. Brain 131:945–961.

Ma J, Leung LS (2007) The supramammillo-septal-hippocampal pathway mediates sensorimotor gating impairment and hyperlocomotion induced by MK-801 and ketamine in rats. Psychopharmacology (Berl) 191:961–974.

Newcomer JW, Farber NB, Jevtovic-Todorovic V, Selke G, Melson AK, Hershey T, Craft S, Olney JW (1999) Ketamine-induced NMDA receptor hypofunction as a model of memory impairment and psychosis. Neuropsychopharmacology 20:106–118.

Oranje B, Geyer MA, Bocker KB, Leon Kenemans J, Verbaten MN (2006) Prepulse inhibition and P50 suppression: commonalities and dissociations. Psychiatry Res 143:147–158.

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.

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

Spencer KM (2011) Baseline gamma power during auditory steady-state stimulation in schizophrenia. Front Hum Neurosci 5:190.

Spencer KM, Nestor PG, Niznikiewicz MA, Salisbury DF, Shenton ME, McCarley RW (2003) Abnormal neural synchrony in schizophrenia. J Neurosci 23:7407–7411.

Spencer KM, Salisbury DF, Shenton ME, McCarley RW (2008) Gamma-band auditory steady-state responses are impaired in first episode psychosis. Biol Psychiatry 64:369–375.

Swerdlow NR, Geyer MA, Braff DL (2001) Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. Psychopharmacology (Berl) 156:194–215.

Swerdlow NR, Geyer MA, Shoemaker JM, Light GA, Braff DL, Stevens KE, Sharp R, Breier M, Neary A, Auerbach PP (2006) Convergence and divergence in the neurochemical regulation of prepulse inhibition of startle and N40 suppression in rats. Neuropsychopharmacology 31:506–515.

Swerdlow NR, Weber M, Qu Y, Light GA, Braff DL (2008) Realistic expectations of prepulse inhibition in translational models for schizophrenia research. Psychopharmacology (Berl) 199:331–388.

Swerdlow NR, Light GA, Breier MR, Shoemaker JM, Saint Marie RL, Neary AC, Geyer MA, Stevens KE, Powell SB (2012) Sensory and sensorimotor gating deficits after neonatal ventral hippocampal lesions in rats. Dev Neurosci 34:240–249.

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

van den Buuse M (2010) Modeling the positive symptoms of schizophrenia in genetically modified mice: pharmacology and methodology aspects. Schizophr Bull 36:246–270.

Yee BK, Feldon J (2009) Distinct forms of prepulse inhibition disruption distinguishable by the associated changes in prepulse-elicited reaction. Behav Brain Res 204:387–395.